

## Kinetics and Mechanism of Reduction of Fe(III)-Acetohydroxamic Acid by Hydroxylamine Hydrochloride at Acidic pH

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### ABSTRACT

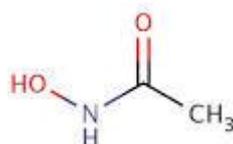
The complexes of Fe(III)-AHA were prepared in acetate buffer of pH 4.5, 5.0 and 5.5. Stopped flow technique was used to study the reduction of these complexes by hydroxylamine hydrochloride. The reaction shows a biphasic behavior which is significantly pH dependent. Rate of both phases increases with hydrogen ion concentration. The rate was found to be first order with respect to [Fe(III)]. The overall rate was neither first order nor second order but there was a pre-equilibrium in mechanism. Slight increase in the values of  $k_{obs}$  might be a consequence of increased reducing power of hydroxylamine hydrochloride with pH.

**Keywords:** Reduction, stopped flow kinetics, pre-equilibrium, Acetohydroxamic acid

### 1. INTRODUCTION

Iron acquisition presents profound difficulties for aerobic microorganism due to insolubility of  $Fe(OH)_3$ . The equilibrium concentration of Fe(III) is  $10^{-18}$  M at pH 7<sup>1</sup>. As a response to this environmental stress, bacteria have developed the strategy of secreting low molecular weight compounds, siderophores, which chelate and solubilize Fe(III) ion for transport into the cell<sup>2</sup>. Their role in microbial metabolism is to acquire iron from the environment, a task that involves three steps; solubilization of Fe(III) through chelation, transport to and across the cell membrane and deposition at the appropriate site into the cell<sup>3</sup>. The mode of iron transport into the bacterial cell is an important area of interest in siderophore physiology. Raymond and Carrano in 1979 first time proposed three mechanisms of microbial iron transport<sup>4</sup>.

Desferrioxamine B, a hydroxamate based siderophore, is currently used for removal of iron from the body in the treatment of patients suffering from  $\beta$ -thalassemia or acute iron poisoning<sup>5</sup>. Synthetic monohydroxamic acids, such as acetohydroxamic acid (AHA) (Figure-1), can serve as a model ligand for the investigation of the hydroxamate-based siderophore-Iron(III) interactions, which were thoroughly studied by Crumblis and his coworkers<sup>6</sup>.



Aceto hydroxamic acid  
**Scheme-1**

Binding of iron is important in biological system. The clinical efficacy of the chelator is very much dependent on the thermodynamic and kinetic factors of iron binding by this chelator<sup>7</sup>. The stability of Fe(II) siderophores is considerably lower than Fe(III) siderophores, and they also tend to be kinetically labile with respect to Fe(II) dissociation. Consequently, reduction of Fe(III) siderophore at the site of deposition is an attractive mechanistic possibility for iron release<sup>8</sup>.

### 2. METHODOLOGY

In our studies acetohydroxamic acid (AHA) has been used as Fe(III) chelator and hydroxylamine hydrochloride as reducing agent for the kinetic study.

The reduced form of the complex, hydroxylamine hydrochloride or its' oxidized form have no absorbance in the visible range whereas Fe(III)-hydroxamate complexes are highly colored. Addition of reductant to a solution of the complex results in the decrease of absorbance of the solution, which enables us to measure the extent of reduction.

Lowering of absorbance of complex is mainly due to two reasons, one is reduction and the other is dilution. Lowering of absorbance due to dilution can be corrected by converting observed absorbance into corrected absorbance as follows;

$$A_{corr} = A_{obs} \times \frac{(V_{oxi} + V_{red})}{V_{oxi}}$$

Where,

$A_{\text{obs}}$  = observed absorbance,  $V_{\text{oxi}}$  = volume of oxidant used,  $V_{\text{red}}$  = volume of reductant added

### 3. EXPERIMENT

All the reagents used were of A.R grade. Distilled deionized water was boiled to free from  $\text{CO}_2$ . Analytical grade reagents and distilled water were used in preparation of the solutions each time.

### 4. PREPARATION OF SOLUTIONS

Fe(III) solution was prepared by dissolving a calculated, accurately weighed amount of  $\text{Fe}(\text{NO}_3)_3 \cdot 9\text{H}_2\text{O}$  in 0.05 M  $\text{HNO}_3$ . Standardization with Fe- Orthophenanthroline method was used to determine actual concentration. The solution was found to be approximately 0.01 M with 6% error. This solution served as stock solution.

Acetohydroxamic acid (AHA) solution was prepared as per requirement, by dissolving calculated amount of AHA in deionized distilled water.

Hydroxylamine hydrochloride solution was freshly prepared by dissolving calculated and accurately weighed amount of hydroxylamine hydrochloride in buffer of desired pH. Different dilutions of the solution were prepared accordingly.

Acetate buffers of pH 4.5, 5.0, 5.5 having  $\mu = 0.2$  M were prepared. The required ionic strength was adjusted by adding calculated amount of NaCl.

Complex solution was prepared by mixing Fe (III) and AHA solution of known concentration. The concentration of AHA was kept 5 times over the concentration of Fe (III). Solutions were made up to mark with required buffer.

### 5. INSTRUMENTATION

$\lambda_{\text{max}}$  of the complex at different pH was determined by monitoring absorption spectra at particular pH on Shimadzu spectrophotometer UV-160A. The molar extinction coefficients ( $\epsilon$ ) of Fe(III)-AHA were calculated (Table 1).

For all pH measurements, Orion pH-meter model SA-720 was used. Absorbance change in visible region was monitored on Spectronic21. The output of Spectronic21 was read in to Pentium I computer interfaced through "Labpro" compatible with "Logger Pro" program distributed by "Vernier". Labpro interface and the Logger Pro program together allowed us to save the records of individual kinetic runs in files with voltage output of Spectronic 21. Kinetic study was followed by stopped flow method. SFA-II is a fast kinetic accessory, which eliminates the problem of long mixing time. Its mixing time is less than 20 milli seconds. This means it can measure half-life down to about 0.05 seconds. The cell IS thermo stated.

### 6. RESULTS AND DISCUSSION

It is evident that high spin  $d^5$  Fe(III) has no spin-allowed d-d transitions, rather LMCT exists in Fe(III)-siderophore complexes which are not readily interpreted as are ligand - field (d-d) transitions [9]. Fe(III)-siderophore complexes possess high  $K_f$ , but with the reduction of  $\text{Fe}^{+3}$  to  $\text{Fe}^{+2}$ , decreases. The iron(II)-siderophore complexes are colorless because of the absence of LMCT. In the pursuit of kinetic studies, discoloration of Fe(III)-siderophore on reduction to Fe(II) enables us to follow the reaction.

In the pursuit of kinetic studies, disappearance of color of Fe(III)-siderophore on reduction to Fe(II) enables us to follow the reaction. Consequently these kinetic studies are helpful in the determination of electron transfer or biological actions of metalloproteins<sup>10</sup>.

#### 6.1 Effect of pH

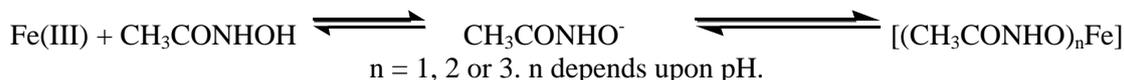
Complex of 1:1 metal to ligand ratio is formed at very low pH. While increasing the pH causes 1:2 & 1:3 complex formations those are much stable and difficult to reduce.

The general trend in reduction process, as reported in the literature is in the order  $\text{FeL}_3 < \text{FeL}_2^+ < \text{FeL}_2^+$ . As the stability of Fe(III)-(AHA)<sub>3</sub> complex increases with PH, it becomes difficult to reduce Fe(III)-(AHA)<sub>3</sub>. Thus the rate of reaction decreases with increasing pH. The reducing power of  $\text{NH}_2\text{OH} \cdot \text{HCl}$  is also very much pH dependent. By increasing pH it's reducing power increases. At higher pH the reducing power is more dominant over the complexation, which is indicated by a slight increase in the values of rate constant.

pH 4.5, 5.0 and 5.5 were chosen for the present study. It has been observed that the  $\lambda_{\text{max}}$  decreases with increasing pH (Table-1).

Table-1: pH dependence of  $\lambda_{\text{max}}$  values

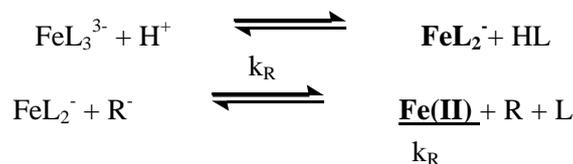
pH	$\epsilon$ $\text{M}^{-1} \text{cm}^{-1}$	$\lambda_{\text{max}}$ (nm)
4.5	1774	449
5	1916	439
5.5	2034	429



Possibility of maximum complexation, i.e. 1:3, increases with increase in pH. Fe(III)-AHA is reduced to Fe(II)-AHA on addition of reducing agent such as hydroxylamine hydrochloride. Fe(II)-AHA is a colorless species.

Basically in the reaction mixture, two species are involved, one is Fe(III)-AHA complex while other is reductant. Both of them have contrary effects on rate with pH rise.

By increasing pH the formation of 1:2 & 1:3 complexes prevails & 1:3 complex is less reducible than 1:2 which is less reducible than 1:1. It means that by increasing pH (or decreasing H<sup>+</sup> concentration) the reducing ability of Fe(III) complex decreases due to different stoichiometric complexes.



Where, L is a bidentate ligand.

k<sub>R</sub> at higher pH is lower than k<sub>R</sub> at lower pH because FeL<sub>3</sub> is less reducible than FeL<sub>2</sub> which is less reducible than FeL<sup>+</sup>.

However nature of reductant like NH<sub>2</sub>OH.HCl is such that, by increasing the pH its E<sup>o</sup> decreases as shown below:

<u>MEDIUM</u>	<u>OXIDATION STATE (-1)</u>	<u>OXIDATION STATE (0)</u>
ACID	NH <sub>3</sub> OH <sup>+</sup> -181	N <sub>2</sub>
BASE	NH <sub>2</sub> OH -3,04	N <sub>2</sub>

Due to less value of E<sup>o</sup><sub>red</sub>, E<sup>o</sup> is greater which is directly related to K value as shown previously.

By applying simplified Marcus equation

$$k_{AB} = \left( \frac{k_{AA} K_{AB} Z_{AB}^2}{Z_{AA} Z_{BB}} \right)^{1/2}$$

$$K_{AB} = (k_{AA} \times k_{BB} \times K_{AB} \times F_{AB})^{1/2}$$

This is the cross relationship in terms of rate constants (12). Here k<sub>AA</sub> and k<sub>BB</sub> are the self exchange rate constants of specie A and B respectively and K<sub>AB</sub> is the equilibrium constant. If F<sub>AB</sub> = 1 then equation reduces to.

$$k_{AB} = (k_{AA} \times k_{BB} \times K_{AB})^{1/2}$$

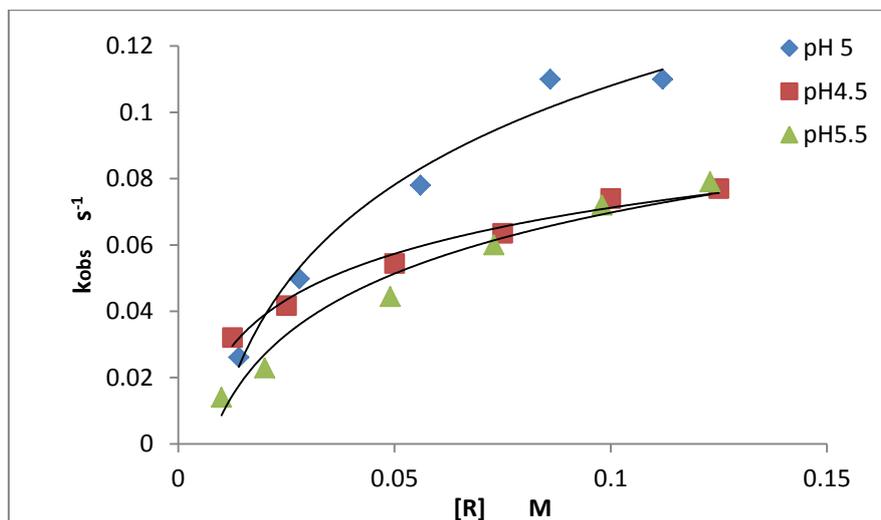
This is called Marcus equation. If all terms k<sub>AA</sub> and k<sub>BB</sub> remain constants then K<sub>AB</sub> depends only on changing K<sub>AB</sub> with changing E<sup>o</sup> values at different pH. So, by increasing pH resulting k<sub>AB</sub> or rate of reduction increases.

The above two contrary effects with respect to complex & reductant cancel each other, therefore no significant increase or decrease is observed by changing pH. But a small increase can be seen by increasing pH, which might be due to the increased reducing power of reductant at higher pH.

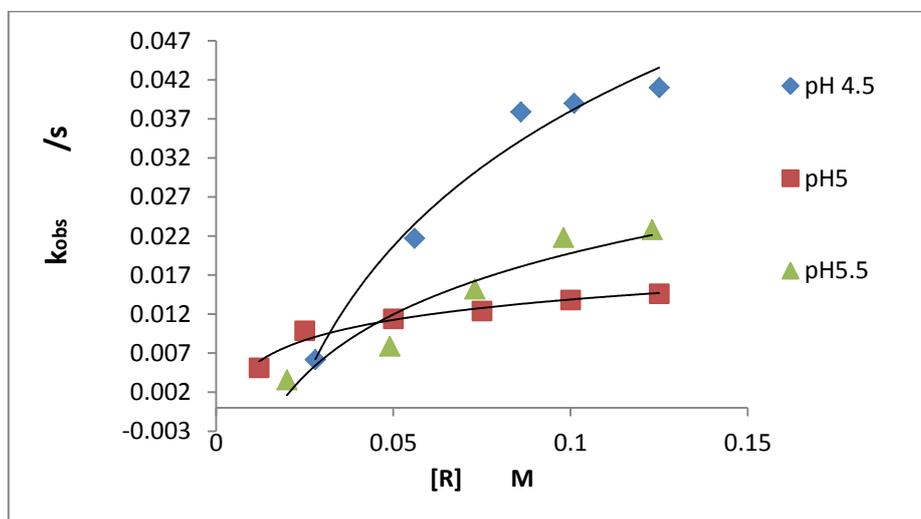
The recent work of Bengtsson and coworkers reveals that the results obtained from the reduction of Fe(III) by hydroxylamine, truly in absence of siderophore, are consistent with a mechanism dependent on the relative iron(III) to hydroxylamine concentration. It has also to be considered that the consistent mechanism identifies the mechanism with two pre-equilibria in that case<sup>[11]</sup>.

Reduction of Fe(III)-AHA by hydroxylamine hydrochloride is a typically biphasic reaction i.e. a fast phase which may be due to the reduction of Fe-(AHA)<sub>2</sub> parallel with slow phase that may be due to the reduction of Fe-(AHA)<sub>3</sub>. It is very difficult to determine the interval at which fast phase terminates and slow phase starts.

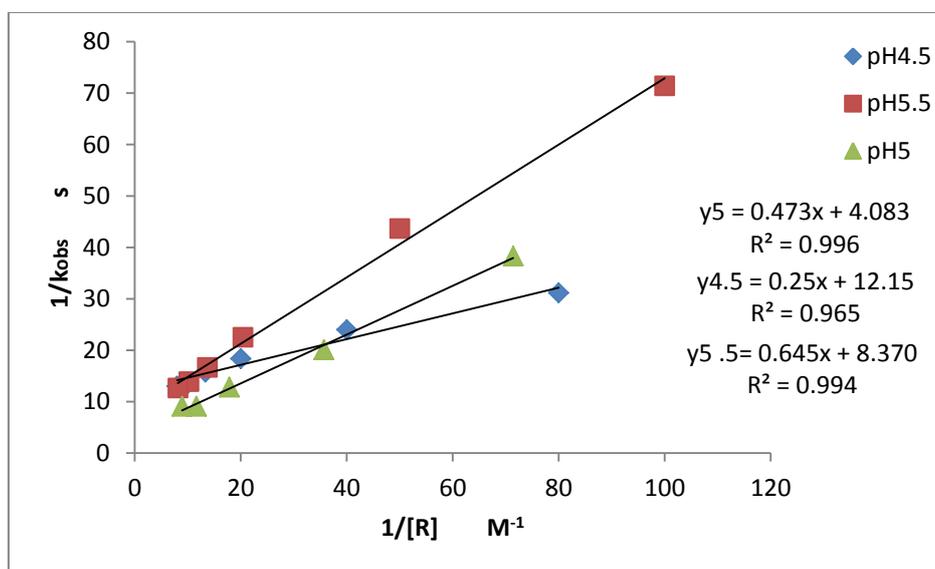
However, the plot k<sub>obs</sub> vs [RED] would have been a straight line, if this reaction was first order in [hydroxylamine]. The clear leveling off describes reaction is not first order in [hydroxylamine] but rather pre-equilibrium exists [Fig:1, 2]. The plots of 1/ K<sub>obs</sub> as a function of 1 / [Red] are given in figures 3 and 4.



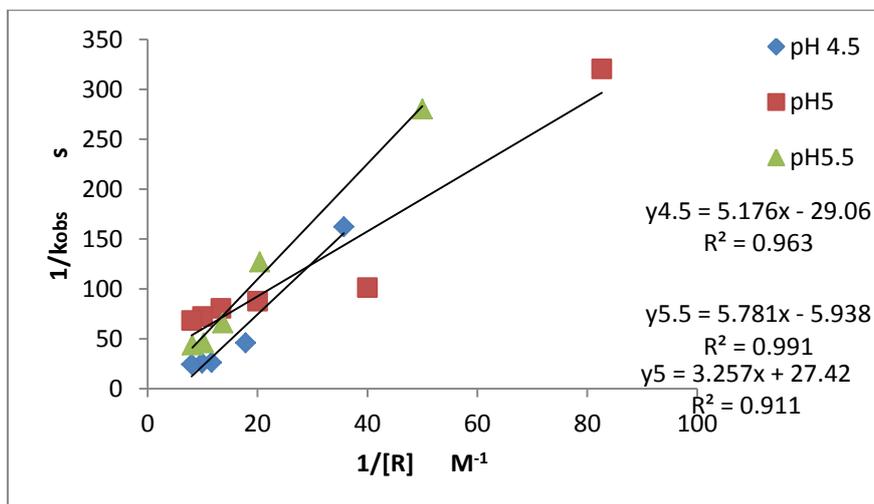
**Fig-1:** Plots of the Observed Rate Constants for the Fast Phase of Reduction of Fe(III)-AHA complex at different pH,  $T=30\pm 0.5^\circ\text{C}$ ,  $[\text{Fe(III)-AHA}] = 5.5 \times 10^{-4}\text{M}$ ,  $\mu=0.2$



**Fig-2:** Plots of the Observed Rate Constants for the Slow Phase of Reduction of Fe(III)-AHA complex at different pH,  $T=30\pm 0.5^\circ\text{C}$ ,  $[\text{Fe(III)-AHA}] = 5.5 \times 10^{-4}\text{M}$ ,  $\mu=0.2$



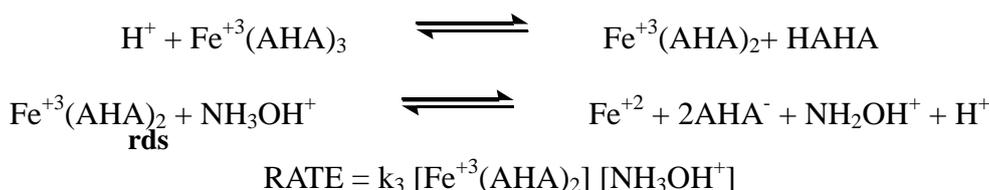
**Fig-3:** Double Reciprocal Plot for Fast Phase of Reduction of Fe(III)-AHA complex at different pH,  $T=30\pm 0.5^\circ\text{C}$ ,  $[\text{Fe(III)-AHA}] = 5.5 \times 10^{-4}\text{M}$ ,  $\mu=0.2$



**Fig-4:** Double Reciprocal Plot for Slow Phase of Reduction of Fe(III)-AHA complex at different pH, T=30± 0.5°C, [Fe(III)-AHA] = 5.5 × 10<sup>-4</sup>M, μ=0.2

The results obtained help us to suggest the following mechanism for both the phases

**FAST PHASE**



By steady-state approximation,

$$k_1 [\text{Fe}^{+3}(\text{AHA})_3] [\text{H}^+] = k_2 [\text{Fe}^{+3}(\text{AHA})_2] [\text{HAHA}] + k_3 [\text{Fe}^{+3}(\text{AHA})_2] [\text{NH}_3\text{OH}^+]$$

$$\text{Rate} = -d \text{Fe}^{+3} (\text{AHA})_3 = k_1 [\text{Fe}^{+3}(\text{AHA})_3] [\text{H}^+]$$

$$[\text{Fe}^{+3}(\text{AHA})_2] (k_2 [\text{HAHA}] + k_3 [\text{NH}_3\text{OH}^+]) = k_1 [\text{Fe}^{+3}(\text{AHA})_3] [\text{H}^+]$$

$$[\text{Fe}^{+3}(\text{AHA})_2] = \frac{k_1 [\text{Fe}^{+3}(\text{AHA})_3] [\text{H}^+]}{(k_2 [\text{HAHA}] + k_3 [\text{NH}_3\text{OH}^+])}$$

$$\text{Rate} = \frac{k_3 k_1 [\text{Fe}^{+3}(\text{AHA})_3] [\text{H}^+] \cdot [\text{NH}_3\text{OH}^+]}{(k_2 [\text{HAHA}] + k_3 [\text{NH}_3\text{OH}^+])}$$

[H<sup>+</sup>] = constant; k<sub>1</sub> [H<sup>+</sup>] = k<sub>4</sub>

$$k_{\text{obs}} = \frac{k_3 \cdot k_4}{(k_2 [\text{HAHA}] + k_3 [\text{NH}_3\text{OH}^+])} \cdot [\text{NH}_3\text{OH}^+]$$

if k<sub>2</sub> [HAHA] » k<sub>3</sub> · [NH<sub>3</sub>OH<sup>+</sup>]

$$\text{then } k_{\text{obs}} = \frac{k_3 k_4 [\text{NH}_3\text{OH}^+]}{k_2 [\text{HAHA}]}$$

if k<sub>3</sub>[NH<sub>3</sub>OH<sup>+</sup>] » k<sub>2</sub> [HAHA]

then k<sub>obs</sub> = k<sub>4</sub> i.e. k<sub>obs</sub> = k<sub>1</sub> [H<sup>+</sup>]

$$k_{\text{obs}} = \frac{k_3 k_4}{(k_2 [\text{HAHA}] + k_3 [\text{NH}_3\text{OH}^+])} \cdot [\text{NH}_3\text{OH}^+]$$

$$\frac{1}{k_{\text{obs}}} = \frac{k_2 [\text{HAHA}]}{k_3 k_4 [\text{NH}_3\text{OH}^+]} + \frac{k_3 [\text{NH}_3\text{OH}^+]}{k_3 k_4 [\text{NH}_3\text{OH}^+]}$$

$$\frac{1}{k_{\text{obs}}} = \frac{k_2 [\text{HAHA}]}{k_3 k_4} + \frac{1}{[\text{NH}_3\text{OH}^+]} + \frac{1}{K_4}$$

$$\text{Intercept} = \frac{1}{k_4} = \frac{1}{k_1 [\text{H}^+]}$$

$$\frac{1}{\text{Intercept}} = k_1 [\text{H}^+]$$

The following changes are expected to occur during the oxidation of hydroxylamine hydrochlorides:



In this way the overall stoichiometry of the reaction is;



## SLOW PHASE

Following mechanism can be assumed for the slow phase of reduction of Fe (III)-AHA by hydroxylamine hydrochloride:



Where R = hydroxylamine hydrochloride

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