

EXPERIMENTAL STUDY

Iron chelating ligand for iron overload diseases

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Iron overloads are a serious clinical condition in the health of humans and are therefore a key target in drug development. In this study, iron(III) complex of 8-hydroxyquinoline-5 sulphonic acid was synthesized and structurally characterized in its solid state and solution state by FT-IR, UV-Vis, elemental analysis, magnetic susceptibility and ¹H-NMR. The catalase activities of complex were investigated. It was showed that the complex has the catalase activity. It is suggested that this type of complex may constitute a new and interesting basis for the future search for new and more potential drugs. The electrochemical behaviour patterns of the ligand and complex were examined as supporting electrolyte and platinum electrode for cyclic voltammetry. The electrochemistry studies showed that the reductions in free ligand and complex take place differently. The cytotoxicity was evaluated by MTT assay. The complex exhibited a very high cytotoxic activity and showed a cytotoxic effect that was much better than that of the ligand. The observed cytotoxicity could be pursued to obtain a potential drug. These results indicate that using the 8-hydroxyquinoline-5 sulphonic acid for this aim in further studies is appropriate (Tab. 1, Fig. 4, Ref. 18). Text in PDF www.elis.sk.

KEY WORDS: iron overload, complex, ligand, 8-hydroxyquinoline-5 sulphonic acid, catalase.

Iron plays a crucial role in the regulation of numerous cellular functions and is thus essential for life. The ability of iron, through redox cycling, to function as both an electron donor and acceptor means that it serves as a co-factor within the active site of several key enzymes in a number of critical biochemical pathways including ATP generation, oxygen transport, cell cycling and DNA synthesis (1, 2, 3, 4). Although iron is essential for the proper functioning of all living cells, it is toxic when present in excess. Iron overload occurs when excess iron accumulates in the body. It is a common clinical problem arising from disorders of increased iron absorption such as hereditary haemochromatosis or thalassaemia intermedia syndromes or as a consequence of chronic blood transfusions for various blood disorders. In the presence of molecular oxygen, the “loosely-bound” iron is able to undergo the redox cycle between its two most stable oxidation states, namely iron (II) and iron (III), thereby generating oxygen-derived free radicals such as hydroxyl radicals. Hydroxyl radicals are highly reactive and capable of interacting with most types of biological molecules including sugars, lipids, proteins and nucleic acids, resulting in peroxidative tissue damage and life-threatening complications such as cirrhosis, hepatocellular cancer, diabetes and heart diseases (5, 6, 7, 8, 9, 10, 11).

The best selection for treatment of iron overload is iron chelation therapy (12). Chelation therapy was introduced in clinical practice in the seventies of last century to defend thalassaemic patients from the toxic effects of iron overload. Among the numerous tasks of iron chelating agents, deeply discussed in several reviews, the main ones consist of creating a favorable equilibrium between transfusional iron assumed by patients and that excreted in a chelated form, protecting against the circulating non-transferrin-bound iron which via Fenton reaction easily leads to ROS production, as well as of scavenging iron stores from organs and tissues where the toxic action takes place (13, 14, 15). Currently, three iron chelators are licensed for clinical use. The most widely-used therapeutic iron chelator in haematology over the past 40 years has been Desferrioxamine-B (DFO) a hexadentate ligand, which suffers the major drawback of being orally inactive (16, 17). The joined research efforts of clinicians, biochemists and chemists to improve the knowledge of iron metabolism and requisites of iron chelators has led to the introduction of two new oral chelators, deferiprone (DFP) and deferasirox (DFX), into clinical use at the beginning of this century. These drugs are extremely useful in the treatment of iron overload, but they too have some disadvantages, the fact of which lends an urgency to develop new chelating agents that would be more suitable from the clinical point of view (18).

The aim of this work is to study the coordination of iron (III) ions with 8-hydroxyquinoline-5-sulphonic acid ligand which may give chelate complexes that may be used in iron overload treatment. In addition, the cytotoxicity and electrochemistry studies of iron (III) complex with 8-Hydroxyquinoline-5-sulphonic acid ligand and its catalase-like activities were investigated in this study.

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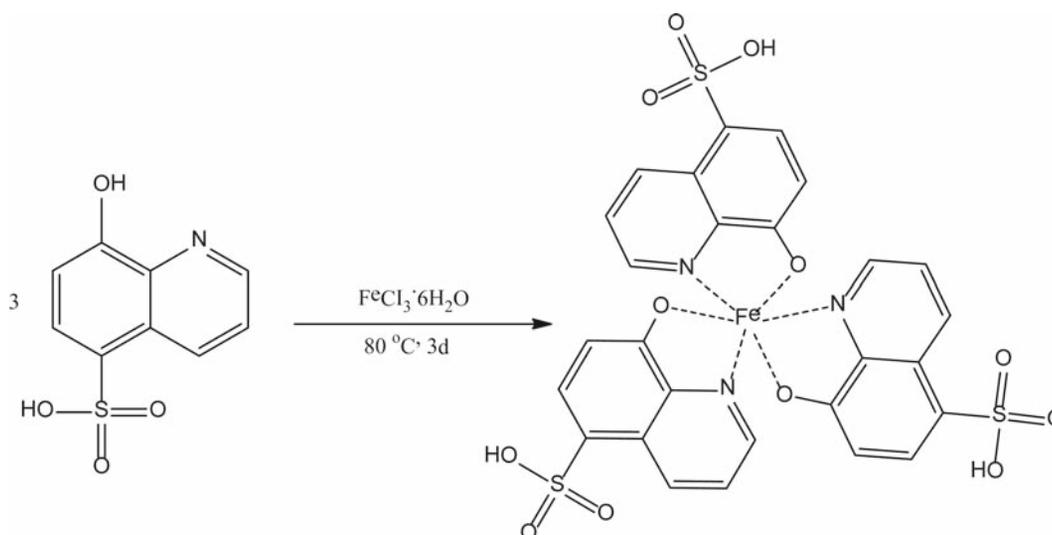


Fig. 1. The synthetic route of complex.

Materials and methods

Synthesis and characterization

The complex was prepared by treating 8-hydroxyquinoline-5-sulfonic acid (0.1 mmol, 0.019 g) with $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$ (0.1 mmol, 0.016 g) in ethanol under solvothermal conditions at 80 °C for four days. Green crystals of complex were harvested. Tris(8-hydroxyquinoline-5-sulfonic acid) iron(III) complex ($\text{Fe}(\text{HQS})_3$) was obtained as a green powder in good yield (0.028 g, 95 %). The results from C, H, N elemental analyses are in excellent agreement with the expected chemical formula (Anal. Calc.) for $\text{C}_{27}\text{H}_{18}\text{FeN}_3\text{O}_{12}\text{S}_3$: C 44.22; H 2.59; N 11.98 %. Found: C 44.24; H 2.63; N 11.84. IR (KBr, cm^{-1}): 1605 (s, $\nu(\text{C}=\text{N})$), 1305 (s, $\nu(\text{S}=\text{O})$), 968 (s, $\nu(\text{N}-\text{O})$), 572 (s, $\nu(\text{Fe}-\text{N})$), 464 (s, $\nu(\text{Fe}-\text{O})$). UV (nm) 259.342.386.438.560. (For the synthetic route) (Fig. 1).

Cytotoxicity assay (Cell culture and MTT assay)

Cytotoxic effects of the compounds were evaluated by MTT test for HUVEC human umbilical vein endothelial cell line. The cells were cultured in a humidified atmosphere with 5 % CO_2 at 37 °C. The cells were grown in 75 cm^2 culture bottles supplied with 15 mL DMEM, and after a few passages, the cells were plated in a 96-multiwell plate. After 24 h of incubation of the cells, the medium was replaced with 100 μL medium containing various doses of ligand and complex (12.50, 25, 50, 100, and 200 μM). After 72 h of treatment, the cell viability was determined by the MTT assay. The proliferation test is based on the color reaction of mitochondrial dehydrogenase in living cells by MTT. At the end of the treatment period, MTT (final concentration 5 mg mL^{-1} PBS) was added to each well, which was then incubated at 37 °C in 5 % CO_2 atmosphere for 2–4 h. The absorbance was measured at 570 nm on microplate reader (Elisa 2100C). Cell proliferation was calculated as the ratio of the absorbance of the treated group divided by the absorbance of the control group, multiplied by

100 to give the percentage proliferation. The cytotoxicity index (CI) was calculated using the following formula compared with the control: $\text{CI}\% (\text{Cytotoxicity index}) = 1 - \text{OD treated wells} / \text{OD control wells} \times 100$. Also, the inhibitory concentration of cell growth (IC_{50} = the concentration of the compound that inhibited 50% cells) was calculated from dose–response curves. Statistical analysis was performed using the Statistical Package for Social Statistics (SPSS).

Catalase activity

Catalase activity was measured using Biodiagnostic Kit which is based on the spectrophotometric method described by Aebi. The examination of catalases-like activities of different concentrations of complex was done by reacting a known quantity of H_2O_2 catalase with a known quantity of hydrogen peroxide, and the reaction is stopped after 1 min with catalase inhibitor. In the presence of peroxidase, the remaining hydrogen peroxide reacts with 3,5-Dichloro-2- hydroxybenzene sulfonic acid and 4-aminophenazone

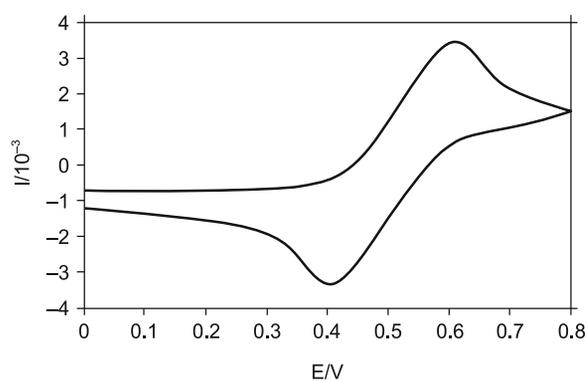


Fig. 2. Cyclic voltammogram of 1 mM $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$ 0.1 M TBATFB in DMSO on Pt electrode, $\nu = 0.1 \text{ V s}^{-1}$ (vs $\text{Ag}|\text{Ag}^+$ electrode).

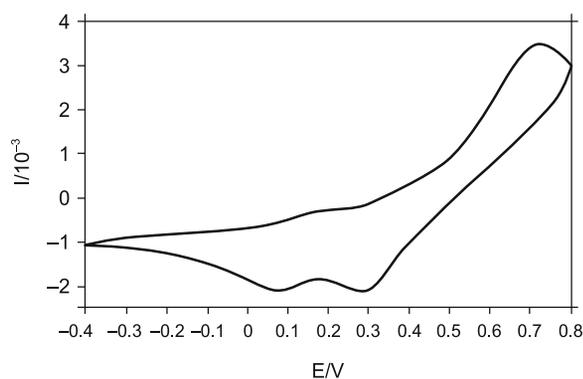


Fig. 3. Cyclic voltammogram of 1 mM Ligand 0.1 M TBATFB in DMSO on Pt electrode, $\nu = 0.1 \text{ V s}^{-1}$ (vs Ag/Ag⁺ electrode).

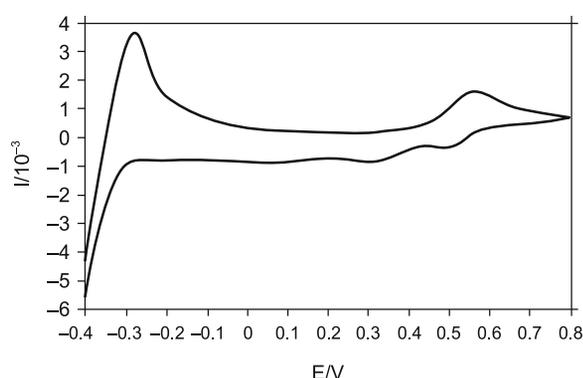


Fig. 4. Cyclic voltammogram of 1 mM Fe (III)-ligand complex in 0.1 M TBATFB in DMSO on Pt electrode, $\nu = 0.1 \text{ V s}^{-1}$ (vs Ag/Ag⁺ electrode)

to form a chromophore with a color intensity inversely proportional to the amount of catalase in the sample. The absorbance was measured at 510 nm.

Cyclic voltammetry

The cyclic voltammetric studies of Fe(III)-8-hydroxyquinoline-5-sulfonic acid complex was conducted at platinum electrode. The cyclic voltammogram of the complex was conducted at a scan rate of 100 mVs^{-1} . The current-potential curves for ligand, and curcumin-iron (III) complex are presented in Figures 2, 3 and 4, respectively. A standard three-electrode assembly was employed: platinum as working electrode, Ag/AgCl as reference electrode, and platinum wire as counter electrode. The CV of the complex is reversible.

Results

Structural characterization

The synthesis complex was characterized by techniques like CHN analysis, FT-IR, ¹H-NMR, UV-vis, and magnetic susceptibility. The results from the C,H,N elemental analyses are in excellent agreement with the expected chemical formula (Anal. Calc.) for C₂₇H₁₈FeN₃O₁₂S₃: C 44.22; H 2.59; N 11.98 %. Found:

C 44.24; H 2.63; N 11.84. The infrared spectrum of the complex recorded the band characteristics of the coordinated ligand. The absence of broad characteristic peak at 3.429 cm^{-1} corresponding to O-H stretching vibration confirmed the coordinate nature of Fe-O and Fe-N bond. Formation of Fe-O, Fe-N bands were further supported by the appearance of band at 464 cm^{-1} and 572 cm^{-1} . All these frequencies confirmed the formation of complex. The chemical shifts of protons of the complex using chloroform as a solvent were recorded and following δ values in ppm with respect to tetramethylsilane are obtained: 7.18 (d,2H); 7.59 (d,2H); 7.97 (t,2H); 8.87 (d,2H); 9.52 (d,2H). The absence of singlet peak at 9.7 δ of hydroxyl proton otherwise present in H-NMR of ligand indicated the formation of Fe-O bond in the complex. The proposed formula was also consistent with the IR spectrum. The UV-Vis absorption spectrum of Fe (III), ligand and complex are shown in Figure 2, in which three characteristic absorption bands were found. The spectrum of 8-Hydroxyquinoline-5-sulphonic acid and its Fe (III) complex were recorded at pH 6–8 in the range of 200–800 nm. The complex shows λ_{max} at 259 nm & 342 nm–386 nm & 438nm–560 nm corresponding to π - π^* , n - π^* , and d-d transition, respectively. Magnetic susceptibility measurements of the complexes were performed at room temperature. The magnetic moment values for the various Fe (III) complexes are in the range of 2.49 BM for octahedral complexes.

Catalase activity

The catalytic activity studies of Fe (III) complex in DMSO towards the disproportionation of hydrogen peroxide were also performed. The study showed that the complex is catalytically active. At a concentration of 8.0 mM, the activity was equivalent to 604.40 U/L. When the coordinate Fe (III) ion is present in structure, the catalytic reactivity greatly enhances.

Cytotoxicity assay

Firstly, the cytotoxic potential of ligand and complex was investigated in HUVEC endothelial cells by the colorimetric MTT assay. The cells were exposed to different concentrations of ligand and complex for 24 and 72 h at 37 °C. The results obtained were IC₅₀ values (Tab. 1). When comparing the cytotoxic effect of the complex and ligand, a significant difference was observed.

While the complex exerted extreme cytotoxicity with IC₅₀ values for 72-h treatment of 4,56 μM (Tab. 1), the ligand did not show a considerable cytotoxic effect. The complex exerted a very high cytotoxic activity while the cytotoxicity increased with the increase in complex concentration. A statistically significant difference in cytotoxicity after 24 h of treatment was noticed compared to that after 72 h of treatment.

Tab. 1. Growth inhibitory effects – IC₅₀ values (μM) of the ligand and its Fe (III) complex on HUVEC cell lines after 24 and 72 h of treatment (mean \pm standard deviation of $n = 8$ experiments).

Compound	HUVEC	
	24 h	72 h
Ligand	254.74 \pm 2.15	400
Fe (III) complex	25.79 \pm 0.43	4.56 \pm 0.06

Cyclic voltammetry

The cyclic voltammetric behavior of Fe (III) complex of 8-HQ-5-SA was studied in DMSO at room temperature. The cyclic voltammograms of Fe (III)-8-HQ-5-SA complex and free ligand were compared first. Then the electrochemical reduction of Fe (III)-8-HQ-5-SA complex was investigated. Then the electrochemical reduction of Fe (III)-8-HQ-5-SA complex was investigated by cyclic voltammetry. The current-potential curves for $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$, ligand and its Fe (III) complex using 0.1 M TBATBF in DMSO are presented in Figures 2, 3 and 4, respectively.

The voltammetric behavior of free ligand is characterized by two reduction peaks in this medium at a platinum surface (Fig. 3). The peak potential is about 0.075 V and 0.28 V (vs. $\text{Ag}|\text{Ag}^+$) at a scan rate of $\nu = 0.1 \text{ Vs}^{-1}$. The peak height corresponds to a two-electron reduction. There appears no oxidation peak in the reverse scan. It is seen that the free ligand reduces irreversibly with two electron transfers on a platinum surface. In contrast to free ligand, the Fe (II)-8-hydroxyquinoline-5-sulfonic acid complex undergoes a quasi-reversible process at the platinum surface. The complex gives a two-electron transfer redox system at 0.3 V and 0.5 V (vs. $\text{Ag}|\text{Ag}^+$). The oxidation peak is also observed in the reverse scan for the complex (Fig. 4). The peak potential is about -0.3 V and 0.56 V. These results indicate that the reductions of free ligand and complex take place differently.

Discussion

Iron overload is a serious clinical condition which can be largely prevented by the use of iron specific chelating agents. Despite the significant improvements made in the cure of iron overload with the introduction of deferiprone and deferasirox, and combined chelation therapy, the clinical results have not been completely satisfactory for various drawbacks presented by these chelators. The failure to find the ideal iron chelator can be ascribed to inherent difficulties deriving from the biological and clinical restraints. Although a number of agents, natural or synthetic, are currently available or being screened to reduce the iron overload, the development of a best ligand is still a challenge in the area of drug discovery and development. The treatment of chronic iron overload is a challenge to modern practitioners, which exposes them to a great dilemma regarding the management of the problem (6, 7, 18). In this study, the iron (III) complex of 8-hydroxyquinoline-5-sulfonic acid was synthesized and structurally characterized. The electrochemical reductions of ligand and Fe (III)-8-hydroxyquinoline-5-sulfonic acid complex were investigated at platinum surface in DMSO medium. The free ligand reduces irreversibly when the complex behaves as a two-electron quasi-reversible redox system. The peak potential is about 0.075 V and 0.28 V (vs $\text{Ag}|\text{Ag}^+$) at a scan rate $\nu = 0.1 \text{ Vs}^{-1}$. The complex gives two-electron transfer redox system at 0.3 V and 0.5 V (vs $\text{Ag}|\text{Ag}^+$). The oxidation peak is also observed in the reverse scan for the complex. The peak potential is about -0.3 V and 0.56 V. These results indicate that the reductions of free ligand and complex take place differently. In conclusion, the ligand and complex show different patterns. The complex shows an important catalase activity. It may constitute a new and interesting basis for

the future search of new and more potential drugs. The cytotoxicity of complex was investigated on HUVEC cell lines using the MTT viability test. The complex exhibited a very high cytotoxic activity and showed a cytotoxic effect that was much better than that of the ligand. The observed cytotoxicity could be pursued to obtain a potential drug. These results indicate that the use of 8-hydroxyquinoline-5-sulfonic acid for this aim in further studies is appropriate. Nevertheless, the claim that 8-hydroxyquinoline-5-sulfonic acid is a promising medicine needs more *in vitro* and *in vivo* studies.

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