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EXPLORING THE POTENTIAL OF MOMORDICIN AS BIO CHELATOR FOR THE COMPLEXATION WITH HEAVY METALS: REMEDIAL APPROACH

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ABSTRACT

Environmental contamination and exposure to heavy metals such as, mercury, cadmium, lead and arsenic, is a serious growing problem throughout the world. These metals are highly toxic and can cause damaging effects even at very low concentrations. Quantitative estimations of these heavy metals in digested human blood samples collected from different areas of Rajasthan were carried out. The concentrations of these heavy metals were observed near or above the permissible limits in almost 50% of the collected samples. In recent years, particular attention has been paid to studies on the complexation of important bio elements with biologically active ligands to avoid the side effects caused by synthetic chelators. In present study, role of momordicin was evaluated in providing better therapeutic outcomes in heavy metal toxicity. Momordicin was extracted from *Momordica charantia* in slightly acidic medium (at pH 6) to react with certain toxic metals (As, Pb, Hg and Cd). The complexation of momordicin with selected heavy metals was carried out at 30^{o}C at pH 5.8. Spectral studies confirmed the presence of metal- oxygen bonding in the synthesized complexes. Spectrophotometric methods were also implemented for evaluation of stability constants of the synthesized complexes. The present study reveals the potential of momordicin to act as a bio chelator**.**

KEYWORDS: chelation, momordicin, *Momordica charantia*, bio chelator.

INTRODUCTION

Heavy metal ions are highly associated with human life from several decades; however some of these are toxic even at low concentrations when they enter into human body. These heavy metals are kept under environmental pollutant category due to their toxic effects in plants, human and food particularly in areas with high anthropogenic pressure.[1] Heavy metal pollution is an inorganic chemical hazard, which is mainly caused by lead (Pb), chromium (Cr), arsenic (As), cadmium (Cd), mercury (Hg), zinc (Zn), copper (Cu), cobalt (Co), and nickel (Ni).[2] Five metals among them, Pb, Cr, As, Cd, and Hg, are the key heavy metal pollutants. These heavy metals are classified as strong carcinogens by the International Agency for Research on Cancer.[3] Heavy metal is an inexact term used to describe more than a dozen elements that are metals or metalloids which cannot be degraded or destroyed, so these are persistent in all parts of environment.[4,5] Naturally occurring levels of heavy metals below toxic limits are usually not harmful to living organisms, but higher concentration can cause toxic effects. These toxic elements enter the human body mostly through food and water and to a lesser extent through inhalation of polluted air, use of cosmetics, drugs, poor quality herbal formulations particularly and even items like toys which have paint containing lead.[6]

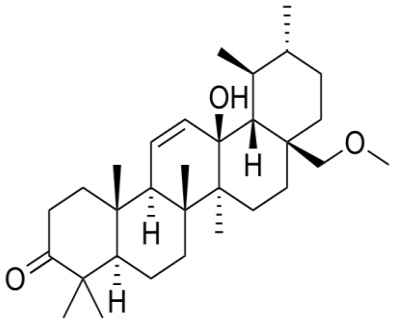
Humans also affect the natural, geological and biological redistribution of heavy metals by altering the chemical form of heavy metals released to the environment. Such alterations often affect the heavy metal's toxicity by allowing it to bio accumulate in plants and animals, bio concentrate in the food chain, or attack specific organs of the body.[7] The main threats to human health from heavy metals are associated with exposure to lead, cadmium, mercury and arsenic. These metals have been extensively studied and their effects on human health regularly reviewed by international bodies such as the WHO.[8]

Several efforts have been made to detoxify the effect of metals once they are administered in the human body. Chelation is considered as the best method used so far.[9] Medicinal treatment of acute and chronic metal toxicity is provided by chelating agents. Mainly atoms like S, N and O function as ligand atoms in the form of functional groups like –SH, –S-S, –NH_2, =NH, –OH, –OPO_3H, or >C=O.[10]

The chelating agents, which have been widely used for metal detoxification are: EDTA, BAL, DMPS, DMSA, D-pencillamine, deferoxamine All these synthetic chelators are reported to show severe side effects.[11]

So, it has become necessary to look for other safe options for heavy metal detoxification. The present study is an effort in this direction. The chelating tendency of momordicin with certain heavy metals (As, Pb, Cd and Hg) has been observed to explore the potential of momordicin as bio chelator. Momordicin is an alkaloid which is a bioactive phyto chemical compound extracted from *Momordica charantia*.[12]

Fruits of *Momordica charantia*



Momordicin

MATERIALS AND METHODS

Equipments

Spectrophotometric measurements were performed on a UV -1700 Shimadzu double beam spectrophotometer (Japan) using matched 10 mm quartz cells. FTIR spectroscopic studies of the synthesized complexes were determined by SHIMADZU, spectrophotometer within the range of 400–4000 cm^{-1}.

Collection of plant materials

The fruits of *Momordica charantia* were collected from local market of Jaipur. The fruit parts were cut in to small pieces, air-dried and pulverized in to course powder by using a dry grinder and passed through the sieve before being stored in closed vessel for further use.

Extraction of momordicin from bitter gourd

Powdered and weighed plant materials (1g) were taken in 100mL Erlenmeyer flasks containing distill water (50mL/g) and 5mL of 0.05 N $H_2SO_4$was added to it. Mixture was macerated for 3-4 hours and boiled gently for 25 minutes. Heavy magnesium oxide (2.5g/g) was added to the mixture and again boiled gently for 20 minutes. It was cooled at room temperature and an equal amount of distilled water was added to make up for loss of distilled water during boiling. Alcohol was added to remove the mucus. Mixture was filtered through

Whatman filter paper.[13] (Kogan *et al*, 1953*)*. Filtrate was evaporated to dryness *in vacuo,* reconstituted in distill water for further analysis.

Spectrophotometric determination of complexes

Continuous Variation Method

A series of solutions were prepared by mixing 1 to 9 ml of 1 M solution of $AsCl_3$, $Pb(CH_3COO)_2$, $CdCl_2$ and $HgCl_2$ with an aliquot of momordicin in such a way that the mole fraction of the solution remained constant. The wavelength of maximum absorbance was noted against the reagent blank for each of the metal complex. All the measurements were made at their respective wavelength for the concerned complex at 30^0C. Each of the molar solution was treated as per the procedure reported in the literature.[14]

Mole Ratio Method

To a series of solutions containing 2 ml of each metal ion ($AsCl_3$, $Pb(CH_3COO)_2$, $CdCl_2$ and $HgCl_2$) increasing amounts of momordicin volumes were added. Each solution was treated as per the developed method.[15] All the measurements were made at wavelength of maximum absorbance for each metal complex.

Complexation of heavy metals with momordicin:

1 M solutions of $AsCl_3$, $Pb(CH_3COO)_2$, $CdCl_2$ and $HgCl_2$ were prepared separately and made to react with extracted momordicin at pH 5.8.

RESULTS AND DISCUSSION

The complexation reactions of momordicin with arsenic chloride, lead acetate, mercury chloride and cadmium chloride were carried out at 30^0 C and at pH 5.8. The absorption spectra were recorded over the wavelength range of 300 to 400 nm. The values of λ_{max} were determined for all the four coordination complexes of arsenic, cadmium, mercury and lead with momordicin which came out to be 340 nm, 320 nm, 360 nm and 300 nm respectively and these values were used for analytical measurements.

Determination of composition and stability constants of heavy metal-momordicin complexes

Variation method

The stoichiometric ratio of heavy metal (As, Cd, Pb and Hg) to momordicin was determined by Job's method of equimolar solutions. The curves for all the four metals – momordicin

complexes displayed maximum absorbance at mole fraction X_{metal} = 0.3, which indicates the formation of complex with metal ion to ligand ratio 1:2. The corresponding equation [16] used in this study for Job's method is as follows:

$$K = \frac{[ML]}{[M]\times[L]}$$

$$K_{st} = \frac{[A_2/A_1]}{[1-A_2/A_1] \times [C_{momordicin} - C_{metal} \times A_2/A_1]}$$

Where A_1 = absorbance at inflection point, A_2= actual absorbance, C_{metal}= concentration of metal ions taken for study, $C_{momordicin}$ =concentration of momordicin

Mole ratio method

Mole ratio is another method for determining stoichiometry of the complexes spectrophotometrically. By using the mole ratio method, at constant metal concentration (for As, Hg, Pb and Cd each) and varying momordicin concentrations at their absorption maximum, a sharp band was observed at 1:2 mole ratio of metal and ligand. The ratio of the units in the complex species is determined by the inflection point in a plot of optical density versus the mole ratio of the reactants. As the complex has metal to ligand ratio equal to 1:2, the suggested general equation for the complexes is:

$$M^{n+}_{metal\ ion} + 2\ L_{momordicin} = [M][L]_2$$

The corresponding equation [17] for the mole ratio method is as follows:

$$K_{st} = \frac{A/\epsilon b}{[C_{metal} - A/\epsilon b] \times [C_{momordicin} - A/\epsilon b]}$$

Where ϵb = molar absorptivity constant , A = absorbance at peak point.

ϵ is dependent upon wavelength and is called molar absorption coefficient and has units of L mol^{-1} cm^{-1}.The use of this symbol specifically requires that the concentration is expressed in units of molarity and sample path length in cm.

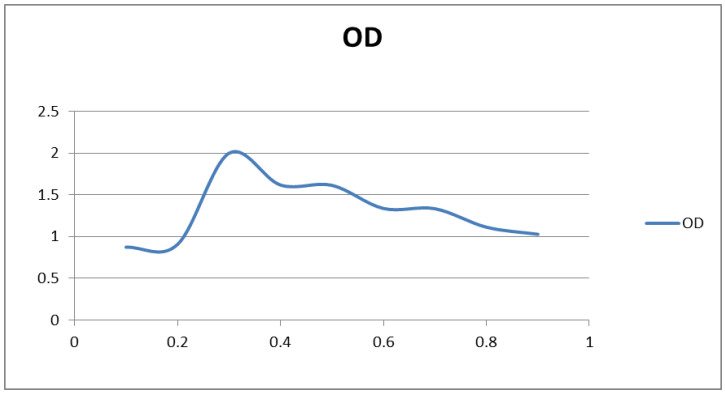


Fig. 1 Arsenic – momordicin curve by Job's method

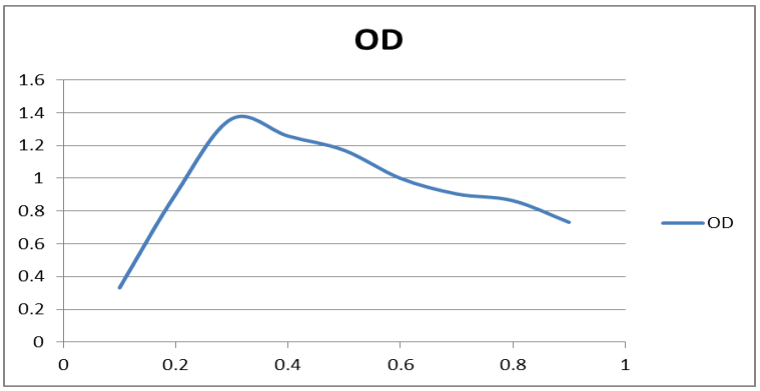


Fig. 2 Cadmium- momordicin curve by job's method

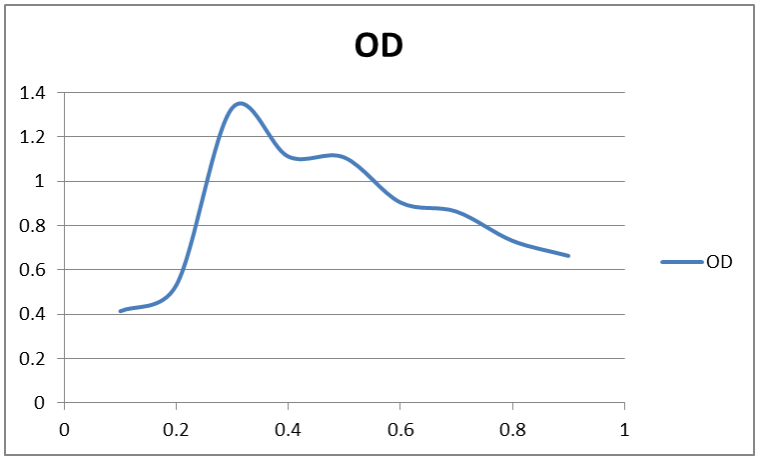


Fig. 3 Mercury – momordicin curve by job's method

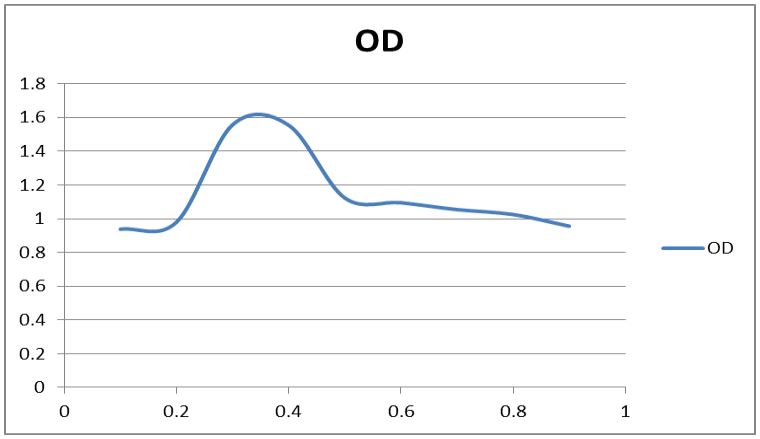


Fig. 4 Lead – momordicin curve by job's method

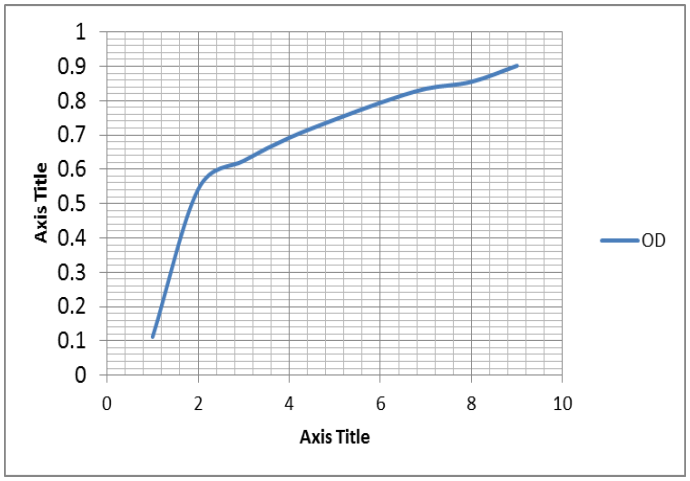


Fig. 5 Arsenic –momordicin curve by mole ratio method

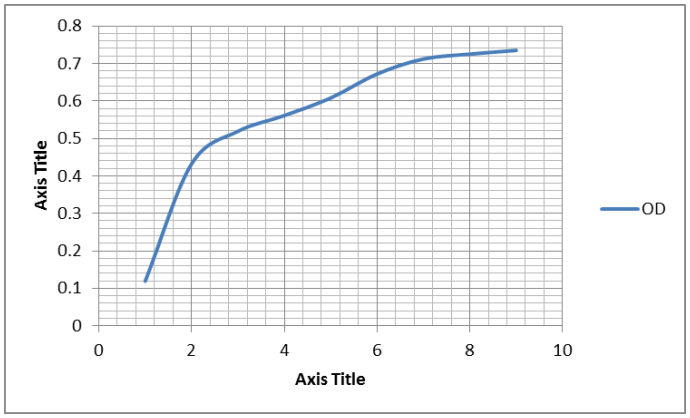


Fig. 6 Cadmium - momordicin curve by mole ratio method

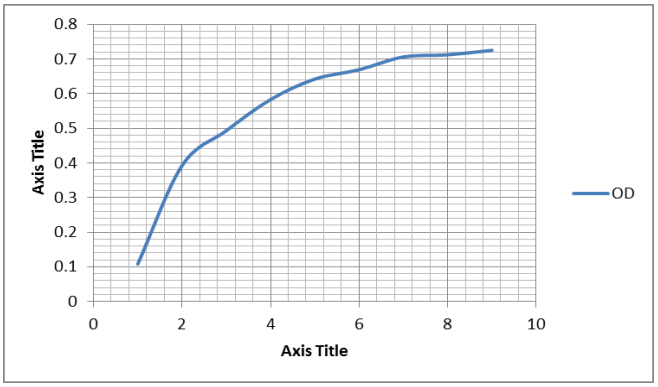


Fig. 7 Mercury – momordicin curve by mole ratio method

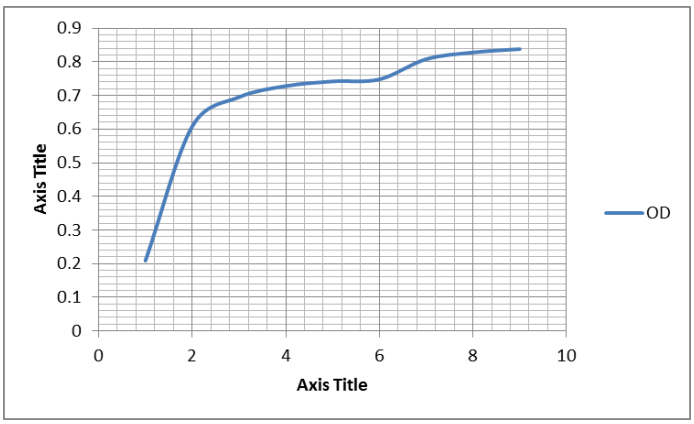


Fig. 8 Lead – momordicin curve by mole ratio method

The stability constants were determined by Job's method, which were further confirmed by mole ratio method.

Table: 1 Stability constants of heavy metal- momordicin complexes

S.No.	Name of the complex	Metal : Ligand	Stability constant
1.	Arsenic-momordicin complex	1:2	4.68×10^{2}
2.	Cadmium-momordicin complex	1:2	1.02×10^{2}
3.	Mercury- momordicin complex	1:2	7.7×10^{3}
4.	Lead-momordicin complex	1:2	3.24×10^{3}

FT-IR analysis

The IR spectral data of momordicin and its complexes with heavy metals (As, Cd, Hg and Pb respectively) are presented in table 2. The IR spectra of the complexes were compared with that of the free ligand in order to determine the coordination sites that may be involved in chelation. The spectrum of free ligand shows a band at 3500 cm^{-1} which corresponds to υ (O-H) stretching. The absorption peaks at 2924 (=C-H) and 1622 (C=C) cm^{-1} suggested the presence of olefin bond in the compound and the band at 1740 cm^{-1} indicates the presence of carbonyl group.[18] In the metal momordicin complexes, there is shifting of bands for OH (str)

and carbonyl group towards lower wave numbers which indicates the involvement in coordination. The appearance of a new non-ligand band around (474-452) cm^{-1} in all complexes may be assigned due to metal oxygen bonding.[19]

Table 2: Comparative table of IR peaks of momordicin and momordicin –metal complexes

	Significant peaks of momordicin (in cm^{-1})	Significant peaks of momordicin –Arsenic complex (in cm^{-1})	Significant peaks of momordicin – Cadmium complex (in cm^{-1})	Significant peaks of momordicin – lead complex (in cm^{-1})	Significant peaks of momordicin – Mercury complex (in cm^{-1})
1.	3500 (-OH)	3436	3390	3406	3445
2.	2924$(=C-H)_{str}$	2924			2923
3.	1740(>C=O)	1594	1626	1569	1613
4.	1622$(C=C)_{str}$	1439	1443	1415	1532
5.	1369				1441
6.	1296		1105		
7.	1013 (C-O-C)	1052		1023	1055
8.		799	746	665	665
9.		667	629	619	
10.	Metal-O	477	454	421	452

CONCLUSION

The article presents the therapeutic potential of momordicin as a detoxifying agent. Complexes of As, Cd, Hg and Pb with momordicin are found to have metal to ligand ratio as 1:2 (ML_2), where M is metal and L is momordicin. The present study envisages the chelating tendency of momordicin. Since it is present in *Momordica charantia*, it is widely available to people. The significance of this study is use of momordicin as a bio chelator as compared to synthetic chelators which have severe side effects. The most important problem concerning the medical use of synthetic chelating agents is their low therapeutic range, which is mainly due to the inherent toxicity of the chelator itself. Momordicin, being a bio chelator, has the possible beneficial effects of removal of toxic metals in people with low-level exposure without overt symptoms of toxicity. So, being a bio chelator and easily available, momordicin can be used in heavy metal detoxification effectively. It may also be recommended that an increase in consumption of fruits of *Momordica charantia* may prove beneficial for people who are facing problem of exposure to heavy metals.

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