A Spectroscopic and Kinetic Investigation on the Substitution of Fe(III) for Ni(II) in a Siderophore Model

Wagner J. Barreto[a], Waléria P. Silva[a], Ieda S. Scarmínio[b], Sônia R. Giancoli Barreto[a], and Luís F. Stucchi Silva[a]

[a] Laboratory of Environmental Physical Chemistry, CCE, Department of Chemistry, Londrina State University, CP 6001, 86051990, Londrina, Paraná, Brasil
[b] Laboratory of Chemometry in Natural Sciences, Departamento de Química, CCE, Universidade Estadual de Londrina, Londrina, 86051-990, Brasil

Reprint requests to Dr. Wagner J. Barreto. Fax: 55-43-33714286. E-mail: barreto@londrina.net

Dedicated to Professor Oswaldo Sala on the occasion of his 80th birthday

A kinetic and spectroscopic study was performed on the substitution of Fe(III) by Ni(II) in the water soluble anion $[Fe(L^{1-})_2 L^{2-}]^-$, a siderophore model (L^- = dopa-semiquinone and L^{2-} = dopa-catecholate). The reaction was followed in the UV/vis range through the appearing of an intense band at 592 nm due to the anion complex $[Ni(L^{1-})_3]^-$ formed which also presents a resonance Raman effect. The overall reaction obeyed a zero-order rate law at 25, 35, 45, and 50 °C, and the rate constants and thermodynamic parameters have been obtained. A chemometric study based on the Imbrie Q-type factor analysis revealed that the reaction occurred with only one kind of intermediate whose UV/vis spectrum has been calculated.

Key words: Siderophore, Raman Resonance, Dopamine, Catecholamine, Iron

Introduction

Siderophores are compounds that typically contain catecholate or hydroxamate groups to coordinate Fe(III) ions octahedrally [1]. Siderophores are produced by aerobic microorganisms and occasionally by anaerobic species and have the function of capturing and transporting Fe(III) as iron complexes with high stability constants [2]. For the tricatecholate siderophores, the thermodynamic stability constants of iron(III) complexes reach up to $K_f > 10^{40}$ [1]. Among the known microorganisms that synthesize siderophores are several enteric bacteria, including those found in humans and animals, fungi (mushrooms) and plant pathogenic bacteria, soil microorganisms, Gram-positive and -negative bacteria, green and blue algae (cyanobacteria) and higher algae, nitrogen-fixing bacteria, and certain plant (phytosiderophores) and yeast species. The selectivity presented by the Fe(III) ion to chelation is due to several factors, including hardness, ion size, *d* electron configuration, high charge density, and relatively high electronegativity. The Fe(III) ion with the d^5 electronic configuration in the high spin state has a ligand field stabiliza-

tion energy (*D*q) of zero characterizing it as a kinetically labile compound despite the high trivalent ion charge density. Lability might increase if the ligand forms a distorted complex, as in the case of chelates with Fe(III). Therefore, the Fe(III) complexes with hexadentate ligands are, at the same time, kinetically labile and thermodynamically stable. This is an ideal situation for the siderophore to work as a Fe(III) chelating agent which can permeate the cellular membrane and transfer iron into the cell [3, 4].

Several studies have been reported on the synthesis, structural characterization, and functional aspects of metal complexes with catecholate ligands [1, 7 – 10]. Barreto *et al.* reported on the preparation and characterization of the mixed ligand complex $[Fe(L^{1-})_2(L^{2-})]^-$ with the dopa-catecholate (L^{2-}) and dopa-semiquinone (L^{1-}) derived from dopamine [11]. The dopamine can form, through oxi-reduction processes, three kinds of ligands: dopa-quinone (L), dopa-semiquinone (L^-) and dopa-catecholate (L^{2-}) (Fig. 1). Iron(III) can form complexes with any of the above ligand species and usually generates stable compounds.

Although siderophores have a great affinity to Fe(III), many other relatively stable complexes can
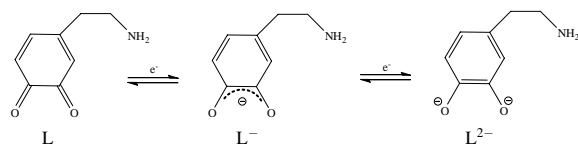
Fig. 1. Possible oxidation forms for dopamine; L = dopa-quinone, L^{1-} = dopa-semiquinone and L^{2-} = dopa-catecholate.

also be formed with Cu(II), Al(III), Cr(III), Ga(III), Mo(III), Ni(II), and Co(II). Emery *et al.* [5] and Khodr *et al.* [6] studied the solubilization of Fe(III) hydroxide by aminochelin, a catecholamine siderophore, at pH = 7 and 25 °C in the absence and presence of molybdate. The addition of molybdate resulted in a significant delay in iron solubilization due to formation of the orange-colored molybdenum complex.

The metal Ni(II) ion is particularly worrisome, because it can be released into the environment by several means, mainly by industries producing or using nickel, its alloys, or salts. In environmental terms, Ni(II) can interfere in the Fe(III) transport by siderophores, thus disturbing or harming plant nutrition [2].

We observed that the complex $[Fe(L^{1-})_2(L^{2-})]^-$ has essentially the same characteristics as a siderophore being very stable in aqueous solution and at neutral pH (thermodynamically stable), and allowing the iron to be replaced by other transition metals present in the aqueous solution (kinetically labile). From an environmental point of view, it is very important to study how a siderophore in natural conditions can be quenched when exposed to an environment contaminated with an excess of other transition metals such as nickel.

The objectives of this work were:
a) to investigate the replacement of Fe(III) by Ni(II) in $[Fe(L^{1-})_2(L^{2-})]^-$ (a siderophore model) in aqueous solution, by spectrophotometry and to determine the kinetic parameters of the reaction; b) to characterize the products formed; and c) to conduct a chemometric study of the reaction.

Results and Discussion

UV/vis Spectra and kinetic results

The anionic complex $[Fe(L^{1-})_2(L^{2-})]^-$ in aqueous solution presented two characteristic bands, a shoulder at 340 and an intense band at 309 nm, assigned to intraligand $\pi \rightarrow \pi^*$ transitions in the catecholate and semiquinone radical, respectively, and a weak band at 598 nm [11]. The spectra obtained dur-
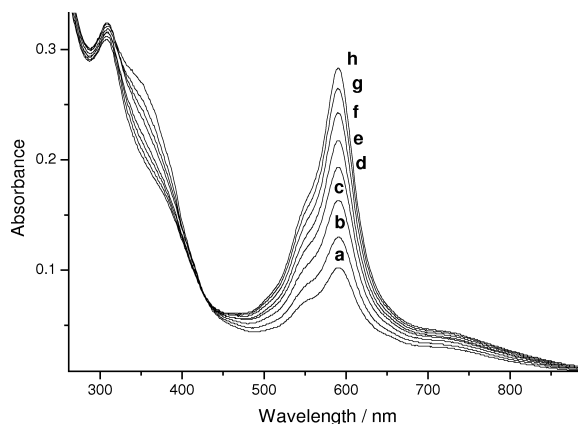

Fig. 2. UV/vis Spectra obtained during the substitution reaction of Fe(III) for Ni(II) in the model siderophore $[Fe(L^{1-})_2(L^{2-})]^-$ at 35 °C for reaction time/min: 60 (a), 90 (b), 120 (c), 150 (d), 180 (e), 210 (f), 240 (g), and 270 (h). L^{1-} = dopa-semiquinone and L^{2-} = dopa-catecholate.

ing the reaction with nickel(II) in aqueous solution (Fig. 2) showed an intense band at 592 nm assigned to an intraligand charge transfer due the anion complex $[Ni(II)(L^{1-})_3]^-$ with $\varepsilon = 4.4 \times 10^3$ L cm^{-1} mol^{-1}. The band at 308 nm, present in all UV/vis spectra, was assigned to an intraligand $\pi \rightarrow \pi^*$ transition expected for semiquinones [12]. As for other temperatures, the spectra showed an isosbestic point around 425 nm; this indicates that the replacement of Fe(III) by Ni(II) occurred quantitatively and through a single reaction. For all reaction mixtures the UV/vis spectra had the same shape profile, independent of the sample temperature. The spectrum of the nickel complex prepared with NiCl$_2$, dopamine, and thiosulfate (Fig. 3a), had bands at 297 (shoulder), 551 (shoulder), and 593 nm (intense), proving that the final compound of the substitution reaction was the same nickel complex (Fig. 3b).

The order rate law for the reaction was determined at each temperature (25, 35, 45, 50 °C) by measuring the band intensity at 592 nm. Regardless of all attempts to achieve a *pseudo* first-order rate law by using a high excess of Ni(II) in solution, the reaction surprisingly followed a zero-order rate law for all temperatures (Fig. 4). As an example we present the concentration of the final product $[Ni(II)(L^{1-})_3]^-$ *versus* the reaction time at 25 °C with a linear fit of $y = 0.0469 + 4.97 \times 10^{-4} x$ and $r = 0.9994$. We also found that upon decreasing the Ni(II) concentration to the ratios 1:200, 1:100 and 1:50, a kinetic zero-order rate law was also obeyed. When equimolar concen-
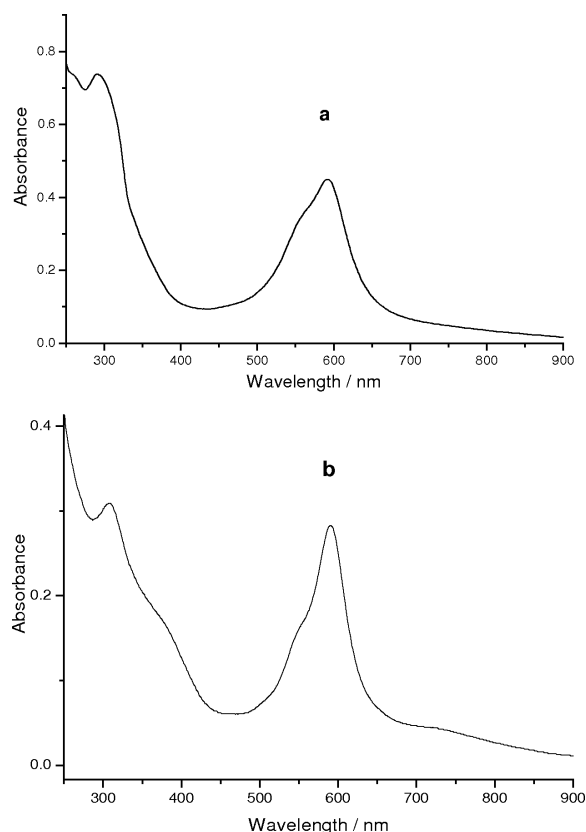
Fig. 3. UV/vis Spectrum of the nickel complex $[Ni(L^{1-})_3]^-$ in aqueous solution: (a) after the exchange reaction of Fe(III) by Ni(II) in the siderophore model; (b) prepared with NiCl$_2$, dopamine, and thiosulfate. L^{1-} = dopa-semiquinone.

trations for the reagents was reached, no reaction was observed even after 30 d. The rate constants obtained using an integrated zero-order rate law for a concentration ratio of 1 : 300 were: k_{25} = 1.82 ± 0.06 × 10^{-9} mol L^{-1} s^{-1}, k_{35} = 3.64 ± 0.03 × 10^{-9} mol L^{-1} s^{-1}, k_{45} = 4.45 ± 0.05 × 10^{-9} mol L^{-1} s^{-1}, and k_{50} = 5.10 ± 0.08 × 10^{-9} mol L^{-1} s^{-1}, where k indicates the rate constant and the subscript, the temperature. The thermodynamic parameters for the reaction were obtained by using the Eyring equation: $\Delta H^{\#}$ = 29 ± 3 kJ mol^{-1}, $\Delta S^{\#}$ = −313 ± 86 J K^{-1} mol^{-1}, and $\Delta G^{\#}$ = 123 ± 28 kJ mol^{-1}. The observed $\Delta H^{\#}$ means that the reaction was endothermic and the low and negative value for $\Delta S^{\#}$ indicates that the intermediate (an activated complex) suffers a small reduction of the molecular volume in relation to the initial reagent, the Fe(III) complex. Therefore, the nickel compound maintains a nearly distorted octahedral structure with hexa-coordination through the phenolate oxygen atoms.
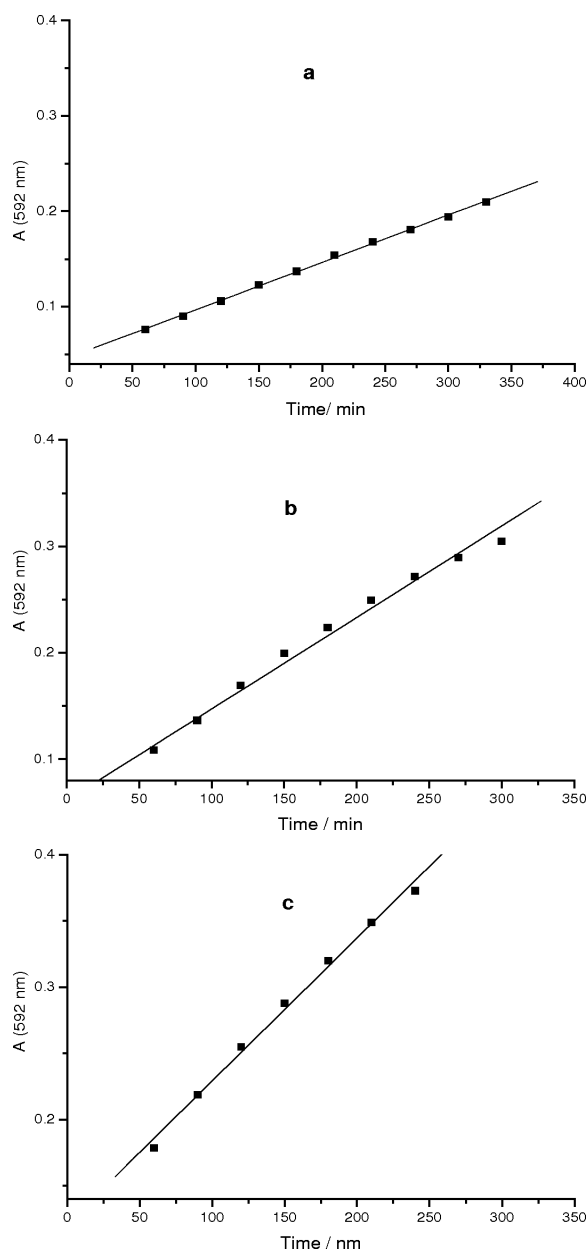

Fig. 4. Absorbance (A) at 592 nm of the $[Ni(L^{1-})_3]^-$ complex as a function of time (min) for the temperatures: (a) 25 °C, (b) 35 °C and (c) 45 °C. L^{1-} = dopa-semiquinone.

Chemometric analysis

A chemometric study based on the analysis of Imbrie Q-type factors followed by Imbrie's varimax and oblique rotations was applied to the spectral data at all four temperatures [13]. The results provide the absorption spectra for each species during the reaction, solv-
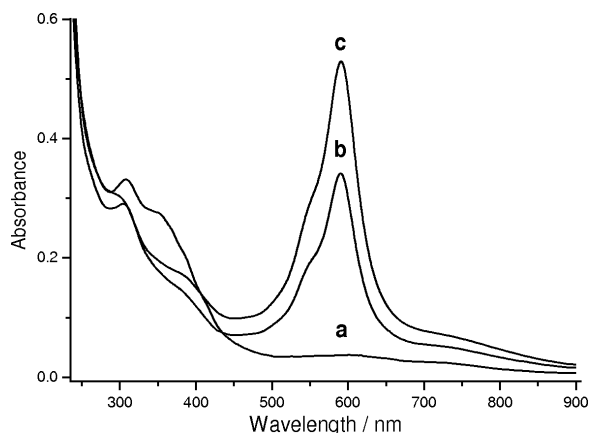
Fig. 5. UV/vis Spectra calculated showing the three chemical species: (a) the reagent; (b) the intermediate; (c) the product, appearing during the reaction at 50 °C.
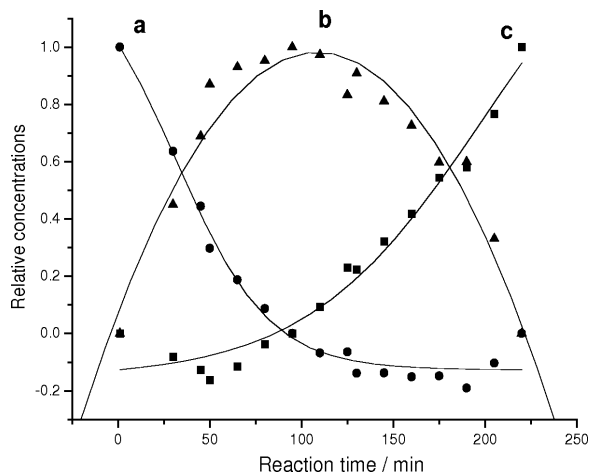

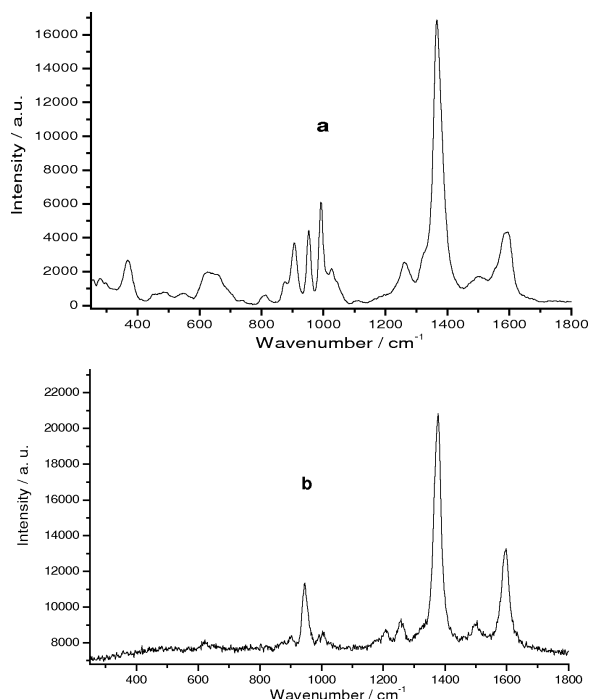Fig. 6. Relative concentrations as a function of the reaction time for (a) the reagent, (b) the intermediate, and (c) the product at 50 °C.

Fig. 7. Resonance Raman spectrum of: (a) $[Ni(L^{1-})_3]^-$ in aqueous solution, the product of the substitution reaction of Fe(III) by Ni(II) in the siderophore model (excitation at λ = 632.8 nm); (b) $[Ni(L^{1-})_3]^-$ prepared with $NiCl_2$, dopamine, and thiosulfate in aqueous solution (excitation at λ = 514.5 nm). L^{1-} = dopa-semiquinone.

ing band superposition. Only three components, the reagent, the intermediate and the product, were determined at all temperatures (Fig. 5). The calculated spectra had bands for the reagent at 307 nm and 353 nm (weak shoulder), for the intermediate at 305, 384 nm (weak shoulder), 545 nm (medium shoulder), and 590 nm (strong) and for the final product at 300 nm, 384 nm (weak shoulder), 545 nm (medium shoulder), and 590 nm (strong). By comparing the spectra, we observed that the band at *ca.* 340 nm, calculated at 353 nm in the reacting mixture and assigned to the $[Fe(L^{1-})_2(L^{2-})]^-$ anion, was absent in the intermediate and in $[Ni(II)(L^{1-})_3]^-$ while the band at 309 remained. The band at *ca.* 340 nm could then be assigned

to an intraligand transition in the catecholates present in the Fe(III) complex, and the band at 309 nm to an intraligand transition in the semiquinone. The spectral similarity between the intermediate and the products, and the presence of an isosbestic point indicated a simple and rapid replacement of Fe(III) by Ni(II). The oxidation of the L^{2-} ligand in $[Fe(L^{1-})_2(L^{2-})]^-$ to L^{1-} in $[Ni(II)(L^{1-})_3]^-$ can involve a step in which Fe(III) is reduced to Fe(II), with high lability and low stability, thus being easily replaced by Ni(II). From the deconvoluted spectra, the relative concentrations for each species could be calculated and plotted as a function of the reaction time. Fig. 6 shows the relative concentrations obtained every 30 min for the reagent, the intermediate and the products at 50 °C. The profiles obtained were as expected for three species in solution.

Resonance Raman study

A Raman spectrum of the solution was obtained to characterize the final reaction products (Fig. 7a). The Raman spectrum was obtained using an excitation ra-

Table 1. A tentative assignment for the frequencies of [CTA][Fe(L^{1-})$_2$(L^{2-})] in the solid state, [Ni(L^{1-})$_3$]$^-$ in completed exchange reaction solution and [Ni(L^{1-})$_3$]$^-$ prepared in solution, compared to calculated frequencies using the PM3 semi-empirical method.[a]

Assignment	[CTA][Fe(L^{1-})$_2$(L^{2-})]$_{(solid)}$	[Ni(L^{1-})$_3$]$^-$ [b]	[Ni(L^{1-})$_3$]$^-$ [c]	Calculated[d]
ν(ring)	1576 s	1590 s	1599s	1594
ν(C$_1$C$_2$), δ(CH)	1360 vs	1368 vs	1375vs	1400
δ(CH), ν(CO)	–	1261w	1257w	1270
δ(CH)	984 w	992s	1006w	1007
δ(CH)	942 m	952m	945s	1004
δ(CH)	901 w	905m	901vw	923
ν(M-ring)	612 m	640 m	623w	719
ν(M-ring)	560 m	540 vw	–	636

[a] CTA = cetyltrimethylammonium cation; Raman wavenumbers in cm^{-1}; s = strong, vs = very strong, w = weak, vw = very weak, m = medium, M-ring = MetalO$_1$O$_2$C$_1$C$_2$, ν = stretching, δ = deformation; [b] Raman wavenumber after exchange reaction; [c] Raman wavenumber for the prepared complex; [d] wavenumbers calculated using the PM3 method [14].

diation of 632.8 nm inside the intense absorption band with the peak at 592 nm. The bands presented a strong intensification of the signal, a phenomenon called Resonance Raman Spectroscopy. The solution with the Fe(III) complex was very diluted and no intense band was seen in the visible spectral range and therefore no detectable Raman signal was observed. The resonance Raman spectrum of the nickel complex prepared with NiCl$_2$, dopamine, and thiosulfate (Fig. 7b) had the same bands as the nickel compound obtained by replacing the iron in the siderophore model. The relative intensities of the bands were different in the two spectra, because each spectrum was obtained using a different incident laser frequency ($\lambda = 632$ nm for the solution after iron replacement, and $\lambda = 514.5$ nm for the prepared nickel complex). The different laser frequencies caused different enhancements of the vibrational modes. Laser excitation at $\lambda = 632$ nm was closer to the maximum of the visible band and provoked greater enhancement than at $\lambda = 514.5$ nm. The bands observed for the nickel complex in aqueous solution and for the iron compound in the solid state are shown in Table 1. The tentative assignment is supported by a semi-empirical calculation using the PM3 method [14]. The intense band at 1368 cm^{-1} was assigned to ν(C$_1$–C$_2$) and δ(C–H), mixing of ligand CC stretching and CH deformation, characteristic of the semiquinone bonded to the metal [15–17]. The absence of an intense band around 1440 cm^{-1} assigned to the stretching mode ν(C=N$^+$), means that there was no cyclization of the ethylamine ramification generating the indol molecule [15, 18].

Conclusion

The use of the anionic complex [Fe(L^{1-})$_2$(L^{2-})]$^-$ as a siderophore model for the uptake of Ni(II) gave kinetic information on the exchange reaction and the nature of the reaction product. The slow exchange reaction surprisingly showed a zero-order equation behavior. The thermodynamic parameters and the chemometric spectral data analyzed by chemometric methods indicated a mechanism involving only one intermediate compound, structurally similar to the reagent molecule. During the exchange reaction, one catecholate ligand in the iron complex suffers oxidation, a process involving reduction of Fe(III) to Fe(II). The Fe(II) complex is likely to be the intermediate which, being thermodynamically unstable and labile, facilitates the exchange of Fe(II) by Ni(II). The nickel compound obtained from the exchange reaction was identified in solution as the anionic complex [Ni(L^{1-})$_3$]$^-$. Our findings show that the siderophore which can be synthesized in water can be used as a model for testing the behavior of natural siderophores in usual conditions, aqueous medium and neutral pH, when the environment is affected by anthropogenic contaminations, like a nickel contamination from industrial sources.

Experimental Section

The Fe(III) complex was prepared with dopamine hydrochloride (C$_8$H$_9$NO$_2$ · HCl, Aldrich Chem. Co., 98%, 0.100 g) and Na$_2$S$_2$O$_3$ (Aldrich Chem. Co. 99%, 0.300 g) added to ultra-pure water (300.0 mL). After complete dissolution of the salts, Fe$_2$O$_3$ (Aldrich Chem. Co., 0.020 g) was added and the mixture was left agitating at 25 °C [11]. The mixture was analyzed by UV/vis spectroscopy in the range 200 to 900 nm (Spectrophotometer Milton-Roy Genesys 2). After the reaction was completed, the mixture was centrifuged (4300 rpm / 60 min) to eliminate the Fe$_2$O$_3$ remaining in suspension. The solution was stored in polyethylene bottles and maintained at 4 °C. UV/vis Spectra were obtained taking 200 μL of the Fe(III) complex diluted to 3.0 mL with

water in a 1 cm quartz cuvette. The final concentration of the Fe(III) complex in solution was $5.8 \ 10^{-4}$ mol L^{-1}. The kinetic experiments were conducted by mixing adequate volumes of the Fe(III) complex and $NiCl_2$ (Aldrich Chem. Co. $\geq 98\%$, 1.76×10^{-1} mol L^{-1}) solutions. The Ni(II) concentration was calculated to be approximately 300 times higher than that of the Fe(III) complex, to obtain a *pseudo* first-order kinetic reaction. The ion strength of the solution was kept constant, $I = 0.27$, during the replacement reactions. Two independent reactions (true duplicate) for each temperature (25, 35, 45, and 50 °C) were performed, yielding a good reproducibility of the results. Samples were maintained in an ultra-thermostatic bath (Microquímica, MQAMA 301) and the 200 μL samples were removed at different reaction times and diluted in 3 mL of water, to obtain the UV/vis spectra scanned between 200 and 900 nm. The nickel complex was prepared by mixing sodium thiosulfate ($Na_2S_2O_3$, Aldrich Chem. Co. 99%, 0.300 g), dopamine (0.100 g), and $NiCl_2$ (0.03 g) in ultra-pure water (500 mL) at 25 ± 2 °C. The UV/vis spectra at several reaction times were recorded until the reaction was finished and the resonance Raman spectrum was obtained. Raman spectra of the solutions were obtained using a Renishaw Raman System 3000 spectrometer, exciting radiation at 632.8 nm (514.5 nm for the prepared nickel complex), with a spectral resolution of 7 cm^{-1}, and equipped with 100 mW laser power focused by using an Olympus microscope. The vibrational frequencies were calculated using the PM3 semi-empirical method, PC Spartan Pro software, Wavefunction, Inc. [14] for a basic iron-complex structure with Fe bonded to oxidized dopamine through the oxygen atoms, $FeOOC_8HNH_9$ as a model. A chemometric method was used to estimate the number of species present as well as to extract the spectra of the species involved and their corresponding concentration profiles. Imbrie Q-mode factor analysis followed by varimax and Imbrie oblique rotations and K matrix treatment were applied to the UV/vis spectral data. The chemometric methods used here permit to deduce the relative concentrations from the observed spectral changes and to resolve the absorption spectra of the species. The methodology used was described in details by Scarminio *et al.* [19 – 21]. The FORTRAN programs used for the Q-mode principal component analysis, varimax and Imbrie's oblique rotations calculations were developed in our laboratory, LCNC-UEL.

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