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SPECTROPHOTOMETRIC ANALYSIS OF CYSTEINE INTERACTION WITH HEAVY METAL SALTS

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ABSTRACT: The contamination from heavy metals has been rising from the last decade due to increase in industrialization. The heavy metals are toxic for living organisms. The accumulation of heavy metals may lead to severe health problems. Many drugs are known to remove heavy metals from living organisms but sometime they may be destructive. The need-of-the-hour; however is to evolve procedures for removal of heavy metals. There is a need to design a drug which has no ill effects. The present study describes an effective and friendly approach to explore the removal of heavy metals using cysteine. The study involves possible potential of designing drug containing cysteine to remove heavy metals. Cysteine is the one of the most reactive amino acid found in proteins due to presence of sulfhydryl (SH) group. It accumulates a large numbers of heavy metals. This study suggests the metal interactions of cysteine with heavy metals such as Murcury, Cobalt, Zinc, Copper and Cadmium etc. The interactions were monitored by using UV-VIS spectrophotometer.

Key words: Heavy metals, Cysteine, Metals toxicity, Sulfhydryl group, Metal interactions

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INTRODUCTION

Heavy metals are present in soil either as natural component or as a result of human activities. Metal-rich mine tailings, metal smelting, electroplating, gas exhausts, energy and fuel production, downwash from power lines, intensive agriculture, and sludge dumping are the human activities that introduce largest quantity of heavy metals into soil. These heavy metals are common and serious pollutant because of its toxicity. If ingested, they can accumulate in body organs, including brain, and may result in various degrees of poisoning. At a high level of exposure heavy metals can cause not only severe damage to the brain and kidneys of children and adults but can also be life threatening. The sulfhydryl-reactive metals (mercury, cadmium, lead, arsenic) are particularly insidious and can affect a vast array of biochemical and nutritional processes (David Quig, 1998). The objective of this study is to evolve designing a drug containing cysteine to remove heavy metals. Cysteine (HO2CH (NH2) CH2SH) is the one of the most reactive amino acid found in proteins due to presence of sulfhydryl (SH) group. The thiol groups have affinity for metals, so cysteine can interact with metals salts (Swaran J.S. Et al., 2010). When metal-binding capacity for various heavy metals by *S. cerevisiae* under different conditions were compared, it was found that Lead and uranium could be removed from dilute solutions more effectively in comparison with other metals. (Jianlong Wang' and Can Chen, 2006). Some vegetables like Onion and garlic contain many sulfur containing active principles mainly in the form of cysteine derivatives. These therapeutic agents possess antidiabetic, antibiotic, hypocholesterolaemic, fibrinolytic and various other biological actions (Augusti KT, 1996).

The normal chromophore activity in cysteine is due to the sulphur in which the transition takes place from non bonding orbital 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 decrease 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 undergo electronic transitions (Stephen L. Upstone, 2000). 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). Among the heavy metal-binding ligands in plant cells the phytochelatins (PCs) and metallothioneins (MTs) are the best characterized. PCs and MTs are different classes of cysteine-rich, heavy metal-binding protein molecules. PCs are enzymatically synthesized peptides, whereas MTs are gene-encoded polypeptides (Christopher Cobbett and Peter Goldsbrough, 2002).

MATERIALS AND METHODS

Metal salt used:

A total no. of five metal salts viz. Copper sulphate ($CuSo_4$), Cadmium chloride ($CdCl_2$), Cobalt chloride ($Cocl_2$), Murcuric chloride ($HgCl_2$)) and Zink sulphate ($ZnSo_4$), 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:

1gm of each salt i.e. Copper sulphate ($CuSo_4$), Cadmium chloride ($CdCl_2$), Cobalt chloride ($Cocl_2$), Murcuric chloride ($HgCl_2$)) and Zink sulphate ($ZnSo_4$), were weighed and dissolved separately in 10 ml of distilled water using orbital shaker.

Cysteine stock preparations (1M):

1.211g of cysteine was weighed and dissolved in 10ml of distilled water in a conical flask and shaken well using orbital shaker to form 1M cysteine stock solution.

Interaction of cysteine with different metal salt solutions and spectrophotometric Analysis: 2 ml Cysteine stock and 8ml of each prepared metal salt solutions were mixed. The mixture of five metal salts and cysteine were then separately analyzed with UV-Vis spectrophotometer at a wavelength range of 200-700nm. Distilled water was used as a reference for these samples. The absorbance values thus obtained in spectroscopic analysis were recorded.

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 (.053) at 584 nm wavelength was observed (TABLE I) and absorbance of cysteine-metal salt solution i.e. effect of metals on cysteine was studied.

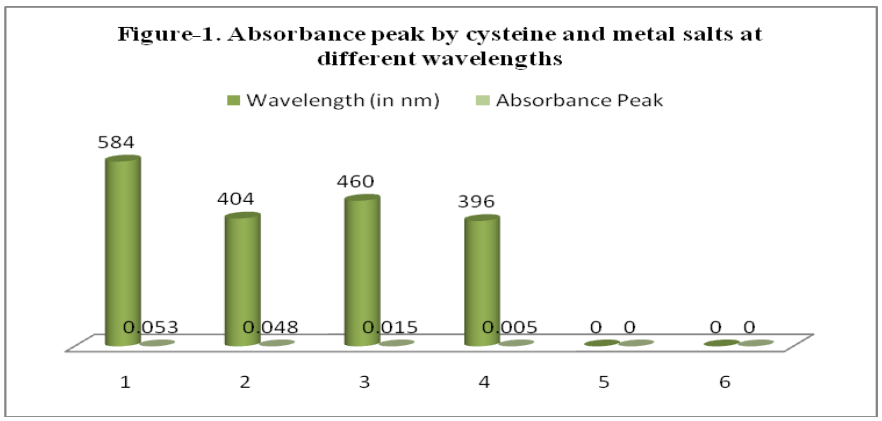


Table-1: Absorbance for cysteine and different metal salt solutions under spectrophotometric analysis-

S. No.	Sample	Wavelength (in nm)	Absorbance Peak
1.	Cysteine	**584**	**.053**
2.	Copper sulphate	**404**	**.048**
3.	Cobalt chloride	**460**	**.015**
4.	Zink sulphate	**396**	**.005**
5.	Cadmium chloride	--	--
6.	Murcuric chloride	--	--

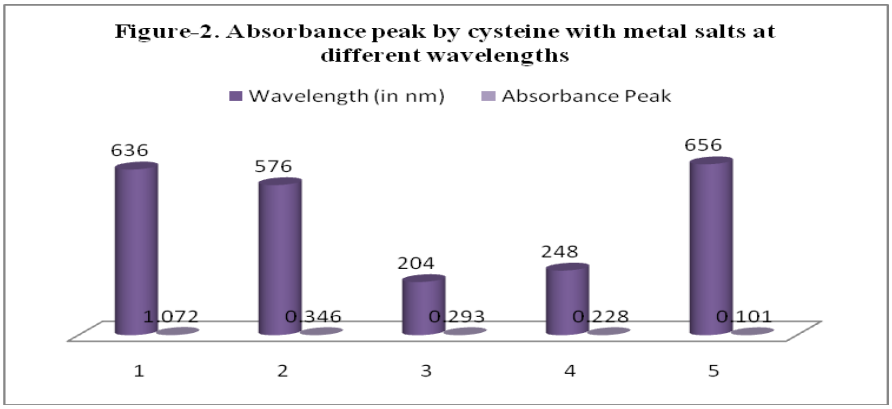
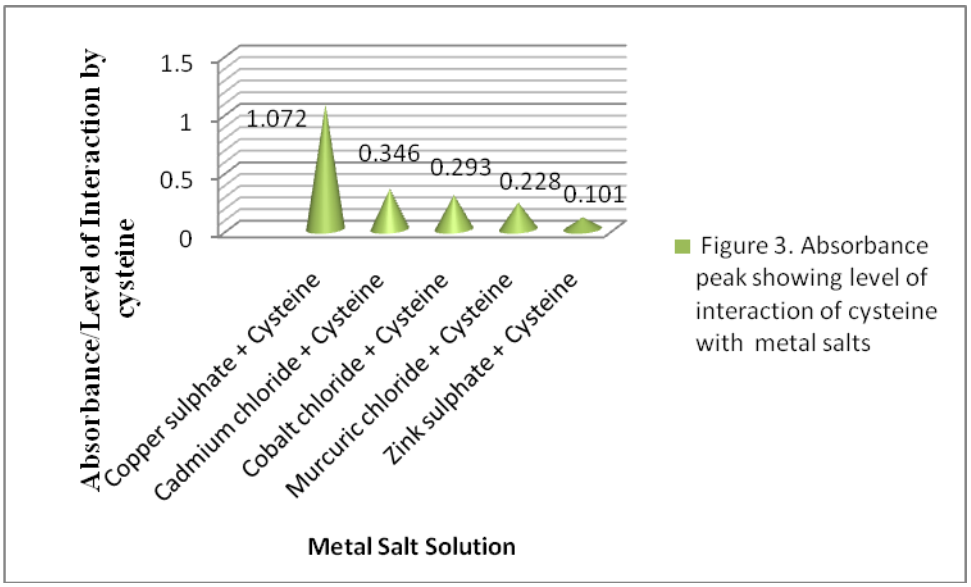


Table-2: Absorbance for mixture of cysteine and different metal salt solutions under spectrophotometric analysis

S. No.	Sample	Wavelength (in nm)	Absorbance Peak
1.	Copper sulphate + Cysteine	**636**	**1.072**
2.	Cadmium chloride + Cysteine	**576**	**.346**
3.	Cobalt chloride + Cysteine	**204**	**.293**
4.	Murcuric chloride + Cysteine	**248**	**.228**
5.	Zink sulphate + Cysteine	**656**	**.101**



Mixture of cysteine with Copper sulphate:
At 636 nm absorbance for Copper sulphate solution with cysteine is 1.072 (Table-2) which is higher than all other metal salt solutions which show higher interaction and change in its chromophore activity.

Mixture of cysteine with Cadmium chloride:
At 576 nm absorbance for solution of Cadmium choride is .346 (Table-2) which is lesser than absorbance of mixture of copper sulphate and cysteine 1.072 (Table-2). But higher than all other metal salt solutions. Change in absorbance after adding cysteine with Cadmium choride shows its interaction and binding with metal salt.

Mixture of cysteine with Cobalt chloride:
At 204 nm, absorbance for mixture of cysteine with Cobalt chloride is .293 (Table-2), while for cysteine stock it was .053 at 584 nm (Table-1). At 460 nm absorbance for Cobalt chloride is .015 (Table-1). It shows interaction of cysteine with Cobalt chloride and change in its chromophore activity. There is increase in absorbance for mixture of cysteine with Cobalt chloride, so there is chelation in cysteine with mixture of Cobalt chloride.

Mixture of cysteine with Murcuric chloride:
At 248 nm, absorbance for solution of Murcuric chloride is .228 (Table-2) which is close to Cobalt chloride absorbance i.e. 0.293 (TABLE II).

Mixture of cysteine with Zink sulphate:
At 396 nm, absorbance for solution of Zink sulphate is .005 (Table-1). Absorbance with cysteine is .101 at 656 nm (Table-2), and for cysteine stock it was .053 at 584 nm (Table-1). It shows interaction of cysteine with Zink sulphate and change in its chromophore activity.

CONCLUSION

The metal binding to that of cysteine was probably due to interaction of thiol group of cysteine which was responsible for the binding of cysteine with metals. The metal binding can be interpreted by the change in the absorbance peak value or the shift in the peak of absorption of cysteine and the cysteine-metal solutions. The peak shift and absorbance 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 shift in the peak intensity was observed. It might be due to the formation of stable complexes of cysteine with the metal ions which absorbs more light at particular wavelength. It can be concluded that range of the peak was specific for different metal salt. When the effect of Copper sulphate ($CuSo_4$), Cadmium chloride ($CdCl_2$), Cobalt chloride ($Cocl_2$), Murcuric chloride ($HgCl_2$)) and Zink sulphate ($ZnSo_4$), was compared, it was found that metal binding can be easily determined by the spectrum data which shows peak shift of same solution. So, finally we can say that the binding capacity of cysteine with Copper sulphate is more followed by Cadmium chloride, Cobalt chloride, Murcuric chloride and Zink sulphate based on the change 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 and to prevent the accumulation of such metals. So we can also use cysteine for water purification, removal of metals from our body considering the fact that cysteine may not have adverse effect in the system. Vegetables like Onion and garlic contain many natural sulfur containing active principles mainly in the form of cysteine derivatives. Based on the above results, we can go for designing a new type of drug containing cysteine which will prevent us from adverse side effects of metals.

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