

RESEARCH ARTICLE**STUDY OF L-ARGININE-D-BLOCK ELEMENTS INTERACTIONS**

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Abstract: The amino acid arginine is an important building block of proteins. Although it can be manufactured by the body with proper nutrition (i.e. it is a 'non-essential' amino acid), studies showing it to be involved in a number of important functions in the human body have led to the increased popularity of arginine nutritional supplements. Arginine creates nitric oxide in the body and helps improve blood flow and maintain a proper nitrogen balance.

The visible spectra of some transitional metals Cu, Fe (II), Fe(III), Cr, Mn, Ni have been studied individually in presence of L-arginine with varying amounts. The spectra are also studied for the effect of varying amounts of metal ion on L-arginine. To observe how arginine itself acts on transitional metal ions. The evaluation of these spectra is carried out for its binding parameters with the help of scatchard plots. The work has revealed certain very significant and interesting data which can have a lot of bearing on many chemical, biological and environmental aspects. It is observed that even arginine is capable of changing some transitional properties of metal ions.

Key words: arginine, nitric oxide, scatchard plot, amino acid.

INTRODUCTION:

Nitric oxide is a free radical with one free electron (NO^*) which makes it reacts readily with other molecules and particularly interacts with transitional metal ions. NO^* is released during exercise by the action of an enzymes, nitric oxide synthase, in cells lining arteries. NO^* causes the arteries to dilate and prolongs vasodilatation thereby fa-

cilitating blood flow to muscle cells. NO^* is used to treat people with obstructive pulmonary disease. L-arginine is one of the non essential amino acids which means, that it can be produced by the body. Amino acids are compulsory for our health and well being and are the basic components in all cells and are also known as the building blocks of protein.

In the body L-arginine is used to make nitric oxide, which reduces blood vessel stiffness, increases blood flow and improves blood vessel function. L-arginine has been used for erectile dysfunction, like the drug sildenafil citrate (viagra), L-arginine is thought to enhance the action of nitric oxide, which relaxes muscles surrounding blood vessel supplying the penis. As a result, blood vessels in the penies dilate increasing blood flow, which helps maintain an erection. The difference in how they work is that Viagra blocks an enzyme we called PDES which destroys nitric oxide and L-arginine is used to make nitric oxide. Despite being a simple molecule, NO is a fundamental player in the field of neuroscience, physiology and immunology and proclaimed "Molecule of the year" in 1992.

L-arginine is used to make the nitric oxide a compound in the body that relaxes blood vessels. Preliminary studies have found that L-arginine may help with conditions that improve when blood vessels are relaxed (called vasodilation) such as atherosclerosis, erectile dysfunction and intermittent claudication.

Hence we feel that it will be interesting to see how arginine itself acts on these transitional metals. So we propose to study metal- arginine interactions and the effects of these interactions on these entities itself. The free radical nitric oxide has direct influence on the spectral properties of transitional metals and particularly it has binding interactions with some of them are observed. Since arginine is precursor of nitric oxide synthesis in human body its interaction with transitional elements becomes important⁽⁷⁻⁹⁾. Since many transitional elements like Cu, Zn, Mg and Mn are required for many biological processes like enzyme activity. Nitric oxide preferentially binds to iron (Fe) atom of heme group in proteins; it can also interact with other metal sites in proteins. NO functions as a neurotransmitter, a macrophage derived defense agent against foreign organism and regulate blood flow as vasodilator. Nitric oxide forms complexes with all transitional metals to give complexes called metal nitrosyls. NO can serve as a one electron pseudo halide. Nitric oxide group can also bridge between metal centers through N-atom in variety of geometries.

EXPERIMENTAL WORK

All the chemicals used for the work are of A.R. grade of S.D.Fine or Merk. Spectral analysis is carried out with Shimadzu model 2450 U.V.-Visible spectrophotometer. Transitional metal ions selected for the work are Cu, Mn, Ni, Fe(II), Fe(III), Cr in the form of CuSO_4 , KMnO_4 , NiSO_4 , $\text{K}_2\text{Cr}_2\text{O}_7$, Fe(II) Ammonium Sulphate, Fe(III) Ammonium Sulphate. These metal ion solutions are studied for its λ_{max} values¹³. To 3.0 ml of these metal ion solution varying amount of (0-0.5 ml) of L-Arginine is added and effect on spectra is studied with scatchard plot¹⁸. The reverse way study is also carried out. The binding parameters are determined with the help of scatchard plot⁽¹⁸⁻²⁰⁾.

Based on the IC_{50} values compared with the standard, the ethanolic extract of *P. ligularis* exerts better free radical scavenging ability. The plant extract play a central role in fascinating and neutralizing the free radicals which is due to the presence of secondary metabolites like alkaloids, flavanoids, phenols and saponins in it.

RESULTS AND DISCUSSIONS

a) Metal ion solutions of Cu, Mn, Ni, Fe(II), Fe(III), Cr were used to find their respective λ_{max} .

b)

Metal ion solution	λ_{max}
CuSO_4	800 nm
KMnO_4	525nm
NiSO_4	406 nm
$\text{K}_2\text{Cr}_2\text{O}_7$	400 nm
Fe(II) Ammonium Sulphate	400 nm
Fe(III) Ammonium Sulphate.	400 nm

c) Metal-L-arginine binding interactions by spectral study and scatchard plots to calculate binding parameters.

Metal ion solution + l-arginine spectra were studied at their respective λ_{max} and the results are given in Figures 1-3.

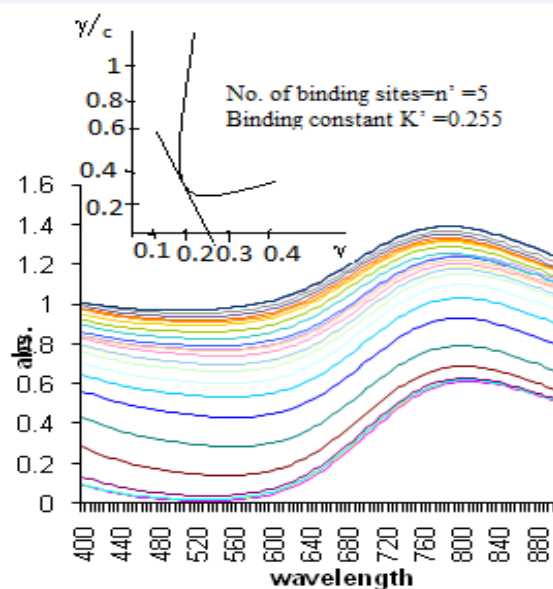


Fig: 1: 3cm³ KMnO_4 + 0.5cm³ L-Arginine

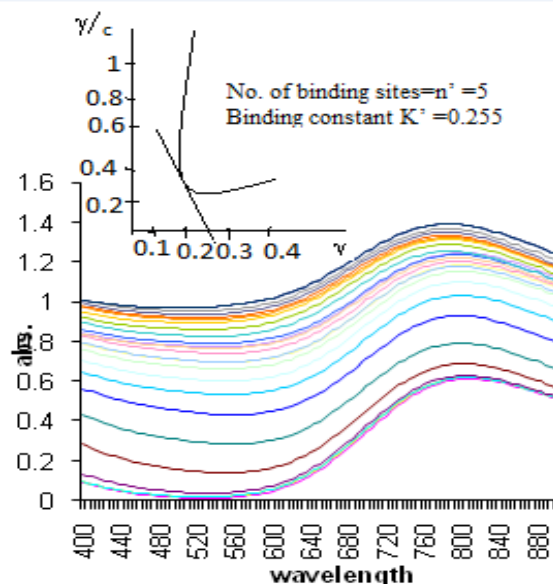


Fig: 2: 3cm³ CuSO_4 + 0.5cm³ L-Arginine

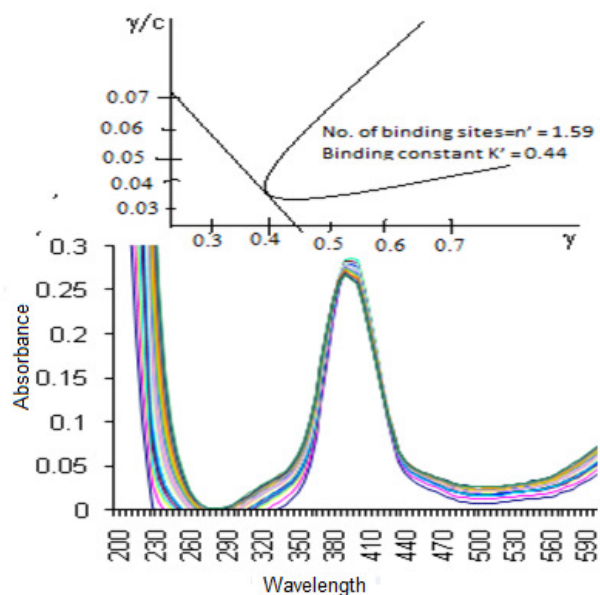
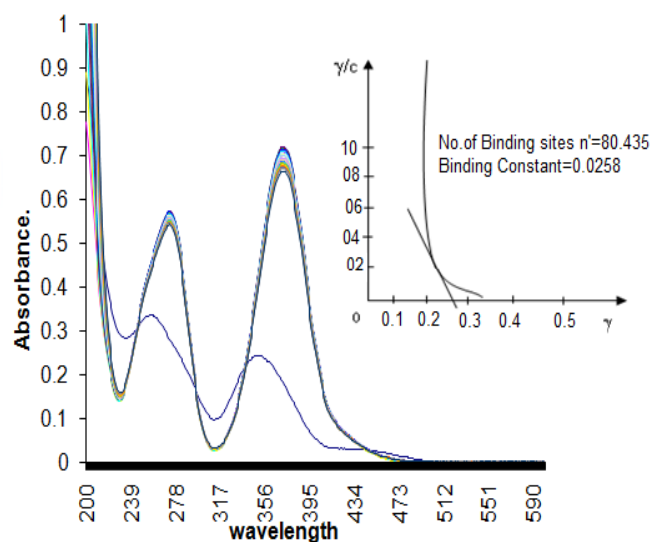
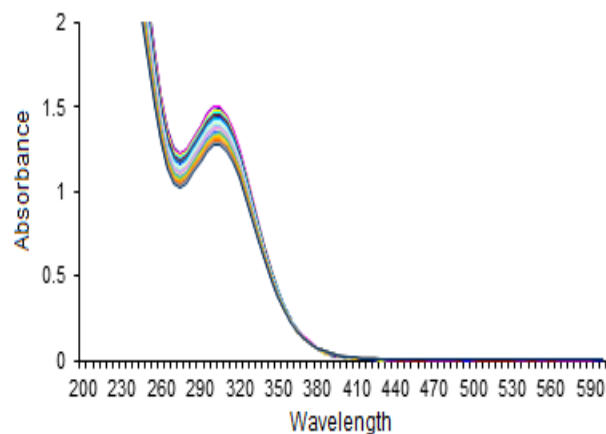
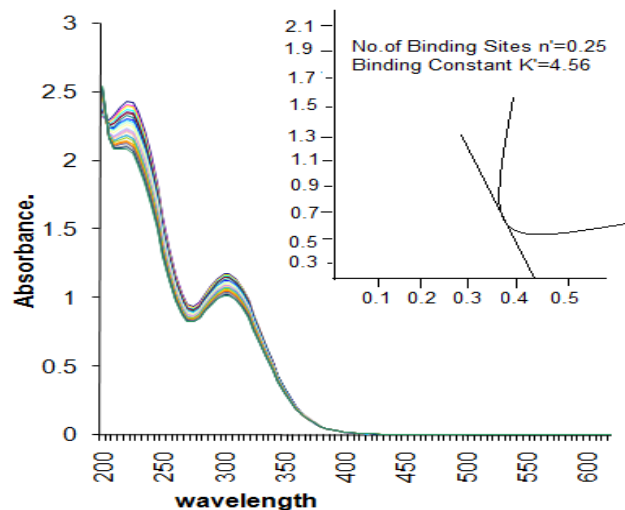
Fig-3: 3cm³ NiSO₄+0.5cm³L-ArginineFig-4: 3cm³ K₂Cr₂O₇+0.5cm³L-ArginineFig-5: 3cm³ Fe(II) A.S.+0.5cm³L-ArginineFig: 6: 3cm³ Fe(III)A.S.+0.5cm³L-Arginine

Fig.1 gives spectra and scatchard plot for KMnO₄, Fig.2 for CuSO₄, Fig.3 for NiSO₄. Fig 4 gives spectra and scatchard plot for K₂Cr₂O₇, Fig 5 for Fe(II) Ammonium Sulphate Fig. 6 gives spectra and scatchard plot for Fe(III) Ammonium Sulphate. Now reverse study is carried out i.e. to 3.0 ml of arginine solution 0- 0.5 ml of CuSO₄ solution is added. Fig 7 gives spectra and scatchard plot for this addition. Fig 8 and 9 for KMnO₄, K₂Cr₂O₇ respectively.

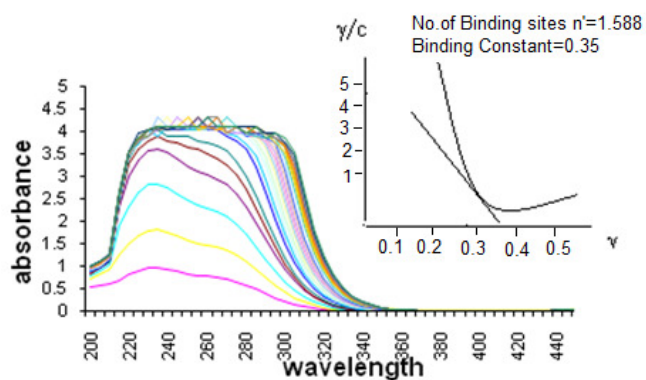
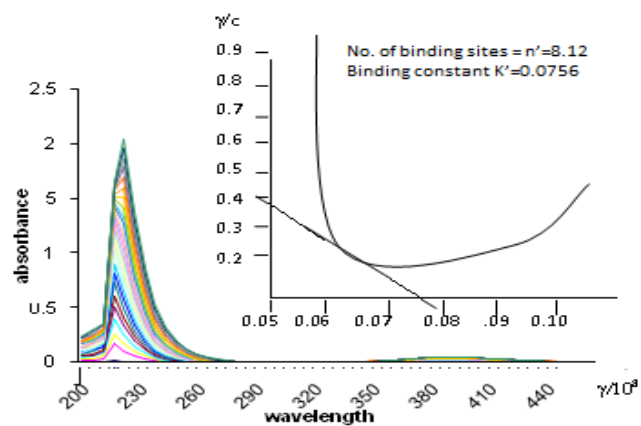
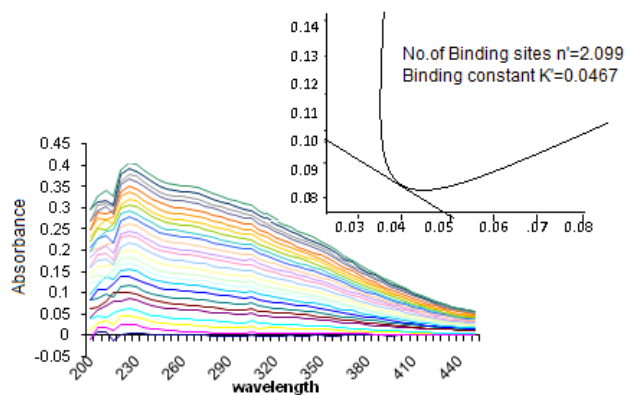
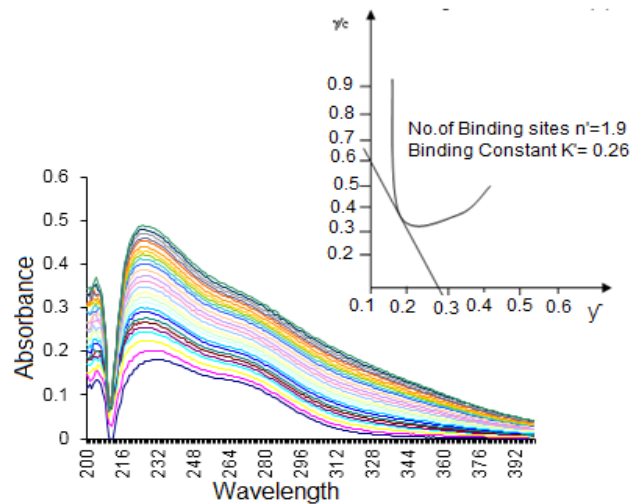
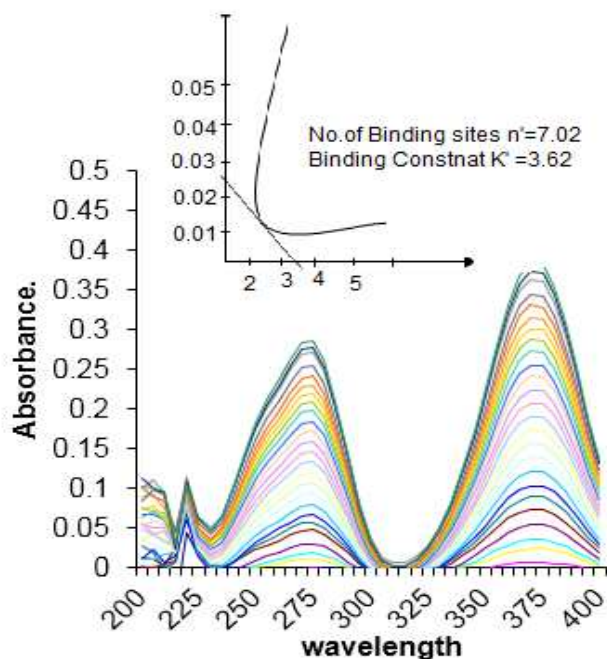
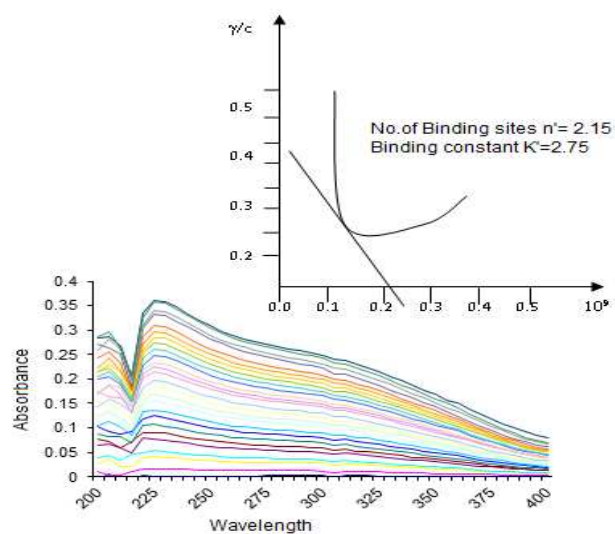
Fig-7: L-Arginine+0.5cm³KMnO₄Fig 10: L-Arginine+0.5cm³NiSO₄Fig-8: L-Arginine+0.5cm³Fig-11: L-Arginine+0.5cm³ Fe(II) A.S.Fig-9: L-Arginine+0.5cm³K₂Cr₂O₇Fig-12: L-Arginine+0.5cm³ Fe(III) A.S.

Fig. 10 gives spectra and scatchard plot for NiSO_4 , Fig 11 and 12 gives spectra and scatchard plot for Fe(II) and Fe(III) Ammonium Sulphate for addition of it to 3.0 ml of arginine.

Most of the results showed a strong curve. Hence the tangent of two types of interactions at the turning point is

taken so as to get the average value.

Calculations of binding parameters:

Table 1 gives values of binding parameters when arginine is added to 3.0 ml of metal ion solution and Table 2 gives values for the reverse way i.e. when 0 to 0.5 ml of metal ion solution is added to 3.0 ml of arginine.

TABLE 1: 3.0 Cm^3 Metal Ion Solution + 0 - 0.5 Cm^3 arginine

SOLUTIONS	λ_{max}	No. of binding sites = n'	Binding constant = K'
3.0 Cm^3 CuSO_4 + 0 - 0.5 Cm^3 arginine	800 nm	0.255	5.0
3.0 Cm^3 KMnO_4 + 0 - 0.5 Cm^3 arginine	525 nm	0.3575	2.436
3.0 Cm^3 NiSO_4 + 0 - 0.5 Cm^3 arginine	406 nm	0.44	1.59
3.0 Cm^3 $\text{K}_2\text{Cr}_2\text{O}_7$ + 0 - 0.5 Cm^3 arginine	400 nm	0.0258	80.435
3.0 Cm^3 Fe(II) A.S. + 0 - 0.5 Cm^3 arginine	400 nm	No Proper graph	
3.0 Cm^3 Fe(III) A.S. + 0 - 0.5 Cm^3 arginine	400 nm	0.25	4.56

TABLE 2: 3.0 Cm^3 arginine + 0 - 0.5 Cm^3 Metal Ion solution

Solutions	λ_{max}	No. of binding sites = n'	Binding constant = K'
3.0 Cm^3 arginine + 0 - 0.5 Cm^3 CuSO_4	196.6 nm	0.35	0.1588
3.0 Cm^3 arginine + 0 - 0.5 Cm^3 KMnO_4	196.6 nm	0.0467	2.099
3.0 Cm^3 arginine + 0 - 0.5 Cm^3 NiSO_4	196.6 nm	0.0756	8.12
3.0 Cm^3 arginine + 0 - 0.5 Cm^3 $\text{K}_2\text{Cr}_2\text{O}_7$	196.6 nm	3.62	7.02
3.0 Cm^3 arginine + 0 - 0.5 Cm^3 Fe(II) A.S.	196.6 nm	0.26	1.9
3.0 Cm^3 arginine + 0 - 0.5 Cm^3 Fe(III) A.S.	196.6 nm	2.15	2.75

From the above study and the results obtained some conclusions can be drawn separately for each metal ion as results are specific in nature.

- **Copper:** with copper sulphate as a base no. of binding sites are 0.255 whereas value of binding constant is only 5.0 with only few positive readings, but with arginine as base no. of binding sites decreased to 0.35 & binding constant 0.1588.
- **Manganese:** used as potassium permanganate no. of binding sites are more with manganese as base indicating more binding sites for manganese to attach to arginine.
- **Nickel:** used as Nickel Sulphate binding constant are much more with arginine as base i.e. 8.12 showing good association but with Nickel Sulphate as base values decreases.
- **Chromium:** used as potassium dichromate, no. binding of sites are much more i.e. 80.435 with potassium dichromate as base whereas binding constants are more with chromium as base. Even with very few positive readings.

- **Ferrous:** For arginine as a base solution Fe(II) interactions are better, but with ferrous as base no graph could be plotted as majority of the readings are negative.
- **Ferric:** used as Ferric Ammonium Sulphate, with metal ion base, binding constants and no of binding sites are almost same. Even with arginine as base the values are also same for both binding parameters.

CONCLUSION

Thus behavior of metal ions is completely different in each case. It appears that every metal has a different interaction with arginine indicating different binding parameters which may have variety of modulation of release of NO in the biosystems and consequent effects. Above results and work shows that there is significant change in transition of metal ions and arginine indicating that arginine is NO donor and modulate the behavior of transitional metal ion in the biological systems

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