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Investigation of deferiprone binding to different essential metal ions using microscale thermophoresis and electrospray ionization mass spectrometry

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ABSTRACT

In this study, the microscale thermophoresis (MST) method was applied to investigate the interaction of deferiprone with Fe^{3+}, Cu^{2+}, Zn^{2+}, Co^{2+}, Ni^{2+}, Mn^{2+}, Mg^{2+}, and Ca^{2+}. Experiments were performed on a MST (Monolith NT.115 LabelFree®) system. Pretest scanning indicated good fluorescence intensity of deferiprone allowing the use of label-free MST experiments. Different concentrations of the intended metal ions in the range of 0.048 to 100.0 μM were titrated against 100 μM fixed concentration of deferiprone dissolved in 0.1 M Tris buffer pH 7.4. MST measurements were performed in standard capillaries at 50% excitation power and 20% MST power. The results indicated significant interactions of deferiprone with Fe^{3+}, Cu^{2+}, Zn^{2+}, Co^{2+}, and Ni^{2+}. The data fitted to the Hill model with Hill coefficients of 1.8, 1.5, 3.2, 1.6, and 1.5 for Fe^{3+}, Cu^{2+}, Zn^{2+}, Ni^{2+}, Co^{2+}, respectively, thus indicating more than 1:1 stoichiometry. EC50 values for the binding of deferiprone to Fe^{3+}, Cu^{2+}, Zn^{2+}, Co^{2+}, and Ni^{2+} were calculated to be 20.6 ± 3.34, 38.1 ± 3.39, 39.5 ± 4.90, 51.1 ± 6.86, and 101.1 ± 22.70 μM, respectively. No binding was observed for deferiprone with Mn^{2+}, Mg^{2+}, and Ca^{2+}. Electrospray ionization mass spectrometry was used as a complementary technique under similar conditions; however, Tris buffer was replaced by ammonium acetate buffer to be compatible with the mass system. Electrospray ionization mass spectrometry confirms the results obtained by MST indicating stable molecular ion peaks for the complexes of deferiprone with either Fe^{3+}, Zn^{2+}, Cu^{2+}, Co^{2+}, Ni^{2+}, and Mn^{2+}, but no binding was observed for deferiprone with either Ca^{2+} and Mg^{2+} under a gas phase state. MST shows a fast and simple approach to study the binding of deferiprone to different essential metal ions. Moreover, the complexes were stable for investigation by mass spectrometry under a soft ionization technique like used electrospray ionization, which aided to confirm binding stoichiometry.

1. Introduction

Essential metals play an important role inside the body and are considered vital for different living systems. They mostly bind with proteins forming catalytic enzymes, or acting as regulators in gene expression and second messenger in signaling pathway or for transportation [1]. The amount of these metals in the living organism varies from kilograms, such as calcium (represents about 1–1.2 kg of the body weight), to ultra-trace amounts, such as cobalt (represents about 1–1.5 mg in the body) [2]. Metals toxicity is concomitant to various disease states, such as *Wilson disease, diabetes mellitus, Alzheimer disease, and β-thalassemia.* Metals accumulation may occur over the years inside body organs and lead to their hypofunctions, fibrosis, mutagenicity, or failure [3,4]. Herein, intervention with chelation therapy is the treatment strategy to protect the organs against metals toxicity. However, this strategy has several threats and needs an intensive monitoring during the treatment plan. For example, iron overload is the biggest problem in this context and is frequently occurring in some clinical situations, such as *β-thalassemia* due to blood transfusion every three to four weeks, which leads to more complications and some organs might be affected, such as the liver, kidney, and heart. Therefore, iron chelators are the most clinically used chelators nowadays either in single or combination therapy [5,6]. Deferiprone (CP20) (3-hydroxy-1,2-dimethylpyridin-4-one) (Fig. 1) is the first used oral iron chelator for patients with *β-thalassemia* to remove excess iron from different organ tissues, especially cardiac myocytes [7]. CP20 is bidentate chelator binds to Fe^{3+} in a 1:3 metal–CP20 ratio (Fig. 2). Regrettably, the chelation effect of CP20 is not an iron-selective process. There are studies that have described the chelation effect of CP20 on different metal ions, such as Zn^{2+},

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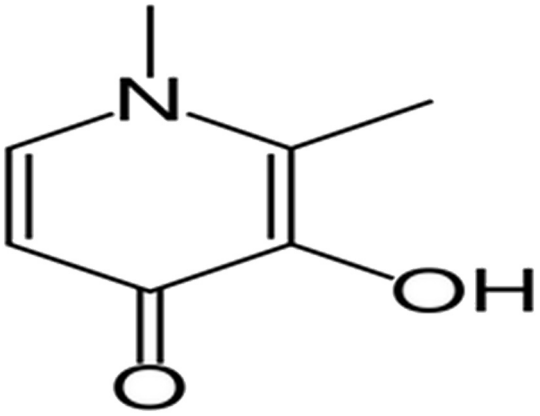

Fig. 1. Deferiprone structure.

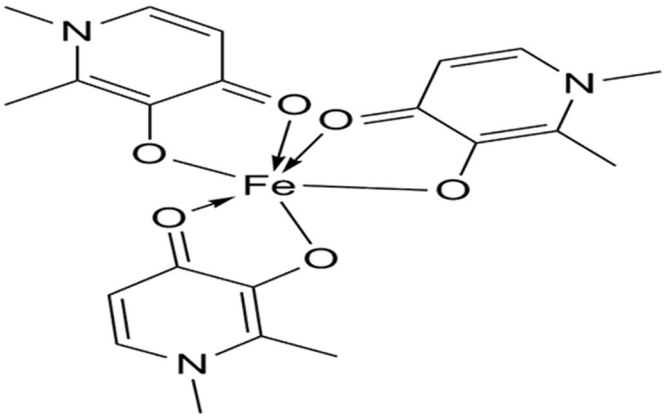

Fig. 2. [Fe-$(CP20)_3$] complex ratio.

Cu^{2+}, Ni^{2+}, Co^{2+}, Mn^{2+}, Mg^{2+}, and Ca^{2+}, which are present in the biological systems, and thus, may affect their functions [8–12]. However, all reported methods were designed to measure the level of metal ions in the experimental animals and the depletion of metal attributed to the chelating effect.

CP20-metal interactions have been studied using classical known techniques such as potentiometry, spectrophotometry and calorimetry for the determination of binding affinity. However, these approaches usually require large sample volume, high sample concentration, complex experimental setup and intensive data analysis and are time consuming [13–17]. Virtually, difficult to use as a fast technique in the screening of metal-chelate binding affinity. MST principle depends on migration in a temperature-gradient which can be used effectively to study binding affinity for several target molecules such as proteins, peptides, small molecules, metal ions [18]. To the author's knowledge, MST has never been used as a screening method for metal-chelate interaction. MST using thermophoresis phenomena to describe the movement of molecules through a temperature gradient is recently used to study the binding of various molecules irrespective of the type or size of the target. Although this phenomenon was described by Carl Ludwig in 1856 [19], MST novelty is implicit in the microscopic scale of temperature gradient by increasing the temperature in a laser spot within a 25-μm heated extension zone. It allows the measurement of any change in a molecular interaction, such as hydration shell, size, and charge. In recent times, MST is one of the most powerful techniques for binding studies. It offers advantages in terms of simplicity, speed of analysis, low sample volume, no buffer or matrix restrictions, and no immobilization procedures required [20].

Although, the optical, electrical, and thermal detecting techniques are accurate and favorable to be used in binding studies, they still provide indirect information about stoichiometry. Mass spectrometry (MS) is a powerful technique to provide direct information about binding stoichiometry and can be used as a complementary technique [21, 22].

This study aimed to screen the binding of CP20 with some selected essential metal ions, such as Fe^{3+}, Cu^{2+}, Zn^{2+}, Co^{2+}, Ni^{2+}, Mn^{2+}, Mg^{2+}, and Ca^{2+}, using MST. Moreover, electrospray ionization mass

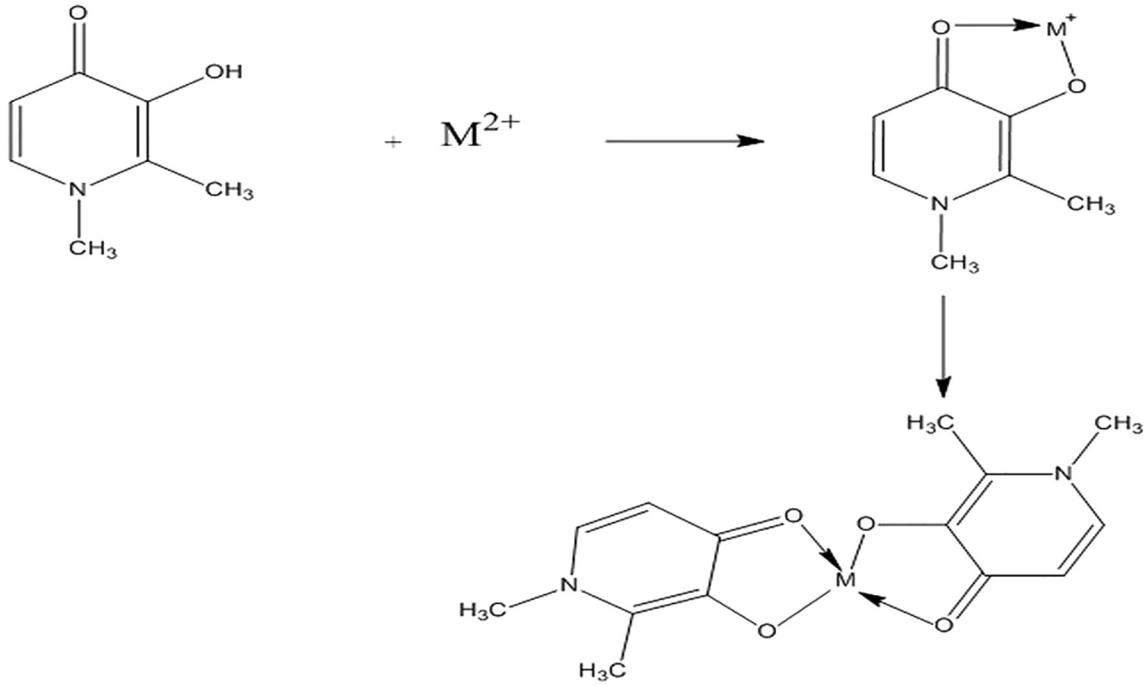

Fig. 3. The chemical reaction scheme for CP20 with divalent essential metal ions.