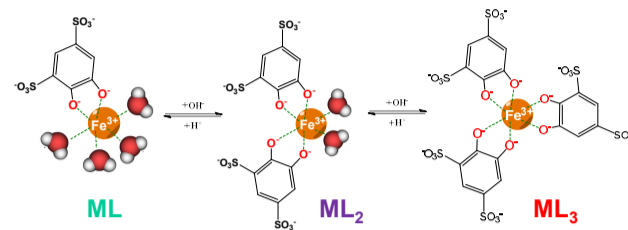
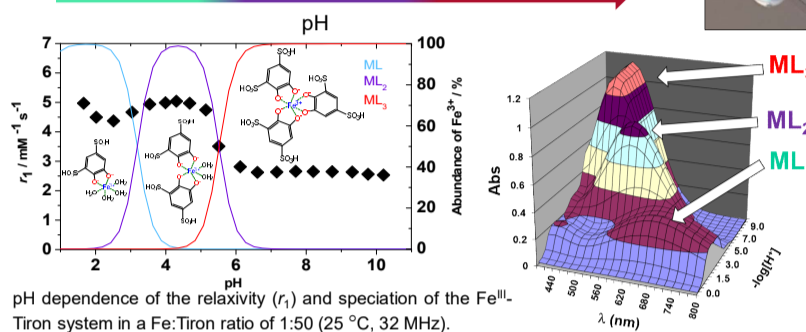
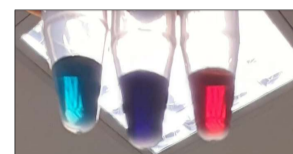


Introduction: Relaxometry is a very powerful technique for investigating the structural and dynamic properties of paramagnetic complexes. Due to their peculiar magnetic characteristics and their great success as diagnostic probes for applications in MRI, many studies have been carried out on Gd(III)- and Mn(II)-based systems. Surprisingly, very few investigations have been reported on Fe(III) complexes despite their considerable importance in various fields of chemistry, starting from bioinorganic chemistry. Fe(III), with five unpaired electrons in the *d* orbitals, a ⁶S configuration and a high magnetic moment, is very well suited to be studied with this technique. In particular, in this work we focused on well-defined type of catecholates complexes of iron(III), Fe-Tiron (Tiron® = disodium 4,5-dihydroxy-1,3-benzenedisulfonate). The formation constants allow for an independent study for each of these complexes by an appropriate choice of starting pH and Tiron concentration [1]. We report for the first time the complete characterization of the various species through a combined ¹H and ¹⁷O NMR relaxometric study. In particular we investigated [Fe(Tiron)(H₂O)₄]⁺ (pH=2.3), [Fe(Tiron)₂(H₂O)₂]⁵⁻ (pH=4) and [Fe(Tiron)₃]⁹⁻ (pH=8). Through a simultaneous fit of ¹H Nuclear Magnetic Resonance Dispersion profiles (from 0.01 up to 500 MHz), ¹⁷O transverse relaxation rates (*R*₂) and shifts ($\Delta\omega$) (measured at 11.7 T as a function of temperature) we were able to obtain accurate value of the structural and dynamic parameters that adequately describe the behaviour of these paramagnetic complexes in aqueous solution [2].



We studied this system as a function of pH by potentiometry, relaxometry and through UV-Vis spectrophotometry.

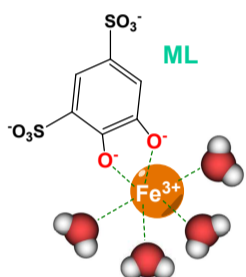


logK _{FeL}	20.32 (1)
logK _{FeL2}	14.49 (2)
logK _{FeL3}	9.83 (2)

Table 1. Stability constants of Fe³⁺-Tiron system (25 °C, I = 0.15 M in NaNO₃). The data are in a good agreement with the literature [3].

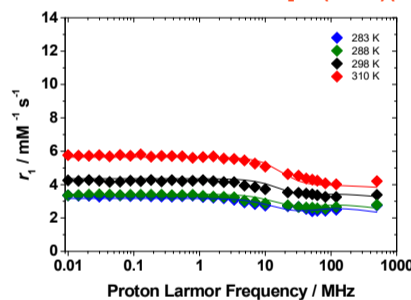
pH dependence of the relaxivity (*r*₁) and speciation of the Fe^{III}-Tiron system in a Fe:Tiron ratio of 1:50 (25 °C, 32 MHz).

[Fe(tiron)(H₂O)₄]⁺

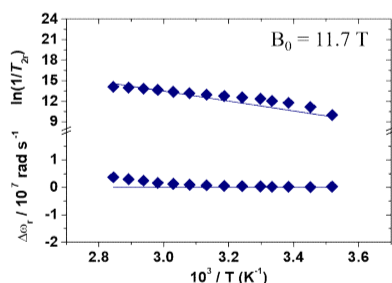


At pH lower than 2.5, the complex is mono-coordinated. In this phase, the complex is characterized by a turquoise-green color and has four water molecules (*q*) in the inner coordination sphere of the metal ion.

¹H NMRD Profiles and ¹⁷O NMR data of [Fe(tiron)(H₂O)₄]⁺



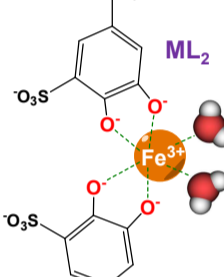
Despite being a tetraaquo complex, the relaxivity values are quite low (*r*_{1p} = 3.4 at 60 MHz and 298 K). This finding, in addition to the fact that relaxivity increases with temperature, suggests that the complex is characterized by a **slow (water) exchange regime**.



Simultaneous fit of the ¹H NMRD profiles and ¹⁷O NMR data allowed us to determine a mean residence life time of the bound water molecules (τ_M) of 18 μ s, which represents the main limiting factor to relaxivity.

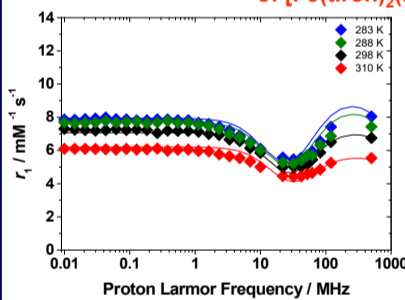
* Parameters	[Fe(tiron)(H ₂ O) ₄] ⁺		
²⁹⁸ <i>r</i> _{1p} / mM ⁻¹ s ⁻¹	3.4 (at 60 MHz), 3.3 (at 120 MHz)		
²⁹⁸ Δ^2 / 10 ²⁰ s ⁻²	5.5 ± 0.1	<i>E</i> _A / kJ mol ⁻¹	3.3 ± 1.7
²⁹⁸ τ_V / ps	9.2 ± 0.5	<i>E</i> _V / kJ mol ⁻¹	1.0 ^a
²⁹⁸ τ_M / s	(1.8 ± 0.7) · 10 ⁻⁵	ΔH_M / kJ mol ⁻¹	57.0 ± 2.5
²⁹⁸ τ_R / ps	34.7 ± 2.5	<i>E</i> _R / kJ mol ⁻¹	15.0 ± 5.0
²⁹⁸ $\tau_{R^{SS}}$ / ps	/	<i>E</i> _{R^{SS}} / kJ mol ⁻¹	/
<i>q</i>	4 ^a	<i>r</i> / Å	2.69 ^a
<i>q^{SS}</i>	0 ^a	<i>r^{SS}</i> / Å	/

[Fe(tiron)₂(H₂O)₂]⁵⁻

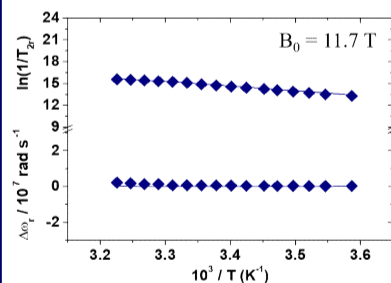


In a narrow pH window between 3 and 5 it is possible to detect the bi-coordinated complex having a *q* = 2, characterized by a strong purple color. Although it is a bi-substituted complex, the catecholic functionalities guarantee a very high stability (log β = 33).

¹H NMRD Profiles and ¹⁷O NMR data of [Fe(tiron)₂(H₂O)₂]⁵⁻

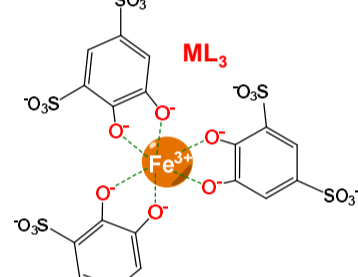


In the case of the bis-aquo complex [Fe(tiron)₂(H₂O)₂]⁵⁻, the *r*_{1p} values are compatible with those of a *q*=2 complex. The four sulphonic groups promotes the formation of a well-defined network of second-sphere solvent molecules, which further increases total relaxivity. From the fit we determined the presence of **two water molecules in the second shell** at a distance *r*^{SS} = 3.4 Å. The ¹⁷O NMR data suggest a faster water exchange regime than the tetraaquo complex with a τ_M of 290 ns.



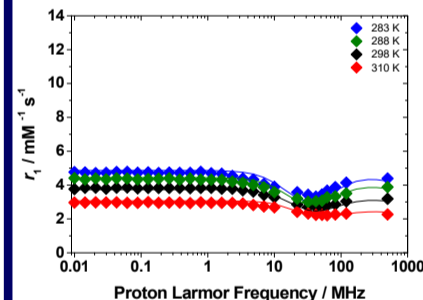
* Parameters	[Fe(tiron) ₂ (H ₂ O) ₂] ⁵⁻		
²⁹⁸ <i>r</i> _{1p} / mM ⁻¹ s ⁻¹	5.4 (at 60 MHz), 6.5 (at 120 MHz)		
²⁹⁸ Δ^2 / 10 ²⁰ s ⁻²	10.8 ± 0.1	<i>E</i> _A / kJ mol ⁻¹	3.0 ± 1.3
²⁹⁸ τ_V / ps	5.7 ± 0.2	<i>E</i> _V / kJ mol ⁻¹	1.0 ^a
²⁹⁸ τ_M / s	(2.9 ± 0.6) · 10 ⁻⁷	ΔH_M / kJ mol ⁻¹	55.0 ± 11.8
²⁹⁸ τ_R / ps	75.0 ± 5.7	<i>E</i> _R / kJ mol ⁻¹	17.0 ± 4.7
²⁹⁸ $\tau_{R^{SS}}$ / ps	20.0 ± 8.4	<i>E</i> _{R^{SS}} / kJ mol ⁻¹	12.0 ± 1.2
<i>q</i>	2 ^a	<i>r</i> / Å	2.70 ^a
<i>q^{SS}</i>	2 ^a	<i>r^{SS}</i> / Å	3.4 ^a

[Fe(tiron)₃]⁹⁻

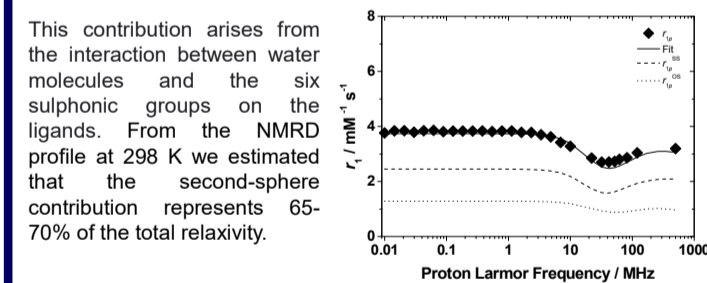


Finally, at pH higher than 7, it is possible to find only the tri-coordinated complex, which is of an intense red color. The stability of this complex is extremely high, with a log β value of 44.

¹H NMRD Profiles of [Fe(tiron)₃]⁹⁻



Even if the complex does not have inner-sphere water molecules, the relaxivity values are extremely high and quite similar to those of the tetraaquo complex. These values are associated with the presence of a strong second-sphere contribution.



* Parameters	[Fe(tiron) ₃] ⁹⁻		
²⁹⁸ <i>r</i> _{1p} / mM ⁻¹ s ⁻¹	2.8 (at 60 MHz), 3.2 (at 120 MHz)		
²⁹⁸ Δ^2 / 10 ²⁰ s ⁻²	17.2 ± 0.1	<i>E</i> _A / kJ mol ⁻¹	5.2 ± 0.2
²⁹⁸ τ_V / ps	4.9 ± 0.2	<i>E</i> _V / kJ mol ⁻¹	1.0 ^a
²⁹⁸ τ_M / s	/	ΔH_M / kJ mol ⁻¹	/
²⁹⁸ τ_R / ps	/	<i>E</i> _R / kJ mol ⁻¹	/
²⁹⁸ $\tau_{R^{SS}}$ / ps	35.3 ± 0.1	<i>E</i> _{R^{SS}} / kJ mol ⁻¹	15.0 ± 1.0
<i>q</i>	0 ^a	<i>r</i> / Å	/
<i>q^{SS}</i>	6 ^a	<i>r^{SS}</i> / Å	3.4 ^a

Tables 2-4 * Parameters obtained from the simultaneous fit of ¹H NMRD and ¹⁷O NMR data (^a = parameters fixed). Additional parameters fixed: ²⁹⁸*D* = 2.24 · 10⁵ cm² s⁻¹; *E*_D = 20 kJ mol⁻¹ and *a* = 3.5 Å).

Conclusions: All the different species of the Fe(III)-Tiron system are amenable to characterization by ¹H and ¹⁷O relaxometric techniques. The global analysis of the data explains the differences in the values of *R*₁ and *R*₂ among the various species based on differences in the state of hydration, in the exchange rate of the water molecules of the inner coordination sphere and in the molecular tumbling. A significant effect of the water molecules hydrogen-bonded to the sulfonate groups of the ligand is highlighted in the case of the complexes with *q* = 2 and *q* = 0.

References:

- [1] W. McBryde, *Can. J. Chem.*, 1964, **42**, 1917
- [2] Aime S., Botta M., Esteban-Gómez D., Platas-Iglesias C., *Mol. Phys.*, 2019, **117**, 898.
- [3] E. Farkas, H. Csóka, *J. Inorg. Biochem.* 2002, **89**, 219–226.