Thermodynamic Study on the Interaction between Fe²⁺(aq) ion and L-Alanine

K. Zare[1,2], F. Keshavarz Rezaie[1,3], F. Soleimani[1] and H. Aghaie[1,*]

1. Department of Chemistry Science & Research Campus, Islamic Azad University, P.O. Box 14515- 775 Tehran, Iran
2. Department of Chemistry, Shahid Beheshti University, Evin, Tehran, Iran
3. Department of Chemistry, Arak Branch, Islamic Azad University, Arak, Iran

ABSTRACT

Using UV-VIS spectrophotometric method, the formation constant for interaction of $Fe^{2+}_{(aq)}$ ion with L-Alanine was experimentally studied at pH = 4.1 ± 0.01 (50mM of potassium hydrogen phthalate buffer), ionic strength of 0.1M potassium nitrate and at 5 different temperatures 15,20, 25,30 and 35 ˚C. The optical absorption spectra of mixtures containing considered cation and L-Alanine were analyzed by using SQUAD software, in order to obtain the formation constant and the stoichiometry of respect complex. The best fitting of our results showed the 1:2 complex respect to the studied system ($Fe^{2+}_{(aq)}$ + L-Alanine) is formed. Regarding the value of formation constants at different temperatures and using Van، t Hoff equation, it is possible to calculate the respective thermodynamic functions of formation such as ΔG°, ΔH°, ΔS°, … of the studied complex.

Keywords: Amino acid; Metal ion complex; Interaction; SQUAD

INTRODUCTION

Alanine plays a major role in the transfer of nitrogen from peripheral tissue to the liver, aids in the metabolism of glucose, a simple carbohydrate that the body uses for energy, guards against the buildup of toxic substances that are released into muscle cells when muscle protein is broken down quickly to meet energy needs, such as what happens with aerobic exercises, strengthens the immune system by producing antibodies [22].

The ability of metal ions to coordinate with bind and then release ligands in some processes, and to oxidize and reduce in other processes makes them ideal for use in biological systems.

The most common metal used in the body is iron, and it plays a central role in almost all living cells. For example, iron complexes are used in the transport of oxygen in the blood and tissues [4].

The formation of metal complexes is often highly dependent on the pH of the solution. This is because there is a competition for the ligand between the metal ion and the proton as they both bind to the same sites of the ligand. For L-Alanine at low pH, the metal ion has to displace a proton from the amine in order to form a stable complex.

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* . Corresponding author:hn_aghaie@yahoo.com

The number of protons displaced through chelate formation can be determined from a titration of ligand and metal ion, in this instance a 2:1 mixture of L-Alanine and $Fe^{2+}_{(aq)}$ ion is used[5,6].

That is why we have studied the formation constant of the complex formed by L-Alanine with $Fe^{2+}_{(aq)}$ ion.

MATERIALS AND METHODS
L-Alanine, iron (II) nitrate with high purities were purchased from Merck Company and were used without further purification.

All considered solutions were prepared using double distilled water.

Potassium hydrogen phthalate 50 mM, (pH = 4.1 ± 0.01) was used as buffer and the ionic strength of 0.1M potassium nitrate was supplied. All of the work solutions were made by dissolving the solid compounds in buffer solution. The metal ion $Fe^{2+}_{(aq)}$ solutions were freshly prepared before spectral analysis and their concentration range was 4.00×10^{-2} – 5.00×10^{-2} M. L-Alanine solution was prepared at room temperature and the concentration range was 0.5-1 M. The titration of considered metal ion solution as a function of L-Alanine concentration was performed at 15, 20, 25, 30 and 35°C. Spectrophotometric measurements were performed on a UV-VIS spectrophotometer (Camspec M350) and a 1.00cm quartz cavetto in the spectral range of 200-800nm with a thermostat cell compartment that controls the temperature around the cell within ± 0.1°C were used.

Using SQUAD software the stoichiometry of the complex and the formation constant were determined by analyzing the optical absorption of considered mixtures ($Fe^{2+}_{(aq)}$ + L-Alanine) at various L-Alanine concentrations.

RESULTS AND DISCUSSIONS
The Absorbance of $Fe^{2+}_{(aq)}$
Figure 1 shows that the maximum absorption band respect to the solutions of Fe^{2+} ion obey the Beer's law over the concentration range of 1×10^{-5} -5×10^{-5} M. Figure 2 shows the absorption spectra

of considered metal ion. The band of $Fe^{2+}_{(aq)}$ is 285-335nm.

All of the considered metal ion solutions were titrated at ionic strength 0.1 M of potassium nitrate and in potassium hydrogen phthalate buffer 50mM, (pH = 4.1 ± 0.01).

Interaction of $Fe^{2+}_{(aq)}$ ion with L-Alanine
The solution of $Fe^{2+}_{(aq)}$ ion was titrated with a stock solution of L-Alanine. It can be assumed that the concentration change due to the adding the titrant is negligible because the total volume change during the titration is less than 6%. Maximum bands respect to $Fe^{2+}_{(aq)}$ were shifted hypochromicity of 20-45%. The representative UV-VIS spectra are shown in figures 3 and 4.

Thermodynamic functions
The standard Gibbs free energy,$\Delta G°$, related to each complex formation is calculated according to equation (1)

$$\Delta G° = –RT \ln K \qquad (1)$$

where K is the equilibrium formation constant of the reaction, T is temperature in Kelvin and R is gas constant. According to the Van؛ t Hoff equation (2)

$$d\ln K/d(1/T) = -\Delta H°/R \qquad (2)$$

a linear plot of lnK versus $1/T$ is observed, if the heat capacity change for the reaction is essentially negligible.

$$\ln K = (-\Delta H°/R)(1/T) + constant \qquad (3)$$

The considered standard entropy change,$\Delta S°$, of the reaction is calculated from equation (4)

$$\Delta S° = (\Delta H° – \Delta G°)/T \qquad (4)$$

The formation constants for the reaction "$2L^- +M^{2+} \rightarrow ML_2$" at different temperatures and their thermodynamic parameters such as $\Delta G°$, $\Delta H°$ and $\Delta S°$ were calculated (table1).

SQUAD program
In order to analyze the spectral data at various concentrations of L-Alanine in titration experiments, 50 wavelengths were selected. The values of absorbances of these selected wavelengths at various L-Alanine concentrations were analyzed in order to calculate equilibrium formation constants, using SQUAD program. Input data were absorbances at 50 different wavelengths of 15 solution spectra. These 15

spectra correspond to 15 various concentrations of L-Alanine. The outputs were the logarithm of equilibrium formation constants, log K_{ij} for the following reaction:

$$i\ L^- + j\ M^{2+} \rightleftharpoons (L^-)_i(M^{2+})_j \tag{5}$$
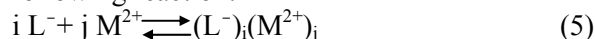
$$K_{ij} = [(L^-)_i(M^{2+})_j]/[(L^-)^i][(M^{2+})^j] \tag{6}$$

The estimated formation constants for the formation of 2:1 complex between $(L^- + Fe^{2+}_{(aq)})$ at various temperatures are listed in table1.

CONCLUSION
Using SQUAD program, the absorbance data obtained from the titration of $(Fe^{2+}_{(aq)} + $ L-Alanine) system were analyzed in order to calculate the respective formation constants.

SQUAD program refines the formation constants by employing a non-linear least square approach. The results represent the formation of 2:1 complex model between considered metal ion and L-Alanine. In Figure 5, a linear plot of lnK versus 1/T is observed respect to $(Fe^{2+}_{(aq)} + $ L-Alanine) system. The positive slopes in the plots represent the exothermicity of $(Fe^{2+}_{(aq)} + $ L-Alanine) reaction and the high correlation coefficient of the line indicate that the standard enthalpy, ΔH^o, and standard entropy, ΔS^o, of the reaction are temperature independent.

ACKNOWLEDGMENT

Table 1. Thermodynamic functions for binding of L-Alanine to $Fe^{2+}_{(aq)}$ in 50mM potassium hydrogen phthalate buffer, pH=4.1 $\pm$ 0.01, and ionic strength of 0.1M potassium nitrate at various temperatures

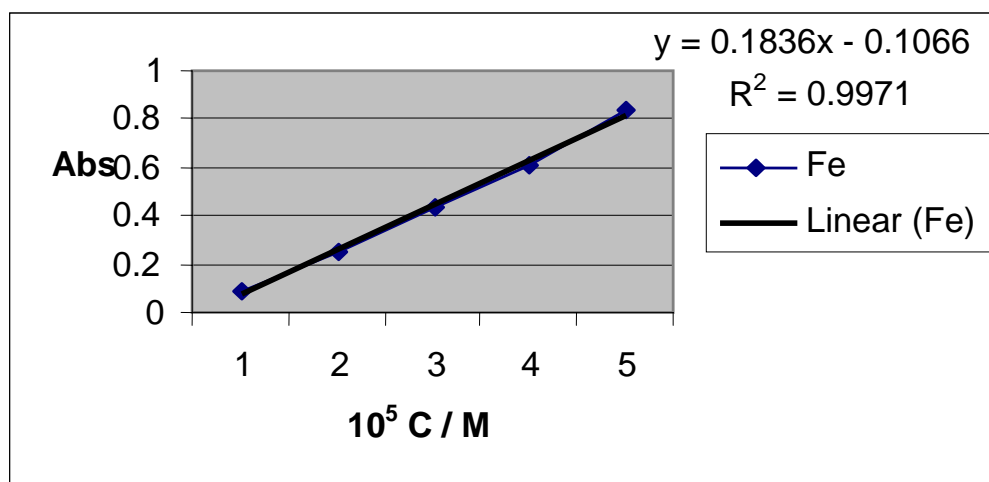| $\theta/\ ^{\circ}C$	$(K\pm\Delta K)\times10^{-5}$	$\Delta G^{\circ} \pm \Delta\Delta G^{\circ}$ /kJmol^{-1}	$\Delta H^{\circ} \pm \Delta\Delta H^{\circ}$ /kJmol^{-1}	$\Delta S^{\circ} \pm \Delta\Delta S^{\circ}$ /Jmol^{-1}K^{-1}
15	3.23594±1.045	-30.394±0.105	-54.08±0.079	-82.2±0.076
20	1.5135±1.022	-29.07±0.053	-54.08±0.079	-85.314±0.102
25	0.8317±1.028	-28.082±0.068	-54.08±0.079	-87.197±0.05
30	0.4466±1.025	-26.986±0.062	-54.08±0.079	-89.375±0.069
35	0.2398±1.049	-25.838±1.22	-54.08±0.079	-91.65±1.26

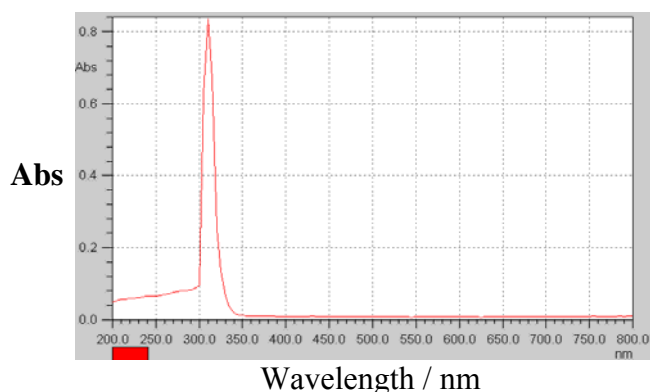Fig.1. Absorbance,Abs, as a function of concentration of $Fe^{2+}_{(aq)}$ at 25 $^{\circ}$C.

Fig.2. Absorption spectra of $Fe^{2+}_{(aq)}$ and its maximum band.

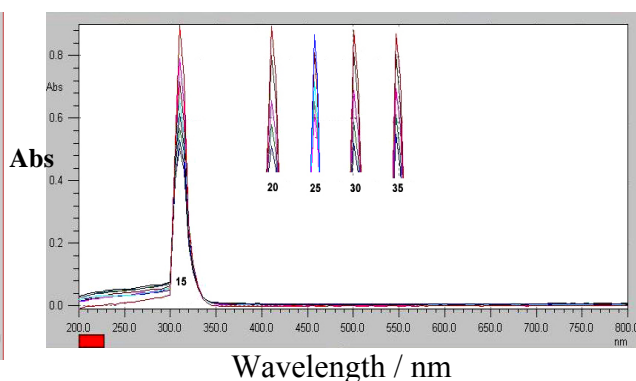**Fig.4.** Absorption spectra of $Fe^{2+}_{(aq)}$ upon titration with L-Alanine in potassium hydrogen phthalate buffer, pH 4.1, and ionic strength of 0.1M KNO_3 at 15˚,20˚,25˚,30˚ and 35˚C.
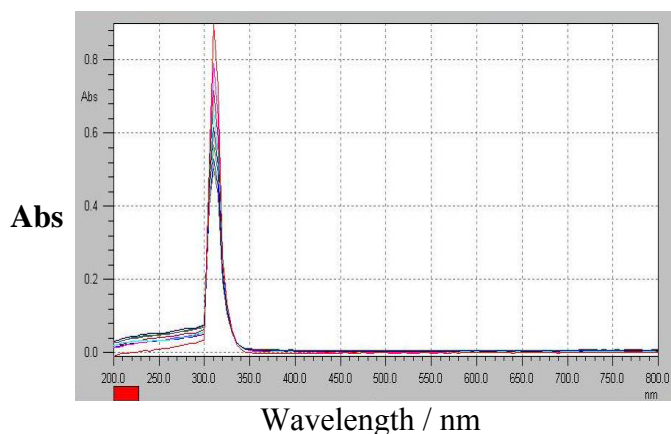

Fig.3. Absorption spectra of $Fe^{2+}_{(aq)}$ upon titration with L-Alanine in potassium hydrogen phthalate buffer, pH 4.1, and ionic strength of 0.1M KNO_3 at 25 ˙C
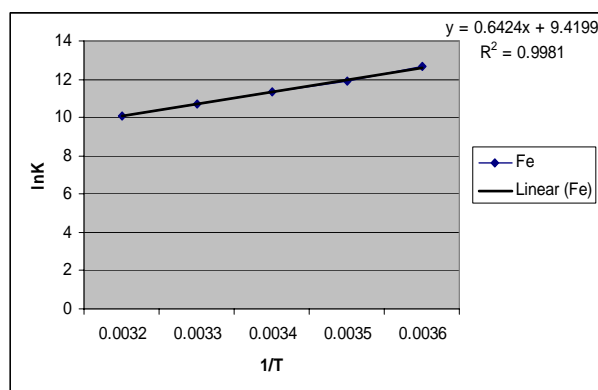

Fig.5. A linear plot of lnK versus 1/T for binding of $Fe^{2+}_{(aq)}$ to L-Alanine in the potassium hydrogen phthalate buffer, pH 4.1, and ionic strength of 0.1M KNO_3.

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