# Compettition of Aluminium on Iron Binding Site in the Biological System

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**Summary**: Aluminum toxicity has been recognized in many ways, when exposure to heavy metals is prolonged, renal function is limited or a previously accumulated bone burden is released in stress and illness. Aluminum is generally found in +3 oxidation state, so sometimes it competes for the binding sites of Fe(III) in the biological system. If the concentration of Aluminum exceeds above the normal, it inhibits the absorption of Iron and Iron deficiency leads to diseases such as Parkinson's and Alzheimer. The complex formations of Al(III) and Fe(III) with salicylic hydroxamate were studied potentiometrically at different temperatures and data were subjected to computer programs. The stability constant (log $\beta$) values and thermodynamic stabilities were calculated. It was found that salicylic hydroxamate forms 1:1 complex at pH 3 and 1: 2 complex at pH 4 with Al(III) and Fe(III), respectively. The stability constant (log $\beta$) and thermodynamic stabilities of Al(III) salicylic hydroxamate complexes are close to Fe(III) salicylic hydroxamate complexes.

## Introduction

Aluminum is the third most abundant element on the earth after Oxygen and Silicon. Aluminum toxicity has been recognized in many ways; where exposure is heavy or prolonged, renal function is limited or a previously accumulated bone burden is released in stress or illness. Aluminum is not implicated in any known biological or metabolic function [1]. It is normally excreted by the kidneys without causing damage to the organism. However, if great quantity of Aluminum is absorbed, it accumulates in tissues impairing their functions. It is absorbed by a mechanism related to that for Calcium. Gastric acidity and oral citrate favor absorption and H-2 reduces absorption. As it is true for several trace elements, transferrin is the primary protein binder and carrier for Aluminum in the plasma, where 80% protein is bound and 20% is free or complexed to small molecules such as citrate. Its toxic effect can be rapidly identified by pathological symptoms mainly anemia, encephalopathy and renal failure. The Iron requirement of biological systems may also be influenced by the presence of Aluminum because Aluminum and Iron both have +3 oxidation states [2]. Among trace metals, Iron is an essential nutrient for micro-organism as well as for other organisms because of its varied functions in biological redox processes. Instead of its importance, Iron could be toxic when it is present in excess quantity. Iron could increase the capacity of transferrin and ferritin. This condition is known as Iron overload [3]. There are many natural mechanism for solubilization or removal of Iron, *e.g.* the micro-organism utilize a well defined Iron acquisition strategy which includes the production of low molecular weight chelating agents called siderophores to solubilize and transport ferric ions in aqueous medium [4]. These siderophores have high affinity towards Fe(III) and they are better chelators for Fe(III) than Fe(II). The stability constants for the ferric siderophore complexes are extremely high formation constant value ($Kf = 10^{36} - 10^{55}$), while Fe(II) has very low formation constant value ($Kf = 10^{8}$) [5]. For the treatment of Iron overload the salicylic hydroxamate appears to be more selective as its stability constant for Fe(III) complex is greater in several orders of magnitude than those for other useful metal ions complexes. Desferrioxamine mesylate, a linear trihydroxamic acid natural siderophore, produced by Nocardia and Streptomyces, has been used for the treatment of Iron overload [6]. In present work, we have established the stability constant, thermo-dynamic stabilities, spectrophotometric studies and

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potentiometric studies of Aluminum salicylic hydroxamate complexes and studied comparison with Iron salicylic hydroxamate complexes.

## Results and Discussion

The potentiometric titrations data at different temperatures for salicylic hydroxamate, Al(III) and Fe(III) complexes were analyzed by the computer program "BEST" [7], which helps to calculate the stability constants values from potentiometric data. The stability constant (log $\beta$) values and thermodynamic stability of Al(III) and Fe(III) complexes are shown in Table-1. It was found that like other hydroxamates, salicylic hydroxamate forms stepwise complexes, one at pH 3 and other at pH 4. Iron showed three stages of complexation (Fig 1), each resulted into highly stable complexes, the third one formed at pH 6 having 1:3 metal to ligand ratio, in which the ligands may behave as bidentate ligand, *i.e.* in addition to the bidentate hydroxamate function, the -OH attached directly at ortno-position becomes capable of binding metal ions [8]. The thermodynamic stabilities and log $\beta$ values of Fe(III) and Al(III) complexes are comparable. From the observed data of stability constant values in Table-1, it can be suggested that for the treatment of iron overload in $\beta$ thelesemic patients on hydroxamate based drugs Al(III) equilibrium may also be disturbed. Significant results were observed, when we compared the potentiometric curves of Al(III) and Fe(III) ions. They are very similar to each other this is the reason, Al(III) ions shows the competition with Fe(III) ions at the binding site in the biological system. Al(III) blocks binding site of Fe(III). Aluminum ion forms very stable complexes with glycine (part of neurotransmitter) so if we use aluminum utensils for household purposes, most of the glycine of food will be destroyed. The presence of Al(III) in biological systems disturbs function of the nervous system and this is the reported cause of Alzheimer disease [9]. If we compare the activities of Al(III) and Zn(II) ions, that Zn(II) ions effect the glycine receptor activity even at low concentration *e.g.* 10 micro mole/L and enhance glycine mediated currents at higher concentrations *e.g.* 1 milli mole/L, have an opposite inhibitory effect [10]. The excesses of Al(III) ions in the system has inhibitory effect and deficiency of Al(III) ions have positive effects on the nervous system [11].

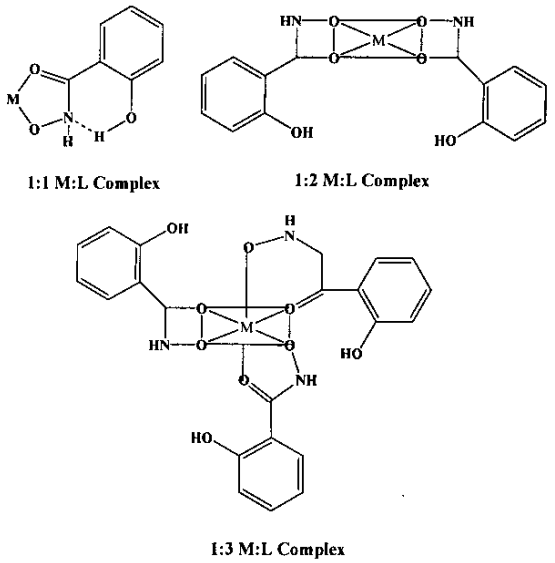


Fig: 1: Complexation of Iron with salicylic hydroxamate in different metal to ligand (M:L) ratios.

Table-1: Log $\beta$ values of (1:1, 1:2 and 1:3 metal to ligand ratios) Al(III) and Fe(III) hydroxamate siderophore at different temperature calculated by computer program.

| Metal to ligand ratio (M:L) | Al(III) | 30 °C | 35 °C | 40 °C | 45 °C | 50 °C |
|---|---|---|---|---|---|---|
| 1:1 | Log $\beta$ 110 | 9.50 | 10.00 | 11.50 | 11.00 | 11.90 |
| 1:2 | Log $\beta$ 210 | 18.00 | 18.50 | 19.53 | 20.25 | 20.00 |
| 1:3 | Log $\beta$ 310 | --- | ---- | --- | --- | --- |
| **Metal to ligand ratio (M:L)** | **Fe(III)** | **30 °C** | **35 °C** | **40 °C** | **45 °C** | **50 °C** |
| 1:1 | Log $\beta$ 110 | 14.8 | 15.12 | 15.52 | 15.95 | 16.60 |
| 1:2 | Log $\beta$ 210 | 24.0 | 24.5 | 24.75 | 24.9 | 25.1 |
| 1:3 | Log $\beta$ 310 | 31.0 | 31.25 | 31.7 | 32.0 | 32.15 |

Stability constants depend upon two factors that is enthalpy and entropy changes as the following equation show:-

$-\Delta G = RT \ln \beta$ or
$\Delta G = \Delta H - T\Delta S$ [7]

When $\beta$ increases $\Delta S$ becomes more positive and $\Delta H$ become more negative when we look through Table-2, it is concluded that our data follow this order.

For Al(III): $\Delta H_2 > \Delta H_1$ $\Delta S_1 > \Delta S_2$
For Fe(III): $\Delta H_3 > \Delta H_2 > \Delta H_1$ $\Delta S_1 > \Delta S_2 > \Delta S_3$

Table-2: Entropy and enthalpy values of Al(III) and Fe(III) hydroxamate siderophore complexes $\Delta H$ = k J $mole^{-1}$ and $\Delta S$ = J $K^{-1}$ $mole^{-1}$.

| | $-\Delta H_1$ | $\Delta S_1$ | $-\Delta H_2$ | $\Delta S_2$ | $-\Delta H_3$ | $\Delta S_3$ |
|---|---|---|---|---|---|---|
| Al(III) | 12.50 | 459 | 8.25 | 310 | --- | --- |
| | $-\Delta H_1$ | $\Delta S_1$ | $-\Delta H_2$ | $\Delta S_2$ | $-\Delta H_3$ | $\Delta S_3$ |
| Fe(III) | 11.75 | 480 | 8.45 | 325 | 5.5 | 115 |

Reactions in which positive ions and negative ligands interact to form complexes of lower charge proceed with large increase in entropy and enthalpy change. Similarity these values are believed to under lie many of similar biological reactions and competitions for binding sites on metalloenzyme transport and storage proteins. When a foreign metal competes with or replaces a functional metal, toxicity results. This is shown in Table-1 with a comparison of the stability constants.

Metal ions behaved as a carrier on drug binding sites in the transportation of different drugs. These types of chelating studies of metal ligand complexes are very important in drug metabolism. Changing the concentration of metal ions abolish activity of drug against the microorganisms. When amino acids are used in metal free system, they are also not absorbed and drug became unaffected. Immune system are also protein which work against antigens, this system is effective when traces of metal ions are present [12]. Self-medication is common among people of Pakistan. They use to take protein extracts for common diseases. People used them without precautions and limits (improper dosage); the result of excessive intake may cause abnormalities in the body. Different types of complexations of amino acids with bio-available trace metals or micronutrients occur in living system. Copper, Iron, Cobalt, Zinc, Nickel, Chromium, Calcium and Magnesium are micro nutrients present in living system. These metal ions are involved in different metabolic reactions of biological processes and they also act as inhibitors for different enzymes. metabolic processes of the body may be disturbed by deficiency of metal ions, such as electrolytic disturbance; alteration in membrane permeability etc. Taking certain precautionary measures can reduce these interactions. Our studies showed that Aluminum has positive interaction with Iron. At stomach pH, complexation do not occur with trace metals, whereas the intestinal pH is alkaline and most of the interactions occur at this pH [13].

The stability constant values depend upon polarizing power *i.e.* charge to radius ratio and hard-soft character of metal ions. A cation with high polarizing power has high stability constant values for complex formation with ligands whose donor groups also have high polarizing power. Tripositive cations (*i.e.* Al(III), Cr(III), Fe(III) etc.) are generally classified as hard acids and form strong complexes with $-O^-$ donor groups *i.e.* carboxylates, oxo groups etc [8]. The tripositive cations of the first transition series decreases in ionic radii along the period, so stability constants for complexation increase. This research revealed that Al(III) ions have higher values of enthalpy and entropy because of smaller ionic radii. The thermodynamics of Al(III) are comparable to Fe(III), so they both can attack on same binding sites in biological system [14].

The stock solutions of hydroxamic acid and metal ions were mixed in different ratios while keeping the total volume of the solution fixed (equal to 10 mL). Metal to ligand ratios were used from 1:9 to 9:1. The absorbances of each solution having different metal to ligand ratios were measured at the selected wavelength (490 nm). Increase and leveling off the absorbance with increase in metal to ligand ratios indicated 1:1 stoichiometry as shown in the Fig 2. It was observed that the equimolar concentration of the hydroxamate and metal ions gave the more stable complex as compare to the other ratios, where concentration of hydroxamate was increased *i.e.* 1:2 and 1:3.

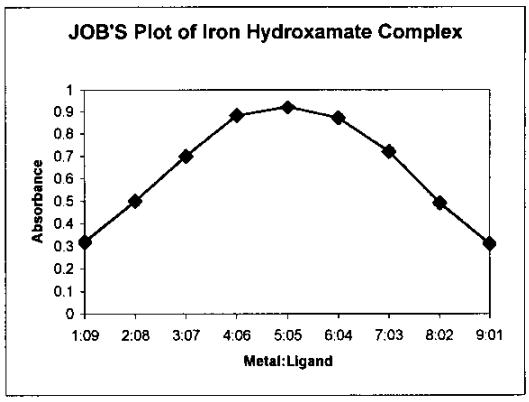


Fig: 2: Graphical representation of JOB'S Plot of Iron Hydroxamate complex.

A chelating agent (salicylic hydroxamate) may be effective in removing a toxic metal from the body [6, 8], it must satisfy second law of thermodynamic that is the free energy change for the

transfer of metal ions from the binding sites to the chelating drug must be negative. To achieve this requirement, stability constant between the toxic metal and chelating drug must be greater than that of the competing ligands with the metal concerned [15].

## Experimental

All reagents were of AnalaR (Analytical Reagents) grade. Solutions were prepared in deionized water (deionizer CSW-300) and carbon dioxide gas was removed by boiling. For all pH measurements Orion pH meter (SA-720) was used. A 0.05 M solution of potassium hydrogen pthalate which has pH value 4.01 at 25 °C was used to calibrate the pH meter alongwith the standard buffer solution, provided by BDH standard chemicals. For potentiometric titrations, a double walled glass cell was used. The temperature of the cell was kept constant throughout the experiment by water circulation. All the titrations were performed at different temperatures *i.e.* 30 °C, 35 °C, 40 °C 45 °C and 50 °C. Twenty milliliters of 0.01 M metal ion solution was mixed with 20 mL of 0.01 M salicyhydroxamic acid solution and titrated against 0.1 M NaOH solution. The change in pH was measured with the addition of Aliquot (0.05 mL) of the base. The solution was stirred with magnetic stirrer continuously. For each metal salicylhydroxamic acid solution, these titrations were performed twice to minimize the probable error. For spectrophotometric measurements, spectra were recorded on Shimadzu (UV 160A) spectrohotometer. The absorbances of the compound at different metal to ligand ratio were measured at 490 nm wavelength.

*Determination of Log β Values Through Potentiometric and Spectrophotometric Method*

The data obtained from pH titrations was utilized for the calculation of stability constant (log $\beta$) values. For this purpose, a computer program BEST [7] was used, which helps to calculate the stability constants values from potentiometric data. An input data file was prepared for each titration with appropriate (log $\beta$) values. Calculated (log $\beta$) values were refined several times, till the sigmafit (an error) values reduced up to 0.023. The data file of this program was required the following informations:-

1. Total volume of the solution.
2. Molarity of the base used for pH titration
3. Change in pH after each step
4. No. of millimole of metal ions present in the solution
5. No. of millimole of ligand present in the solution

The whole calculation in this program was based on the expected (log $\beta$) values for each species present in the solution by refining these values to get sigmafit values. The accuracy of sigmafit was reflected on accuracy of equilibrium constant values. The equilibrium constant values of the complexes at different temperature were used to study the thermodynamics of complexes [5].

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