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Comparison of L-Arginine and Hydroxyurea Interactractions with Transitional Metal Ions.

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Short Communication

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ABSTRACT

No* is a free radical with one free electron and as such it is very highly reactive and particularly it interacts with transitional metals. Nitric oxide, gas is an important signaling molecule in the body of mammals, including humans and is an extremely important intermediate in chemical industry In biological systems there are many enzymes, which contain transitional elements like iron, copper and manganese, which are the most probable sites for nitric oxide to react. Such type of interactions results in considerable modification of the enzyme functions resulting pathological and even genetic disorders. This needs a critical amount of nitric oxide in the system for proper functioning. To observe the effects of NO*, various NO* donor compounds are used. Hydroxyurea (HU) is shown to increase the levels of NO*.L-arginine is one of the non- essential amino acids. In the body L-arginine is used to make nitric oxide, which reduces blood vessel stiffness, increases blood flow and improves blood vessel function. The visible spectra of some transitional metals Cu, Fe(II),Fe(III),Cr, Mn, Ni have been studied individually in presence of hydroxyurea (HU) with varying amounts .The spectra are also studied for the effect of varying amounts of metal ion on hydroxyurea. To observe how arginine itself acts on transitional metal ions. Even effect of Hydroxyurea on metal-arginine binding is also studied. 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.

INTRODUCTION

Nitric oxide – small inorganic molecule which was probably best known to general public as pollutant in car exhaust, became biological molecule of 1990's. Its importance was recognized by the award of a Nobel prize to Furchgott, Ignarro and Murad in 1998'for their discoveries concerning nitric oxide as a signaling molecule in the cardiovascular system'

Nitric oxide plays major role in human physiology. NO functions as a neurotransmitter, a macrophage derived defense agent against foreign organism and regulate blood flow as vasodilator [1,2,3,4,5,6] Nitric oxide forms complexes with all transitional metals to give complexes called metal nitrosyla. 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 [7].

Nitric oxide is produced in the body by enzyme called nitric oxide sysnthase, which converts the amino acid L-arginine to nitric oxide and L-citrulline. There are three types of nitric oxide synthase: brain, endothelial and inducible. NO plays several major roles in human physiology. Nitric oxide functions as a neurotransmitter, a macrophage-derived defense agent against foreign organisms and regulates blood flow as vasodilator. Nitric oxide has great physiological importance and forms the basis of several current or considered therapeutic methodologies. Nitric oxide has good site and a bad side, while excess Nitric oxide in septicaemia, inflammation and stroke causes severe damage, while localized production of small quantities of nitric oxide is essential for normal body function.

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Hydroxyurea represents an approved treatment for sickle cell anemia and acts as nitric oxide donor under oxidative conditions in vitro. Treatment of hydroxyurea with hydrogen peroxide and copper (II) sulphate produces 'NO-LIKE' species capable of nitrosating morphine that eventually decomposes to nitrite (NO^{-2}) and nitrate (NO^{3-}), the stable oxidative decomposition products of nitric oxide.

Hydroxyurea reduces the incidence of painful crises in patients with sickle cell disease and has recently been approved for the treatment of this condition. A number of in vitro studies show that the oxidation of hydroxyurea results in the formation of nitric oxide, which also has drawn considerable interest as a sickle cell disease therapy. While patients on hydroxyurea demonstrate elevated levels of nitric oxide-derived metabolites, little information regarding the site or mechanism of the in vivo conversion of hydroxyurea to nitric oxide exists.

In the body L-arginine is used to make nitric oxide, which reduces blood vessel stiffness, increases blood flow, and improves blood vessel functions. L-arginine is an amino acid that has numerous functions in the body. It helps the body get rid of ammonia (a waste product), is used to make compounds in the body such as creatin, L-glutamate and L-Proline and can be converted to glucose and glycogen if needed.L-arginine is used to make the nitric oxide a compound in the body that relaxes blood vessels.

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 [7,8,9,10]. 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 heam 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 nitrosyla. 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 [11,12,14,15,16,17].

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 $CuSO_4$,$KMnO_4$, $NiSO_4$, $K_2Cr_2O_7$,Fe(II) Ammonium Sulphate,Fe(III) Ammonium Sulphate. These metal ion solutions are studied for its λ_{max} values [13].

To 3.0 ml of these metal ion solution varying amount of (0-0.5 ml) of Hydroxyurea is added and effect on spectra is studied with scatchard plot [18].The reverse way study is also carried out. The study is carried out in presence of other binding substance i.e. to the metal ion L-arginine is added and to it varying amount of Hydroxyurea is added. The binding parameters are determined with the help of scatchard plot [18,19,20].

RESULTS AND DISCUSSIONS

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

Metal ion solution	λ_{max}.
$CuSO_4$	800 nm
$KMnO_4$	525nm
$NiSO_4$	406 nm
$K_2Cr_2O_7$	400 nm
Fe(II) Ammonium Sulphate	400 nm
Fe(III) Ammonium Sulphate.	400 nm

b) Metal-L-arginine binding interactions by spectral study and scatchard plots to calculate binding parameters.
c) Metal ion solution + l-arginine spectra were studied at their respective λ_{max} as follows.

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Fig 1 gives spectra and scatchard plot for $CuSO_4$, Fig 2 for $KMnO_4$, Fig 3 for $NiSO_4$

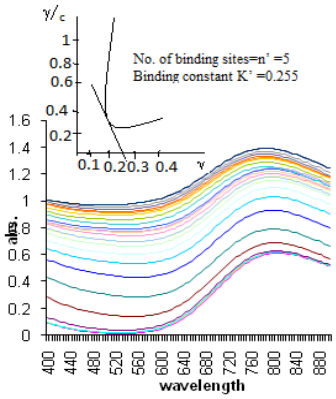

Fig 1

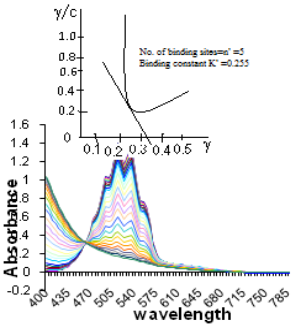

Fig 2

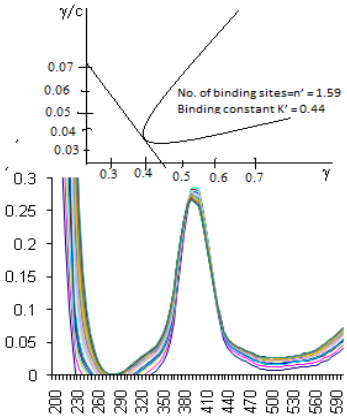

Fig 3

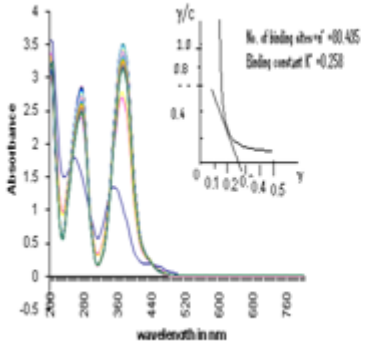

Fig 4

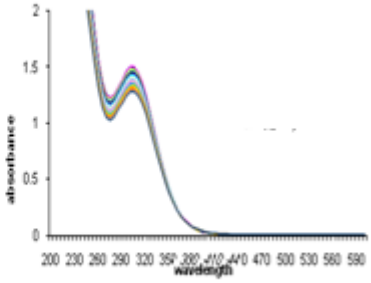

Fig.5

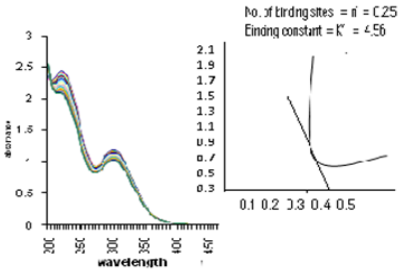

Fig 6

Fig 4 gives spectra and scatchard plot for $K_2Cr_2O_7$,Fig 5 for Fe(II) Ammonium Sulphate Fig. 6 gives spectra and scatchard plot for Fe(III) Ammonium Sulphate.

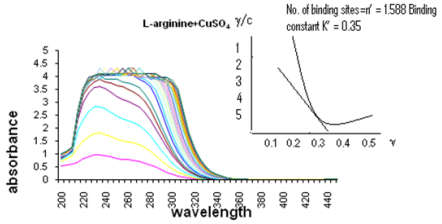

Fig. 7

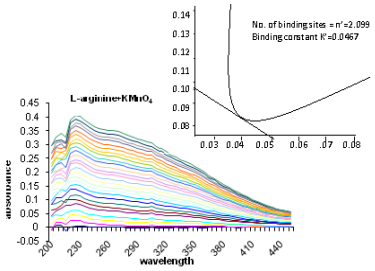

Fig 8

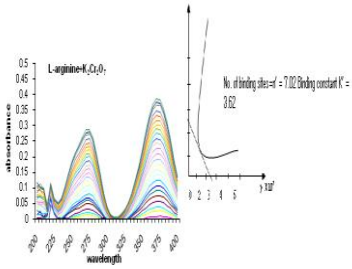

Fig 9

Now reverse study is carried out i.e. to 3.0 ml of arginine solution 0- 0.5 ml of $CuSO_4$ solution is added.
Fig 7 gives spectra and scatchard plot for this addition.Fig 8 and 9 for $KMnO_4$, $K_2Cr_2O_7$respectively.

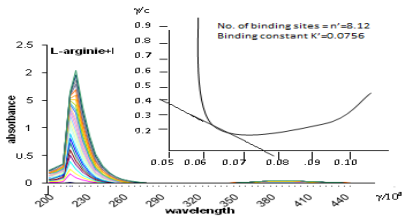

Fig 10

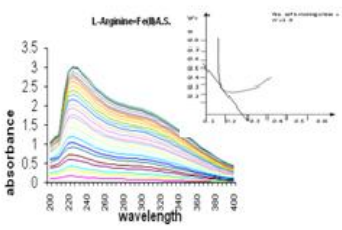

Fig 11

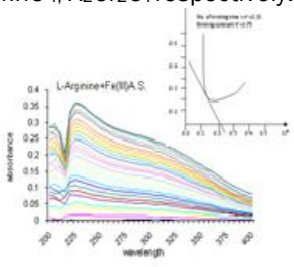

Fig 12

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.

The study is also carried out for Hydroxyurea .To the various metal ion solutions variable amount of Hydroxyurea is added ,the binding parameters are determined with the help of scatchard plot as follows.

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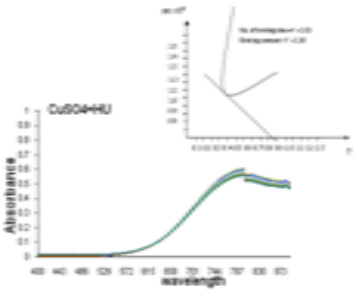

Fig 13

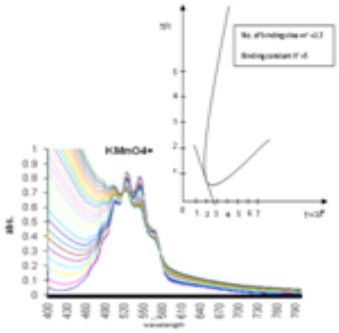

Fig 14

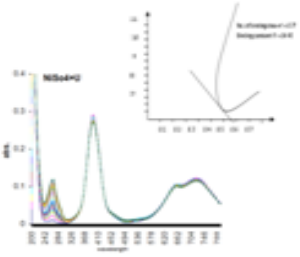

Fig 15

Fig 13 gives spectra and scatcahrd plot for CuSO4, similar way Fig. 14 and Fig. 15 for $KMnO_4$and $NiSO_4$respectively.

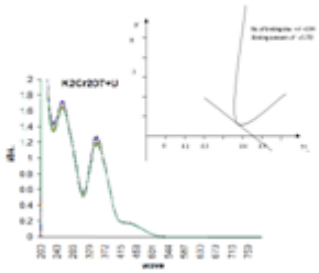

Fig 16

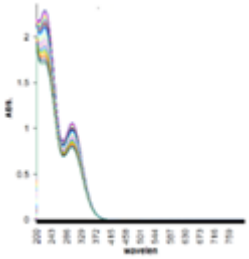

Fig 17

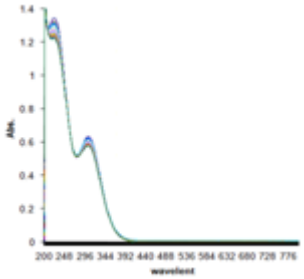

Fig. 18

Fig 16,17& 18gives spectra and scatchard for$K_2Cr_2O_7$, Fe(III) Ammonium Sulphate and Fe (II) Ammonium Sulphaterespectively.

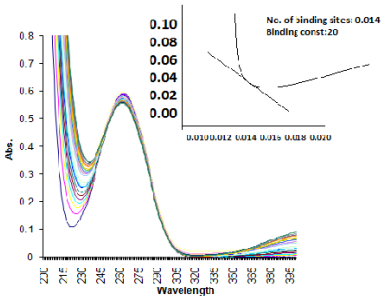

Fig. 19

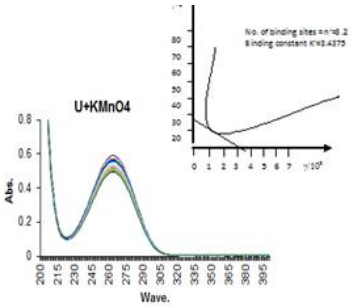

Fig.20

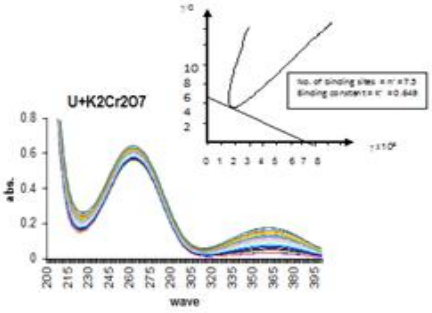

Fig.21

When reverse study is carried out i.e. to 3.0 ml of Hydroxyurea varying amount of metal ion solution is added(0-0.5ml) the spectra are reflected in Fig.19, 20 &21 for $CuSO_4$,$KMnO_4$& $K_2Cr_2O_7$respectively.

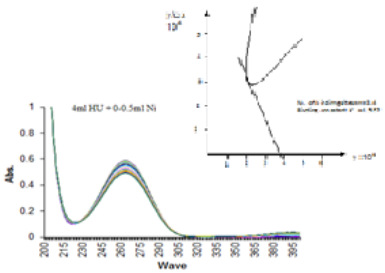

Fig. 22

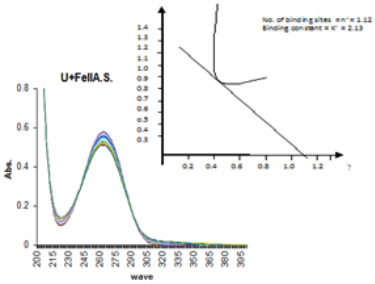

Fig. 23

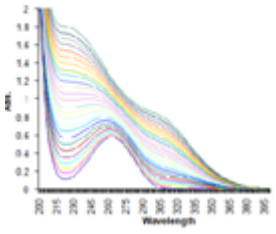

Fig. 24

Fig 22, 23 & 24 for spectra and scatchard plot for $NiSO_4$& Fe(II) andFe(III) Ammonium Sulphaterespectively.

Table 1 gives comparison of binding parameters between L-arginine and Hydroxyurea, when they are added to 3.0 ml of metal ion solution.

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Table 1: Comparison of Binding Parameters of Arginine and Hydroxyurea with Transitional Metal Ions

Metal solutions 3ml of	λ max nm	0 - 0.5 Cm^3 L-arginine No. of binding sites = n'	0 - 0.5 Cm^3 L-arginine Binding constant = K'	0 - 0.5 Cm^3 HU No. of binding sites = n'	0 - 0.5 Cm^3 HU Binding constant = K'
$CuSO_4$	800	0.255	5.0	2.3	5.0
$KMnO_4$	525	0.3575	2.436	1.02	1.28
NiSO4	406	0.44	1.59	0.77	26.43
$K_2Cr_2O_7$	400	0.0258	80.435	0.64	5.733
Fe(II)A.S.	400	No Proper graph	No graph	No Proper graph	No Proper graph
Fe(III)A.S.	400	0.25	4.56	No Proper graph	No Proper graph

Table 2 gives binding parameters, when to 3.0 ml of L-arginineor Hydroxyureavarying amounts of i.e.0 - 0.5ml of metal ion solutions are added. All these readings are taken at respective λmax.

Table 2: Comparison of Binding Parameters Of Arginine And Hydroxyurea With Transitional Metal Ions.

Metal solutions 0-0.5ml of	3.0 Cm^3 L-arginine at 208 nm No. of binding sites = n'	3.0 Cm^3 L-arginine at 208 nm Binding constant = K'	3.0 Cm^3 HU at 199nm No. of binding sites = n'	3.0 Cm^3 HU at 199nm Binding constant = K'
$CuSO_4$	0.35	0.1588	0.714	6.308
$KMnO_4$	0.0467	2.099	3.2	3.4375
NiSO4	0.0756	8.12	3.6	1.557
$K_2Cr_2O_7$	3.62	7.02	7.5	0.649
Fe(II)A.S.	0.26	1.9	1.12	2.13
Fe(III) A.S.	2.15	2.75	No Proper graph	No Proper graph

From the graph it is observed that the binding interactions are different for different transitional metal ions as follows.

For copper the binding tendency is different for hydroxyurea and arginine. It's more with hydroxyurea than arginine.
For chromium no. of binding sites are more with arginine but binding constant is more with hydroxyurea. Whereas hydroxyurea and arginine have almost same no. of binding sites with chromium.

For manganese no. of binding sites are comparatively less with HU and arginine .But with HU as base the binding parameters are more than arginine.

For Nickel binding constant are much more with HU. Same is the case with HU as the base, with arginine the no. of binding sites are more.

For Ferrous no. of binding sites are more with HU. But no such graphs are obtained for ferric indicating either all ferric ions are getting bound completely indicating saturation point.

CONCLUSION

Thus behavior of metal ions is completely different in each case.Above results and work shows that there is significant change in transition of metal ions and Hydroxyurea indicating that HU is NO donor and modulate the behavior of transitional metal ion in the biological systems. Even arginine is capable of changing some transitional properties of metal ions.

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