Study the Molar Ratio of the Reaction between Thalassemia s Drug (Deferoxamine) with Iron Over loud

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Abstract

Iron-Deferoxamine complex have been synthesized in vitro by the reaction of deferoxamine (which are use as therapeutic agent for removing iron overload from the bodies of thalassemia patients) with Fe(II) salt in aqueous medium. The geometry of the prepared complex as well as the mode of bonding were determined through FTIR, UV-Vis, mass spectral studies, metal content, and molar conductance. These studies revealed octahedral geometry for the complex with the general formula $[Fe^{II}(DEF)]Cl_2$. Complex formation via molar ratio method and variation continuous method in aqueous solution have been also investigated and results were consistent to those found in the solid complexes with a ratio of (M:L) as (1 : 1). Conditional formation constant could also be calculated for $[Fe^{II}(DEF)]Cl_2$ as 2.0×10^3. The effect of time on the formation of Iron – Deferoxamine complex has been also studied. This study showed that each 1×10^{-3} M of deferoxamine needs 2.5 – 3 hrs to remove 1×10^{-3} M of Iron (II) by formation coordination complex.

Keywords: Thalassemia, Deferoxamine, Iron Over loud.

Introduction

Iron is stored in the body in the form of the protein complexes, ferritin and hemosiderin, and is transported in the plasma via the protein complex, transferring[1]. It has been estimated that, under normal physiological conditions, about a third to a half of transferrin's iron capacity is utilized. The excess of iron leads to saturation of the transferrin and ferritin and results in toxicity as the iron overloud levels the bloodstream and accumulates in tissues. In principle, this condition can be treated by administration of some of iron chelating agent remobilizes iron and permits its excretion [2].

Hydroxamic acids repersent one of the most important organic bioligands. Firstly the physiological role of these weak acids was used as siderophores by microorganisms [3,4]. Nevertheless, siderophores are also used as pharmacological and agrochemical agents in addition to it uses in clinical therapy, in the sequestration or scavenging of metallic ions [5]. Deferoxamine is an example of hydroxamic siderophore that employed in the treatment of patients constantly submitted to blood transfusion. Iron accumulation in these cases can reach toxic. Deferoxamine administration is an effective way to remove iron excess by water soluble complex formation that is eliminated through bile or urine [6,7]. In general, hydroxamic siderophores may be considered in infection treatment, such as malaria, tuberculosis, leprosy and cholera due to ability to access infected cells and remove iron from intracellular parasites. In this paper, Iron-Deferoxamine complex has been synthesized in vitro by the reaction of deferoxamine with Fe(II) salt in aqueous medium. The mode of bonding and geometry of the prepared complex were determined by used different techniques FTIR, UV-Vis, mass spectral studies, metal content, and molar conductance. Complex formation via molar ratio method and variation continuous method in aqueous solution have been also investigated and the formation constant of the complex has been calculated.

Experimental
Materials and Measurements

All reagents were commercially available (Aldrich Co.) and used without further purification. All manipulations in the synthesis of $[Fe^{II}(DEF)]Cl_2$ was performed under air. Electronic spectra were measured in the region (200–900) nm for solutions in distilled water at room temperature using a Shimadzu, 100 UV–visible spectrophotometer. Infrared spectra were recorded using a Shimadzu 8400 FTIR

spectrophotometer. Mass spectrum was recorded using Waters 996 Micromass. Conductivity measurements were made with distilled water solution using a Jenway 4071 digital conductivity meter.

Synthesis of [Fe(DEF)]Cl$_2$

To a solution of deferoxamine (5.6 ×10^{-3} g, 0.01 mmol) in 10 ml distilled water was added the solution of FeCl$_2$.XH$_2$O (1.27 × 10^{-3}g, 0.01 mmol) in 10 ml distilled water. The solution was not stirred and left undisturbed overnight. The complex was obtained by slow evaporation of solvent at room temperature. Yield 87%, m.p = 100 C°.

Molar Ratio

Complex formation by molar ratio of Deferoxamine to Fe(II) was studied in distilled water. A series of solutions containing constant concentration of Fe(II) (10^{-3} M) was treated with the same volumes of various concentrations of Deferoxamine. The results of L: M titrations were obtained by plotting the correct absorbance of solution mixtures at 430 nm of the complex against [L]/[M].

Continuous Variation Method

Complex formation by continuous variation method was also studied in distilled water. A series of solutions was prepared, each containing the same total number of moles of deferoxamine and Fe(II), but a different ratio of Fe(II) moles to deferoxamine moles. The results of L: M titrations were obtained by plotting the correct absorbance of solution mixtures at 430 nm of the complex against $V_{Fe(II)}/V_{Fe(II)} + V_{DEF}$.

Result and Discussion

Deferoxamine is a trihydroxamic siderophore with a terminal primary amine group (Scheme (1)).
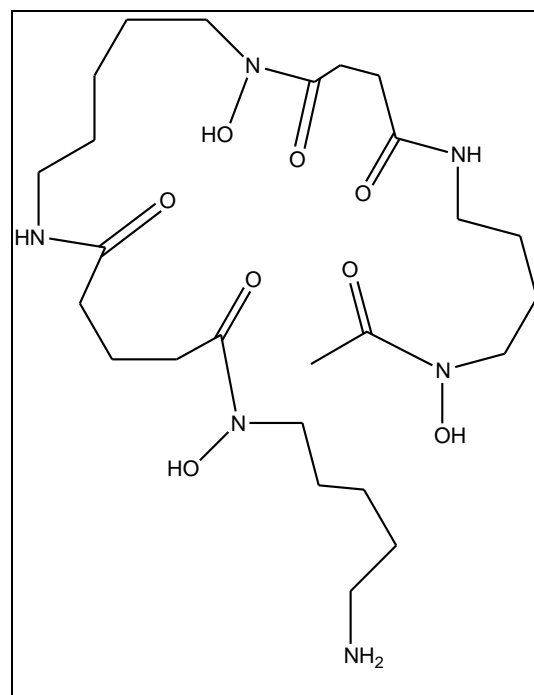
Scheme (1): General structural formulae of Deferoxamine.

It has medical applications as a chelating agent used to remove iron loud from the body [8]. Deferoxamine is used to treat acute iron poisoning in small children. This agent is usually frequently used to treat hemochromatosis, a disease of iron accumulation that can be both genetic and acquired hemochromatosis is common in patients with types of chronic anemia (e.g. thalassemia and myelodysplastic syndrome) who require a blood transfusions, which can increase the amount of iron in the body [9]. Administration for chronic conditions is basically accomplished by subcutaneous injection (SQ) over a period of 8–12 hours daily. The effect of deferoxamine after acute intoxication may color the urine a pinkish red, a phenomenon termed "*vin rose* urine".

Synthesis of Iron – Deferoxamine Complex

Iron–Defroxamine complex was synthesized by the reaction of equimolar amounts of Deferoxamine and FeCl$_2$.XH$_2$O in aqueous media under nitrogen atmosphere. Complex with general formulae [Fe DEF]Cl$_2$ was obtained, (Scheme (2)).
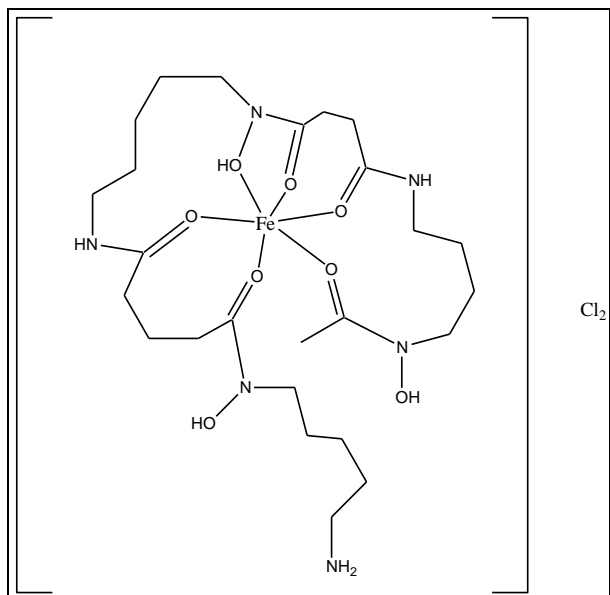
***Scheme (2): Proposed structure of Iron –
Deferoxamine complex.***

Despite numerous attempts, have been unable to obtain crystals suitable for x-ray structural diffraction. The complex is soluble in water and other common organic solvents. Deferoxamine has a high selectivity for Fe(III) may be explained by the principle of hard and soft Lewis acid and base, where acids are electron acceptor atoms and bases are the electron donor ones in a chemical bound. Hard acids (small, compact and scarcely polarizable atoms), like Fe(III), present high affinity for hard bases, like oxygen. Therefore, deferoxamine complexes present different stabilities depending upon the hardness of the metal. The highest affinity of Fe(III) for deferoxamine can be also explained by d electron configuration, high charge density and high electronegativity presented by this ion [5,10]. The carbonic chain length and the structure that connects the hydroxamic groups as well as the number of chelating groups also affect complex stability [11,12].

Characterization of Iron–Deferoxamine complex

The complex was characterized by different techniques (FTIR spectrophotometer, UV–Vis spectrophotometer, Mass spectrophotometer, Atomic absorption spectrophotometer, Molar conductivity, Magnetic Susceptibility). The analytical data agree well with the suggested formulae. The positive (EI) mass spectrum of the complex, Fig.(1) showed the parent ion

peak at m/z 688 corresponding to (M+). The FTIR spectrum exhibited band at 3313 cm^{-1}, which is assigned to υ (NH) vibration. The bands due to υ (C - H) and υ (C=O) vibrations are located in the regions 2931 and 1626 cm^{-1}, respectively [13]. At far infrared region the complex showed band at 530 cm^{-1}, which could be assigned to the vibration of υ(Fe - O) [14]. The UV – Vis spectrum of deferoxamine displayed an intense absorption peak related to the (π–π*) transition, at 48543 cm^{-1}. The (n–π*) transition may be obscured by this absorption band, Fig.(2). The electronic spectrum of Iron – Deferoxamine complex showed the (π–π*) transition at 47846 cm^{-1}. The spectrum also displayed abroad band around (18181 – 30303 cm^{-1}) which could be attributed to CT and spin allowed transition in octahedral geometry, (^5T_2g – ^5A_1g) and (^5T_2g – ^5Eg) [15 – 17]. The molar conductivity of the complex is 235 *S cm^2 mol^{-1}*. This value indicates the electrolytic nature of Iron–Deferoxamine complex [18].
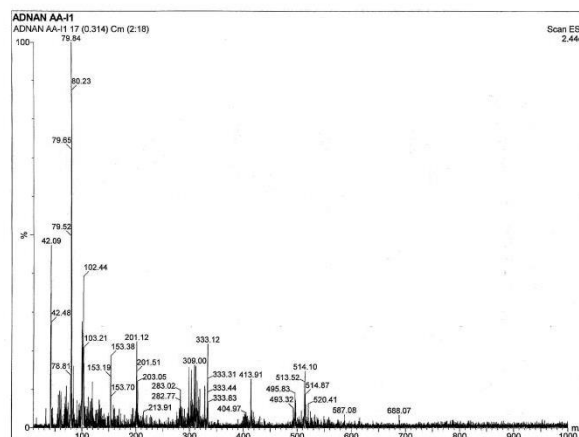

***Fig.(1): Mass spectrum of Iron –
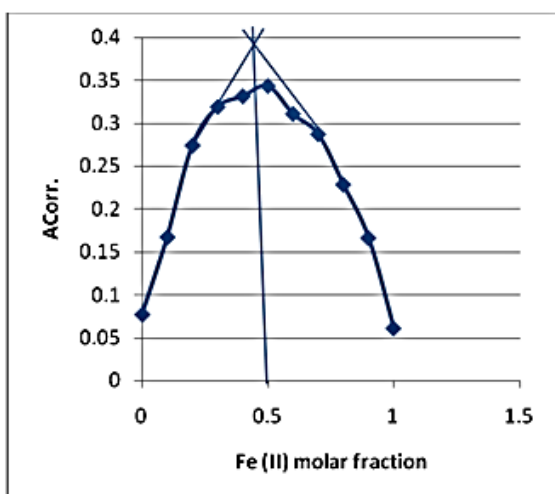Deferoxamine complex.***

Continuous Variation Method

The method of Continuous variations has been used frequently to determine the formulas and formation constants of complexes since its introduction by Ostromisslensky [19] and Job [20]. The method is based on plotting measured absorbances, corrected for absorbances of reactants assuming no complexation, against mole fractions of the two constituents of a complex. Thus, a series of solutions is prepared by mixing different volumes of equimolar solutions of

deferoxamine and Fe(II) and diluting to a constant volume to give solutions having identical total molar concentrations but different mole fractions. Firstly deferoxamine's and Fe(II) electronic spectra were acquired in order to evaluate the best wavelength to perform the complex absorbance measurements Fig.(2).
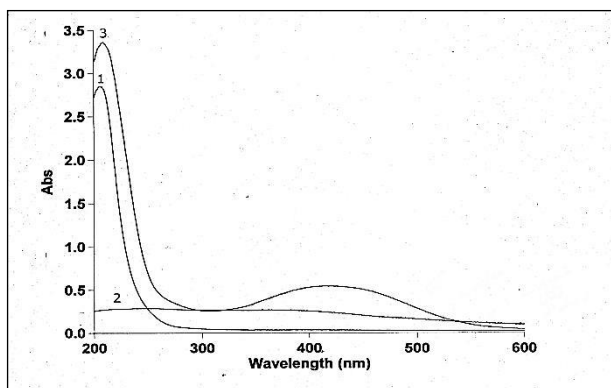
Fig.(2): U.V – Vis spectra 1) Deferoxamine 2) FeCl$_2$.XH$_2$O 3) Iron – Deferoxamine complex.

The selected wavelength was 430 nm due to the neglecting absorbance of deferoxamine and the minimal contribution of Fe(II). Despite the Fe(II) small contribution at 430 nm, the complex absorbance was corrected by Fe(II) subtraction in this wavelength (Acorr.). The Job's plot presented in Fig.(3) indicates that the complex stoichiometry is M:L=1:1.This result agrees well with the data of the mass spectrum of the complex. Formation constant of the complex has been calculated by using Job's methods, it found to be 2.0×10^3.
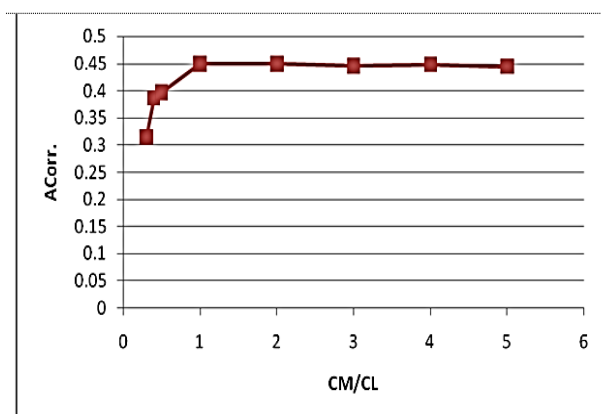

Fig.(3): Job's plot of Iron – Deferoxamine complex.

Molar Ratio

Aiming to compare the obtained results by continuous variation method, molar ratio method was carried out with the well established spectrophotometric technique.

Series of solutions containing constant concentration of Fe(II) (10^{-3} M) was treated with the same volumes of various concentrations of Deferoxamine. The molar ratio plot presented in (Fig. (4)) indicates that the complex stoichiometry is M:L=1:1. This result corroborates with the result obtained by Continuous variation method.

Fig.(4): Molar ratio plot of Iron – Deferoxamine complex.

Effect of time on the formation of Iron – Deferoxamine complex

In order to study the effect of time on the formation of Iron–Deferoxamine complex, and so to determine the exact time which is deferoxamine needs to remove all of Iron (II) overload, 1×10^{-3}M solution of deferoxamine was mixed with 1×10^{-3}M solution of Iron (II) salt. The absorbances of complex were obtained at 430nm and at different time, Fig.(5). This study showed that Iron–Deferoxamine complex formed immediately and increase with time. So, it can be seen from Fig.(5) that each 1×10^{-3}M of deferoxamine needs 2.5 – 3 hrs to remove 1×10^{-3}M of Iron (II) overload.
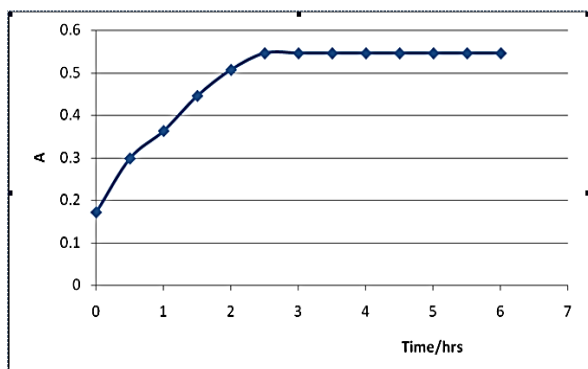
Fig.(5): Effect of time on the formation of Iron – Deferoxamine complex.

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الخلاصة

تم تحضير معقد (ديفيروكسامين–الحديد) خارج النظام الحياتي من تفاعل الديفيروكسامين والمستخدم كمادة علاجية تعمل على إزالة الحديد الزائد من أجسام مرضى الثلاسيما مع ملح الحديد في وسط مائي. طبيعة التاصر والشكل الهندسي للمعقد تم تحديد من خلال استخدام تقنية الأشعة تحت الحمراء البعيدة ومطايف فوق البنفسجية–المرئية ودراسة طيف الكتلة ومحتوى المعقد من عنصر الحديد(III) ودراسة التوصيلة المولارية وكشفت الدراسة إن شكل المعقد هو ثماني السطوح وان الصيغة العامة للمعقد هو [[Fe^{II} (DEF)]CI2 تكون المعقد بطريقة النسب المولارية وطريقة النسب المتغيرة تم التحقق منها في الوسط المائي وكانت النتائج متطابقة مع الحالة الصلبة للمعقد كذلك وكانت النسب بين الايون المركزي والليكند (L: M) يمثل (1:1) وثابت التكوين المقاس للمعقد [[Fe^{II} (DEF)]CI2 هي 2.0×10^3 وقت تكوين المعقد ديفروكسامين–الحديد تم دراسته أيضا وأظهرت الدراسة إن كل 1×10^{-3} مولاري من ديفروكسامين تحتاج 2,5–3 ساعة لإزالة 1×10^{-3} مولاري من الحديد (II) عن طريق المعقد ألتناسقي.