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The Role of the π Acceptor Character of Polypyridine Ligands on the Electrochemical Response of Co(II) Complexes and its Effect on the Homogenous Electron Transfer Rate Constant with the Enzyme Glucose Oxidase

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Abstract. In this work the electrochemical behavior of Co(II) complexes with substituted bidentate and tridentate polypyridine ligands [CoL₃](BF₄)₂ and [CoL'₂](NO₃)₂ in 0.1M phosphate buffer pH 7.2 was studied. A reversible electrochemical process Co(II)L_n → Co(III)L_n + 1e⁻ was observed. A linear relationship between the redox potential (E°) and the pKa of the non-coordinated ligand was found. It was demonstrated by DFT calculations the use of pKa value as a descriptor of the π acceptor character of a ligand. The electrochemical response in the presence of glucose oxidase (GOx) was also studied. It was possible to establish a tendency between the homogeneous electron transfer rate constant (ks) and the redox potential (E°) for the compounds studied in this work and other examples taken from the literature.

Key words: Co(II) complexes; polypyridine ligands; electrochemistry; redox mediator; glucose oxidase; DFT calculations.

Resumen. En este trabajo se estudió el comportamiento electroquímico de complejos de Co(II) con ligantes polipiridínicos bidentados y tridentados sustituidos [CoL₃](BF₄)₂ y [CoL'₂](NO₃)₂ en soluciones tampón de fosfato pH 7.2 0.1 M. Se observó un proceso electroquímico reversible Co(II)L_n → Co(III)L_n + 1e⁻, encontrándose una relación lineal entre el potencial redox (E°) y el pKa del ligante no coordinado. Mediante cálculos DFT se demostró el uso del valor pKa como descriptor del carácter aceptor π de los ligantes. Se estudió la respuesta electroquímica en presencia de glucosa oxidasa (GOx). Se estableció una tendencia entre la constante de velocidad de transferencia de electrónica homogénea (ks) y el potencial redox (E°) para los compuestos estudiados en este trabajo y otros ejemplos tomados de la literatura.

Palabras clave: Complejos de Co(II); ligantes polipiridínicos; electroquímica; mediadores redox; glucosa oxidasa; cálculos DFT.

Introduction

Octahedral Cobalt complexes with polypyridine ligands have been widely used in several areas of chemistry with technological applications. In the field of dye sensitized solar cells (DSSCs) families of Co(II)/Co(III) complexes with substituted bipyridine, terpyridine and phenanthroline ligands have been used as redox mediator for dye regeneration, due to their advantages in comparison with the typical I₃/I⁻ electrolyte, such as non-corrosiveness, non-volatility, negligible light absorption, simple electron transfer mechanism and tunable redox potential that increase the maximum open circuit potential (Voc) [1-18].

The non-covalent interactions of the tris-(1,10-phenanthroline)-Co(III) complex with DNA, demonstrated through cyclic voltammetry, has motivated different research groups to develop new electrochemical biosensors based on DNA hybridization. Detection of human immunodeficiency virus (HIV), *Escherichia coli*, Hepatitis B virus and the chemotherapeutic agent for lymphoblastic leukemia, 6-Mercaptopurine, are some examples of these biosensors [19-35].

In the area of renewable energies using solar radiation, particularly in water splitting to produce H₂ and O₂, also called artificial photosynthesis, Co(II) complexes with polypyridine ligands have also drawn attention due to the formation of hydride species Co(II)-H and Co(III)-H, required for hydrogen

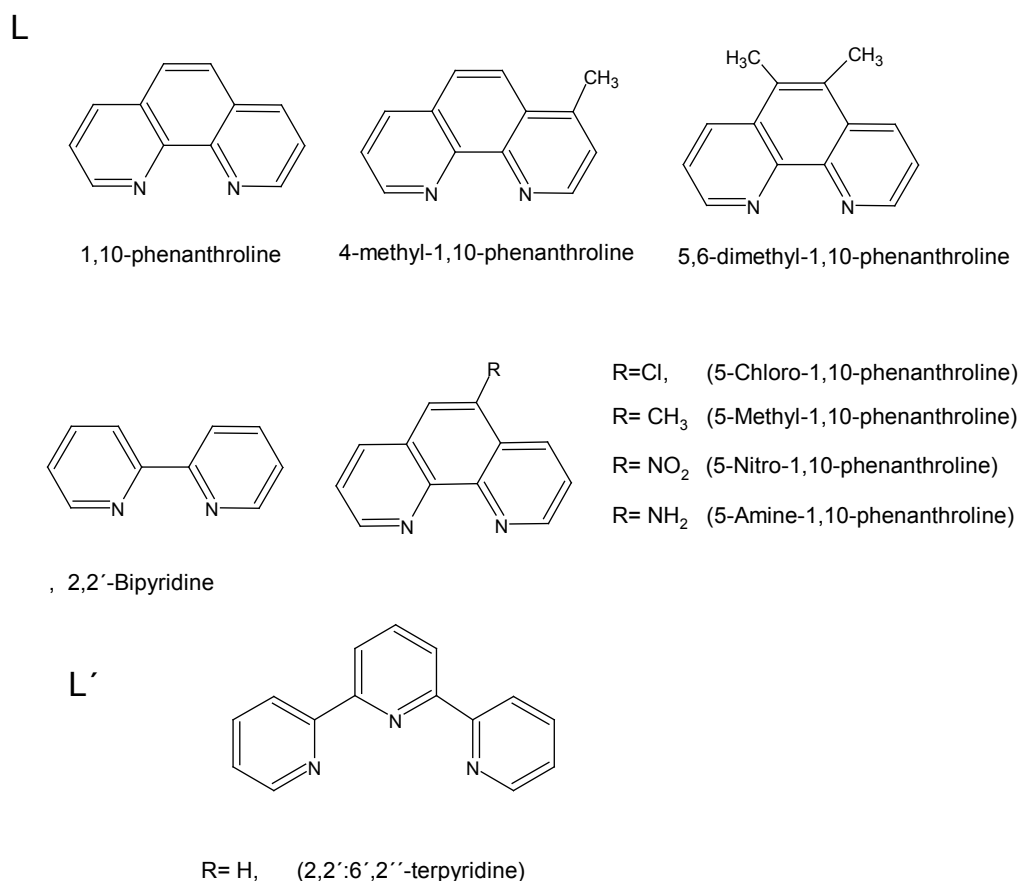
evolution [36-41]. In the realm of molecular electrocatalysis, Co(II)-polypyridine complexes have been proven useful in the electrochemical reduction CO_2 and H_2O as a potential strategy for energy storage [42-48].

In all these applications a great stability of Co(II) and Co(III) oxidation states either in non-aqueous solvents or in acid and basic media is a necessary condition. Despite the aforementioned versatility and chemical properties of Co(II) polypyridine complexes, these systems have not been used as redox mediators for the development of new reliable and, cheap amperometric glucometers for the control of Diabetes Mellitus. These complexes in comparison with typical redox mediators used in commercial devices, present advantages such as simple methods of preparation and high stability [49]. In the amperometric glucose biosensors the sensibility is controlled by the homogenous electron transfer rate constant (k_s) between the oxidized form of the so called "redox mediator" and the reduced form of Glucose oxidase (GOx), where a high rate constant value implies lower amount of enzyme that could lower the cost of the device. Different transition metal complexes, including those with polypyridine ligands have been used as redox mediators [50-61]. It has been suggested that the rate constant (k_s) obeys the Marcus cross relation, where one important factor is the difference between the redox potentials of

the couples $\text{M}^{\text{II}}\text{L}_n / \text{M}^{\text{III}}\text{L}_n$ and FAD/FADH_2 (-0.22 V vs ENH) for the redox mediator and the active site of the GOx enzyme respectively [55-61]. Nevertheless this topic is still a controversy, because the Marcus cross relation was proposed for metal complexes with outer sphere homogenous electron transfer.

On the other hand, for octahedral low spin Ru(II), Fe(II), Os(II), Mn(II), Cr(II), Rh(II), and Re(II) complexes with polypyridine ligands the redox potential is modulated by the π acceptor character of the ligands [62]. However for Co(II) derivatives, this has not been studied, due the lack of an accurate π acceptor descriptor of high spin complexes. Despite that tunable E° values are desired for specific applications, including their use as redox mediators in glucose biosensors.

Therefore in this work we decided to study the redox behavior of a series of polypyridine Co(II) complexes $[\text{CoL}_3](\text{BF}_4)_2$ and $[\text{CoL}'_2](\text{NO}_3)_2$ ($\text{L} =$ substituted 1,10-phenanthroline, 2,2'-bipyridine, $\text{L}' = 2,2':6',2''$ -terpyridine), see scheme 1, in order to establish the role of the π acceptor character of the ligand, using an accurate descriptor, on the modulation of the redox potential (E°) and its effect on the homogenous electron transfer rate constant (k_s) between the oxidized form of a redox mediator and the reduced form of GOx. The obtained results are intended to contribute to the understanding of molecular aspects on the design of redox mediators for glucose biosensors



Scheme 1. Ligands L or L' used for the $[\text{CoL}_3](\text{BF}_4)_2$ and $[\text{CoL}'_2](\text{NO}_3)_2$ complexes studied in this work.

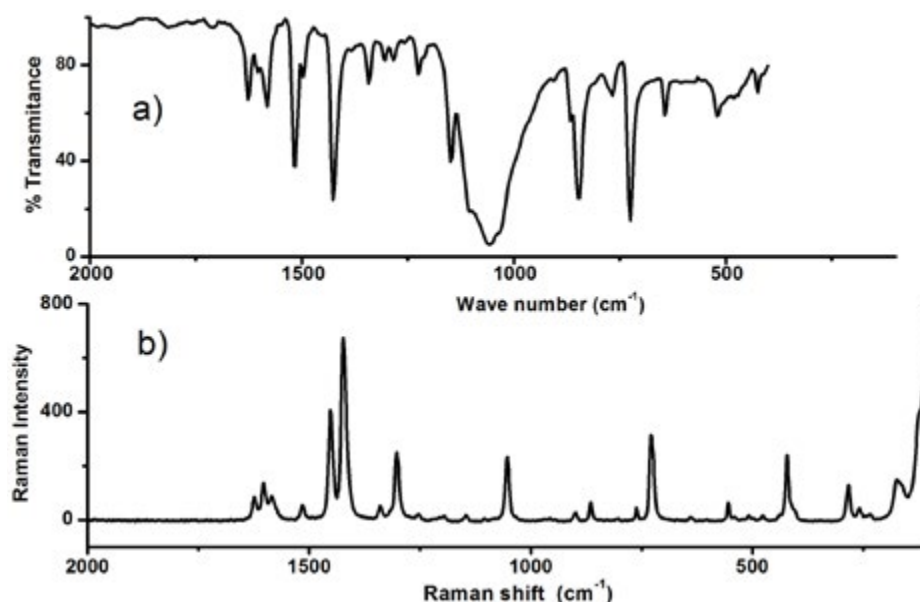


Fig. 1. IR (a) and Raman (b) spectra of the complex $[\text{Co}(\text{1,10-phenanthroline})_3](\text{BF}_4)_2$.

based on the modulation of the redox potential for the electron transfer $\text{Co(II)} \rightarrow \text{Co(II)}^+ + 1e^-$, that can be extrapolated to other transition metal complexes.

Results and discussion

Characterization of complexes

The mixture of the starting salt $[\text{Co}(\text{H}_2\text{O})_6](\text{BF}_4)_2$ or $\text{Co}(\text{NO}_3)_2 \cdot 6\text{H}_2\text{O}$ with the polypyridine ligand L or L' (see scheme 1) in a molar ratio (1:3) yield yellow powders corresponding to the series of complexes $[\text{CoL}_3](\text{BF}_4)_2$ and $[\text{CoL}'_2](\text{NO}_3)_2$. The IR spectrum of the $[\text{Co}(\text{1,10-phenanthroline})_3](\text{BF}_4)_2$ complex shows typical signals of polypyridine ligand, see fig. 1a. A medium absorption bands at 3068 and 3024 cm^{-1} attributable to aromatic stretching ν ($=\text{C-H}$) are observed. The absorptions bands corresponding to the out of plane C-H bending are registered at 846, 724 cm^{-1} . The stretching bands ν ($\text{C}=\text{C}$) + ν ($\text{C}=\text{N}$) occurs at 1604, 1627, 1582 cm^{-1} . A broad signal around 1060 cm^{-1} characteristic for BF_4^- anion was also observed. Similar signals are observed in Raman fig. 1b. Additionally vibrational frequencies related to the stretching ν (M-N) were recorded at 274 and 226 cm^{-1} , which are in the expected range reported (180-290 cm^{-1}) for Co(II) complexes [63].

To propose the geometry around the Co(II) center, NIR diffuse reflectance spectra was obtained for this complex, see fig. 2. Three typical electronic transitions for octahedral Co (II) complexes $\nu_1 = {}^4\text{T}_{1g}(\text{F}) \rightarrow {}^4\text{T}_{2g}(\text{F})$; $\nu_2 = {}^4\text{T}_{1g}(\text{F}) \rightarrow {}^4\text{A}_{2g}(\text{F})$; $\nu_3 = {}^4\text{T}_{1g}(\text{F}) \rightarrow {}^4\text{T}_{1g}(\text{P})$ at 11260, 21770 and 26800 cm^{-1} respectively were detected [64]. Considering this spectrum and the effective magnetic moment $\mu_{\text{eff}} = 4.03 \text{ BM}$ (3 unpaired electron), it can be stated unequivocally the formula

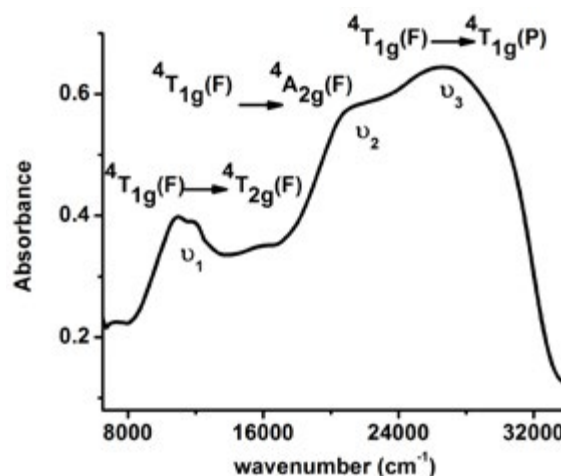


Fig. 2. Typical NIR diffuse reflectance spectra of complex $[\text{Co}(\text{1,10-phenanthroline})_3](\text{BF}_4)_2$.

$[\text{Co}(\text{1,10-phenanthroline})_3](\text{BF}_4)_2$. Spectroscopic and magnetic characterization for the other complexes allow us to propose a similar octahedral Co(II) complexes.

Electrochemical behavior of Co(II) complexes with polypyridine ligands in 0.1 M phosphate buffer pH 7.2

In order to explore the electrochemical behavior of the series of Co(II) complexes in physiological conditions, cyclic voltammetry in 0.1 M phosphate buffer pH 7.2 was carried out. Fig. 3a shows a typical voltammogram for $[\text{Co}(\text{1,10-phenanthroline})_3](\text{BF}_4)_2$ obtained with a glassy carbon electrode at 10 mVs^{-1} . One oxidation process (I_a) was detected with a potential peak (E_{pa}) at 0.178 V vs Ag/AgCl. Its corresponding reduction process (I_c)

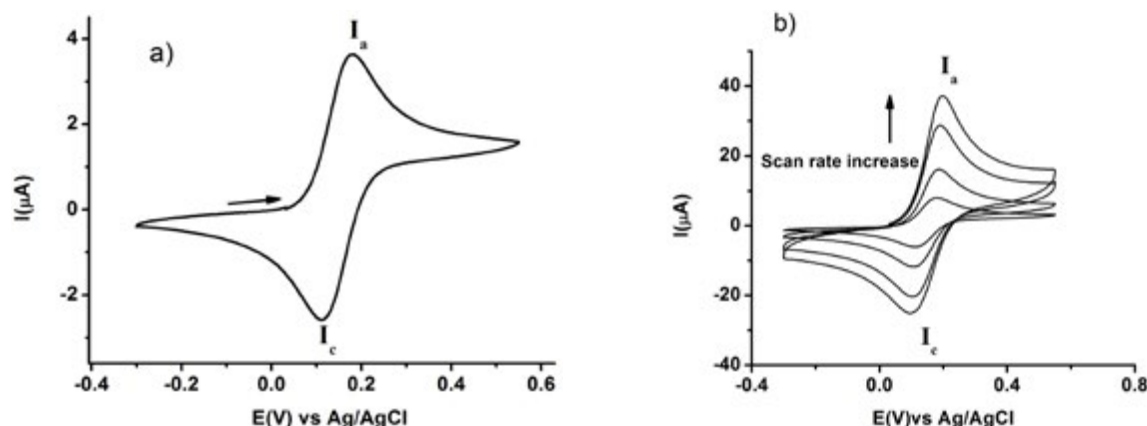


Fig. 3. Cyclic voltammograms of 1.0 mM $[\text{Co}(1,10\text{-phenanthroline})_3](\text{BF}_4)_2$ in 0.1M phosphate buffer pH 7.2, at a) scan rate of 10 mVs^{-1} and b) variable scan rate ($10\text{-}1000 \text{ mVs}^{-1}$). The working electrode was glassy carbon and the reference electrode was Ag/AgCl.

presented a cathodic peak (E_{pc}) at 0.112 V vs Ag/AgCl. A potential peak difference ΔE_p of 0.066 V was calculated. When the scan rate was increased, the anodic peak current (I_a) showed a proportional relation with the $v^{1/2}$. The behavior mentioned before gives evidence to propose a reversible one electron transfer $[\text{Co(II)}\text{L}_3]^{2+} \rightarrow [\text{Co(III)}\text{L}_3]^{3+} + 1e^-$, with a redox potential value $E^\circ = 0.145 \text{ V}$ vs Ag/AgCl [65,66].

One step chronoamperometry experiment stepping the potential from OCP to a value where the process is limited by diffusion was carried out. A typical $i(t) \text{ vs } t^{-1/2}$ plot with a linear relationship $i(\mu\text{A}) = 7.64(\mu\text{A s}^{1/2}) t^{-1/2} - 0.152(\mu\text{A})$, $r = 0.999$, was obtained. From the slope of this equation and according to the Cottrell law, a diffusion coefficient value (D_O) of $3.955 \times 10^{-6} \text{ cm}^2 \text{ s}^{-1}$ was calculated [66].

The electrochemical behavior of the other complexes was explored in the same way than that described for the compound $[\text{Co}(1,10\text{-phenanthroline})_3](\text{BF}_4)_2$. Similar redox responses were observed with differences in redox potential values being attributed to the electronic properties of the ligands, a summary is presented in table 1. The complex with the highest redox potential corresponds to that where the ligand with the electron-withdrawing substituent (5-nitro-1,10-phenanthroline) is present. On the other hand complexes containing ligands with electron-donating substituents, such as the 5,6-dimethyl-1,10-phenanthroline, decrease the redox potential. In order to understand the electronic properties that have influence over the E° value, we decided to explore a simple and reliable linear relationship.

Linear relationship between the redox potential and the pKa of non-coordinated ligands

It is reported in the literature, that the pKa of a non-coordinated polypyridine ligand is related with its π acceptor character in metal complexes [67-69]. With this criteria linear relationships between E° and pKa have been described for Cu(II), Fe(II) and Ru(II) complexes, but not for Co(II) compounds. In this work we found this type of relationship for the reversible electron

transfer $[\text{Co(II)}\text{L}_3]^{2+} \rightarrow [\text{Co(III)}\text{L}_3]^{3+} + 1e^-$, see table 1, with the equation $E^\circ = -0.1603 \text{ pKa} + 0.9264$ ($r=0.999$), see fig. 4. Complexes containing ligands with high π acceptor character (low pKa) present high redox potentials. The metal complex with the ligand 2,2'-bipyridine in this relationship gives a low correlation coefficient. This can be a consequence of different electronic and resonant effects due to the presence of only two aromatic rings or low spin/ high spin equilibrium already reported for Cobalt-2,2'-bipyridine complexes [8,10]. Hence we obtained a simple model that predicts the redox behavior of Co(II) complexes, just by knowing the pKa of the free ligand.

Theoretical studies

In order to verify the use of pKa of a non-coordinated ligand as a descriptor of the π acceptor character, theoretical calculations using DFT were carried out. Based on reliable results reported in literature we decided to use the reactivity and response indexes; chemical hardness (η), chemical potential governing charge donating process (μ^-), chemical potential governing charge accepting process (μ^+), electro-donating power (ω^-)

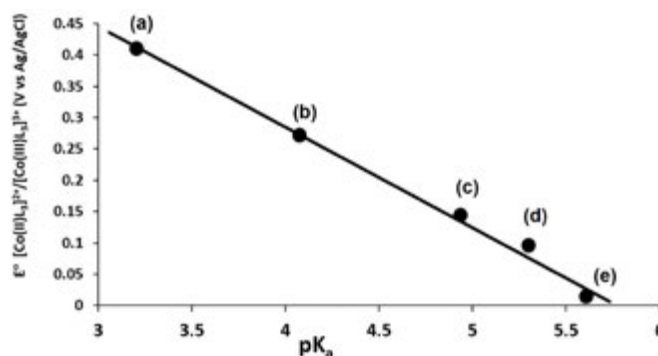


Fig. 4. Plot $E^\circ [\text{Co(II)}\text{L}_3]^{2+}/[\text{Co(III)}\text{L}_3]^{3+}$ vs pKa of non-coordinated ligand. The redox potential was calculated in 0.1 M phosphate buffer pH 7.2. L= (a) 5-NO₂-1,10-phen, (b) 5-Cl-1,10-phen, (c) 1,10-phen, (d) 5-CH₃-1,10-phen and, (e) 5,6-di-CH₃-1,10-phen.

Table 1. Redox potential, diffusion coefficient and pKa (no coordinated ligands) for Co(II) complexes with polypyridine ligands obtained in this work.

Compound	ΔE_p	^a E° (V)	D_0 (cm ² s ⁻¹)	^b pKa
[Co(1,10-phenanthroline) ₃](BF ₄) ₂	0.066	0.145	3.955×10^{-6}	4.93
[Co(5-methyl-1,10-phenanthroline) ₃](BF ₄) ₂	0.070	0.094	8.257×10^{-6}	5.3
[Co(4-methyl-1,10-phenanthroline) ₃](BF ₄) ₂	0.067	0.041	3.694×10^{-6}	n.r.
[Co(5,6-dimethyl-1,10-phenanthroline) ₃](BF ₄) ₂	0.076	0.011	6.864×10^{-6}	5.6
[Co(5-chloro-1,10-phenanthroline) ₃](BF ₄) ₂	0.072	0.274	5.586×10^{-6}	4.07
[Co(5-nitro-1,10-phenanthroline) ₃](BF ₄) ₂	0.065	0.411	5.419×10^{-6}	3.2
[Co(5-amino-1,10-phenanthroline) ₃](BF ₄) ₂	0.074	0.026	8.596×10^{-6}	5.78
[Co(2,2'-bipyridine) ₃](BF ₄) ₂	0.060	0.086	4.427×10^{-6}	5.28
[Co(2,2':6',2''-terpyridine) ₂](NO ₃) ₂	0.060	0.043	6.930×10^{-6}	n.r.

^a Recorded in 0.1M phosphate buffer pH 7.2 vs Ag/AgCl, scan rate 100 mVs⁻¹. ^b = Value for non-coordinated ligand. n.r. = not reported value

and electro-accepting power (ω^+), which depends on the vertical ionization energy (I) and vertical electron affinity (A), see equations 1-5 [70]

$$\eta = \frac{1}{2}(I - A) \quad (1)$$

$$\mu^- = \frac{-1}{4}(3I + A) \quad (2)$$

$$\mu^+ = \frac{-1}{4}(I + 3A) \quad (3)$$

$$\omega^- \equiv \frac{(\mu^-)^2}{2\eta} \approx \frac{(3I + A)^2}{16(I - A)} \quad (4)$$

$$\omega^+ \equiv \frac{(\mu^+)^2}{2\eta} \approx \frac{(I + 3A)^2}{16(I - A)} \quad (5)$$

It can be observed that for a low pKa the chemical potential governing charge accepting process (μ^+) and electro-accepting

power (ω^+) presented high values, which confirms the ability of ligands with electron withdrawing substituents to participate in a π back bonding (high capacity to accept charge, due to a high π acceptor character). For the chemical potential governing donation process (μ^-) and electro-donating power (ω^-), these values are increased when the pKa of the ligand is also increased. This value is related to the σ character of the ligand, a necessary condition to participate in the π back bonding [63]. Results are summarized in Table 2.

On the other hand, ligands containing electron donating groups such as methyl or amino group decrease the redox potential of the complex, due to low ionization energy (I). The opposite is observed for ligands with electron withdrawing groups (-Cl and -NO₂) where high redox potential values are present (it is more difficult to remove an electron from Co(II) center) as a consequence of the higher ionization energy (I). Fig. 5a, shows a typical HOMO plot of 1,10-phenanthroline ligand, which is very similar for all the calculated derivatives. It can be observed the localization of the molecular orbital over donor atoms. The ligands 2,2'-bipyridine and 2,2':6',2''-terpyridine do not present the same tendency, because they have a

Table 2. Theoretical parameters obtained: ionization energy (I), electron affinity (A), chemical hardness(η), chemical potential governing donation (μ^-) and accepting (μ^+) process, electrodonating (ω^-) and electroaccepting (ω^+) powers. All the results are in eV.

Ligand	^a E°	I	A	η	μ^-	μ^+	ω^-	ω^+	pKa
5,6-dimethyl-1,10-phen	0.011	7.91	-0.18	3.86	-5.98	-2.12	4.63	0.58	5.6
5-amino-1,10-phen	0.026	7.52	0.18	3.67	-5.69	-2.01	4.40	0.55	5.78
2,2':6',2''-terpyridine	0.043	8.16	-0.03	4.07	-6.13	-2.06	4.62	0.52	n.r.
4-methyl-1,10-phen	0.067	8.12	-0.26	3.93	-6.16	-2.23	4.82	0.63	n.r.
2-2'-bipyridine	0.086	8.87	-0.13	4.37	-6.68	-2.31	5.11	0.61	5.28
5-methyl-1,10-phen	0.094	8.07	-0.21	3.93	-6.10	-2.18	4.74	0.60	5.3
1,10-phen	0.145	8.26	-0.23	4.02	-6.25	-2.24	4.87	0.62	4.93
5-chloro-1,10-phen	0.274	8.30	-0.53	3.88	-6.35	-2.47	5.20	0.79	4.07
5-nitro-1,10-phen	0.411	8.84	-1.49	3.86	-7.00	-3.33	6.67	1.51	3.2

^a Reported vs Ag/AgCl, scan rate 100 mVs⁻¹ in 0.1 M phosphate buffer pH 7.2

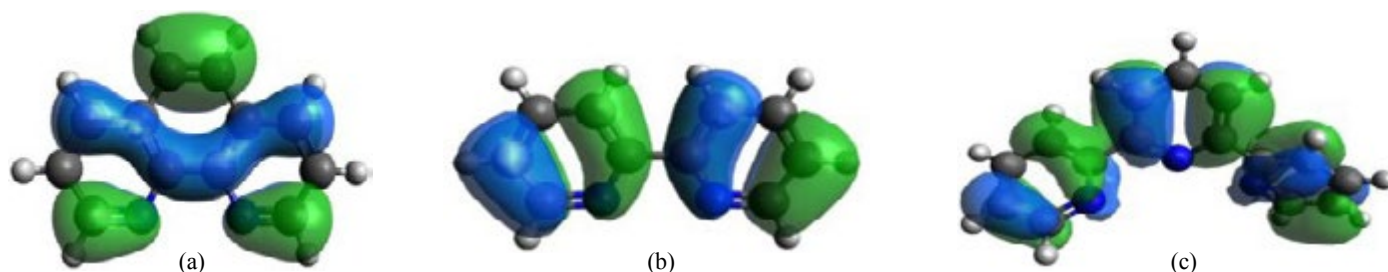


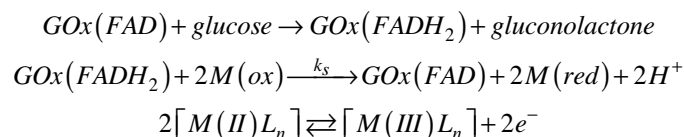
Fig. 5. Highest occupied molecular orbital, HOMO, corresponding to (a) 1,10-phenanthroline, (b) 2,2'-bipyridine and (c) 2,2':6',2''-terpyridine.

different electronic delocalization in comparison with 1,10-phenanthroline derivatives, see figures 5b and 5c [69].

It was demonstrated that is possible to tune the redox potential with a reliable π acceptor description (pKa of non coordinated ligand). To continue with the aim of this work electrochemical experiments of Co(II) complexes in the presence of GOx are presented in the next section.

Electrochemical behavior of Co(II) complexes with polypyridine ligands in the presence of Glucose Oxidase

Second generation amperometric glucose biosensors, require the presence of a redox mediator, because the active site of Glucose oxidase (FAD) is embedded in the protein domain, and no direct electron transfer between the enzyme and the electrode is recorded [58, 71,72]. The reactions that describe the operation of this type of glucose biosensors are listed below [73]:



The sensibility of these type of biosensors is evaluated with the homogenous electron transfer rate constant (k_s) between the reduced active site of the Glucose oxidase (FADH_2) and the oxidized mediator $[\text{M}(\text{III})\text{L}_n]$. It has been suggested in literature that this rate constant obeys the Marcus cross relation, see equation 6 and 7, where k_{11} and k_{22} values correspond to the self-exchange homogenous electron transfer rate constants for the species involved in the reaction. Whereas the K_{12} value is the equilibrium constant for the reaction, calculated from the difference of redox potentials between the couples $[\text{M}(\text{II})\text{L}_n] / [\text{M}(\text{III})\text{L}_n]$ and FAD/FADH_2 (-0.41 V vs Ag/AgCl). The f factor is a parameter that relates k_{11} and k_{22} with the collision rate for the reactants, and has a value close to the unity. It should be highlighted that the Marcus cross relation was proposed for outer sphere homogenous electron transfer rate constant for metal complexes. Therefore we decided to study the electrochemical response of three representative cobalt complexes $[\text{Co}(2,2'\text{-bipyridine})_3](\text{BF}_4)_2$, $[\text{Co}(1,10\text{-phenanthroline})_3](\text{BF}_4)_2$ and $[\text{Co}(5\text{-chloro-1,10-phenanthroline})_3](\text{BF}_4)_2$ with different π acceptor ligands in the presence of GOx to evaluate their

corresponding k_s values and to explore its relationship with the redox potential. Data taken from literature for Os (II) and Ru (II) complexes with the same ligands were also considered in this analysis [50].

$$\log(k_s) = 2 \log f_{12} + 2 \log k_{11}k_{22} + 2 \log K_{12} \quad (6)$$

$$\log K_{12} = \frac{(E_2^0 - E_1^0)n_2n_1}{0.059} \quad (7)$$

Fig. 6 shows a typical voltammogram of a 1mM solution of $[\text{Co}(5\text{-Chloro-1,10-phenanthroline})_3](\text{BF}_4)_2$ in 0.1 M phosphate buffer pH 7.2 with 0.05 M of Glucose in the absence and in the presence of GOx 3.4 μM at scan rate of 1 mVs^{-1} . When no enzyme was added to the solution a typical Current (I) vs Potential (E) profile for a diffusion control process was recorded, with a maximum current value (I_d), fig. 6a. On the other hand in the presence of GOx the voltammogram presented an S shape catalytic response, with the anodic and cathodic traces superimposed and with a plateau current (I_k) characteristic of ECi' mechanism [74]. To confirm the mechanism and to

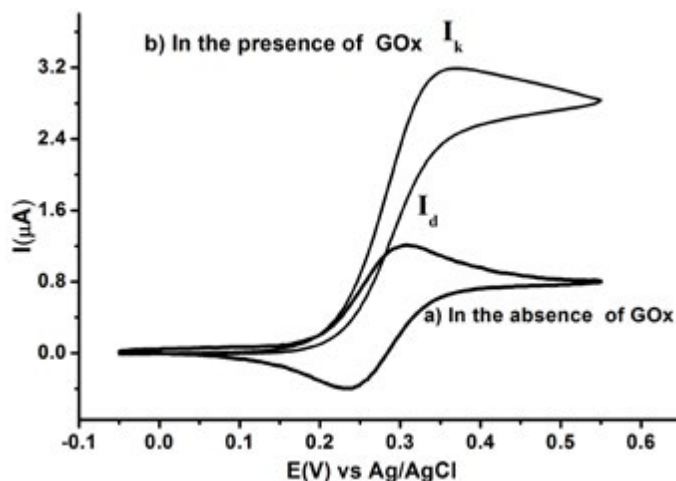


Fig. 6. Cyclic voltammograms of 1.0 mM $[\text{Co}(5\text{-Chloro-1,10-phenanthroline})_3](\text{BF}_4)_2$ in 0.1M phosphate buffer pH 7.2 + 0.05 M Glucose a) in the absence and b) in the presence of 3.04 μM GOx, scan rate 1 mVs^{-1} . The working electrode was glassy carbon and the reference electrode was Ag/AgCl.

calculate ks experiments with variable scan rate from 1 mV s^{-1} to 100 mV s^{-1} were carried out, see fig. 7. The increase in the scan rate makes that the voltammogram pass from an S shape catalytic response (pure kinetic condition) to a partially reversible wave (no substrate consumption) and finally to a reversible wave (no catalysis) [74,75]. According to the method reported by Nicholson and Shain, using a working curve Ψ than depends on the ratio of the catalytic and diffusion currents (I_k/I_d) at different scan rates in the presence and absence of GOx respectively. A pseudo-first order catalytic rate constant (k_f') was calculated from the slope of Ψ vs v^{-1} plot, see equation 8 [74].

$$\Psi = [k_f' / (nFv / RT)]^{1/2} \quad (8)$$

$$k_s = k_f' / [\text{GOx}] \quad (9)$$

A ks value of $1.75 \times 10^3 \text{ M}^{-1} \text{ s}^{-1}$ was calculated for the homogenous electron transfer between the reduced GOx and $[\text{Co}^{\text{III}}(5\text{-Cl-1,10-phenanthroline})_3]^{3+}$, using equation 9. The Enzyme concentration was calculated from value for Ferrocene carboxylic acid, obtained in the same experimental conditions described above and with its corresponding k_s value taken from literature [73]. The same experiments were performed for $[\text{Co}(2,2'\text{-bipyridine})_3](\text{BF}_4)_2$ and $[\text{Co}(1,10\text{-phenanthroline})_3](\text{BF}_4)_2$, see figures 8 and 9. A summary of this data with the equilibrium constant K_{I2} corresponding to the difference between the redox potential of the mediator and the active site of the enzyme are shown in table 3. Similar analysis with Os (II) and Ru (II) complexes are also present in this table.

A detailed inspection of table 3, shows that Co(II) complexes, with low redox potential (E°) present low homogenous electron transfer rate constants (ks) and low equilibrium constants K_{I2} . High values for both parameters are observed in

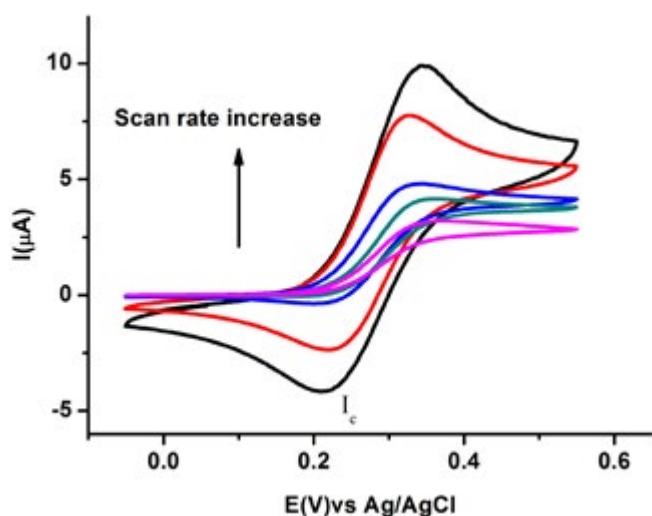


Fig. 7. Cyclic voltammograms of $1.0 \text{ mM } [\text{Co}(5\text{-Chloro-1,10-phenanthroline})_3](\text{BF}_4)_2$ in 0.1 M phosphate buffer $\text{pH } 7.2 + 0.05 \text{ M}$ Glucose 0.05 M in the presence of $3.04 \text{ } \mu\text{M}$ GOx, variable scan rate ranging from 1 to 100 mV s^{-1} . The working electrode used was glassy carbon, the reference electrode was Ag/AgCl .

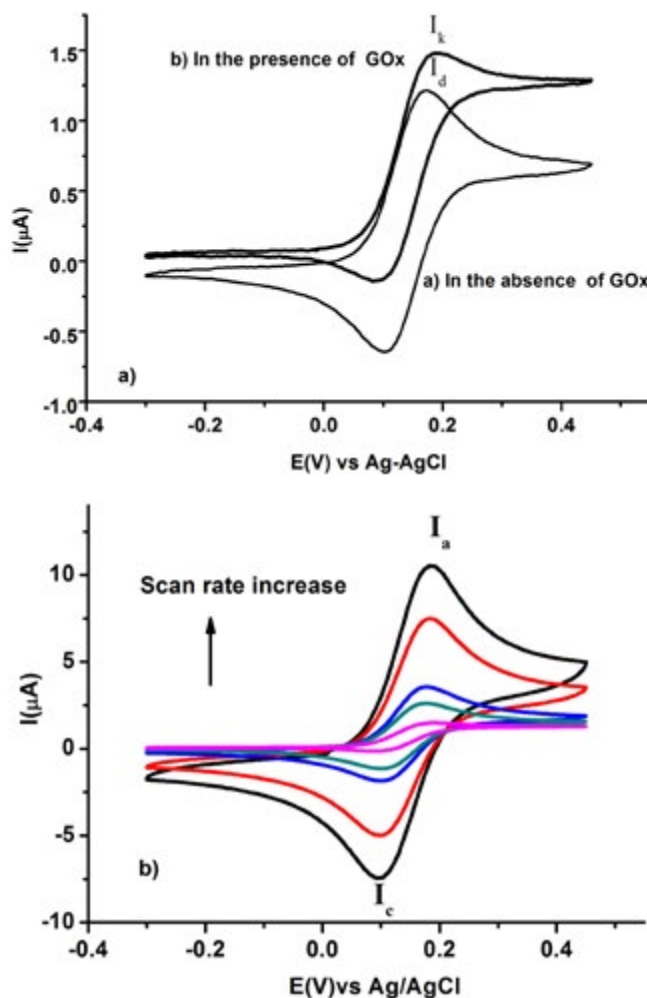


Fig. 8. Cyclic voltammograms of $1.0 \text{ mM } [\text{Co}(1,10\text{-phenanthroline})_3](\text{BF}_4)_2$, in 0.1 M phosphate buffer $\text{pH } 7.2 + 0.05 \text{ M}$ Glucose a) in the absence (blue line) and in the presence (red line) of $3.04 \text{ } \mu\text{M}$ GOx, scan rate 1 mV s^{-1} . b) Experiments obtained in the presence of $3.04 \text{ } \mu\text{M}$ GOx 0.05 M Glucose at variable scan rate. The working electrode used was glassy carbon, the reference electrode was Ag/AgCl .

Ru(II) complexes and intermediate behavior is observed in Os(II) complexes. Despite no linear relationships between these parameters can be calculated a tendency is observed. The remarkable change in the redox potential values among Co(II) , Os(II) and Ru(II) compounds could be explained in terms of simple crystal field theory, keeping in mind the low spin behavior configuration $(t_{2g})^5(t_{2g})^2$ for Co(II) complexes and the configuration $(t_{2g})^6(t_{2g})^0$ for Ru(II) and Os(II) complexes. This consideration implies less energy to oxidize the Co(II) compounds due to lower Crystal Field Stabilization Energy CFSE [63]. Regarding (ks) values, there is not a linear relationship between $\log ks$ and $\log K_{I2}$. In this case a rough tendency can be observed, which indicates that the Marcus cross relation, equation 6, did not describe correctly this homogenous electron transfer. Actually it is observed that when the $\log K_{I2}$ value is increased the parameter $\log ks$ presented an asymptotic value around 6.0 . This fact suggests that there should be additional

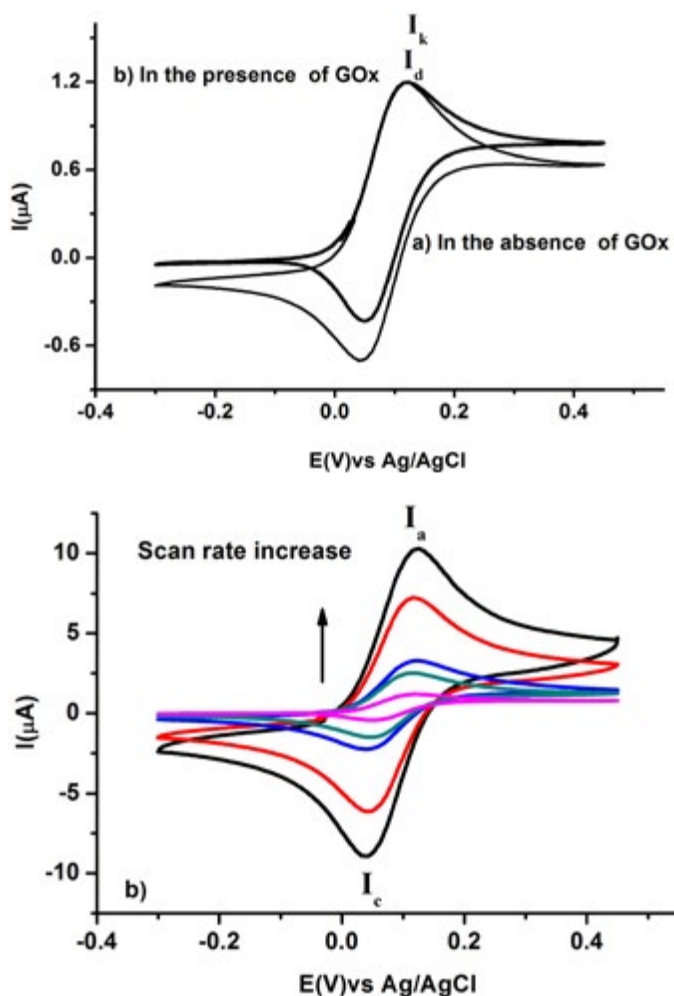


Fig. 9. Cyclic voltammograms of 1.0 mM $[\text{Co}(2,2'\text{-bipyridine})_3](\text{BF}_4)_2$ in 0.1M phosphate buffer pH 7.2 + 0.05 M Glucose a) in the absence (blue line) and in the presence (red line) of 3.04 μM GOx, scan rate 1 mVs^{-1} . b) Experiments obtained in the presence of 3.04 μM GOx 0.05 M Glucose at variable scan rate. The working electrode used was glassy carbon, the reference electrode was Ag/AgCl.

factors that control the whole process, such as electrostatic interactions or long distance electronic communication between the active site of the enzyme and the mediator. It should be highlighted that metal complexes with high redox potential (E°) values present high homogenous electron transfer rate constant (k_s). Co(II) complexes with a strong π acceptor ligand could be used as a good redox mediator for this kind of amperometric biosensor. These compounds have the advantage of being cheaper compared with their corresponding Os(II) and Ru(II) analogous. The results also show that there is no necessity to use a redox mediator with a high redox potential value due to the limit asymptotic k_s value.

Conclusions

A linear relationship between the redox potential E° and the pKa of the non-coordinated ligand for the reversible electron transfer $[\text{Co(II)}\text{L}_3]^{2+} \rightarrow [\text{Co(III)}\text{L}_3]^{3+} + 1\text{e}^-$ with polypyridine ligands was obtained, with the equation $E^\circ = -0.1603 \text{ pKa} + 0.9264$ ($r = 0.999$). It was demonstrated by DFT calculations that the pKa of the non-coordinated ligand is a good descriptor for the π acceptor character of a polypyridine ligand. Co(II) complexes containing ligands with high π acceptor character (low pKa) increase the redox potential E° and the homogenous electron transfer rate constant (k_s), which indicates that these compounds could be useful as good redox mediators for glucose biosensors.

Experimental section

Chemicals

All chemicals and solvents in this work were used as received from Aldrich Chemical Co., Acros Organics and J.T. Baker.

Table 3. Homogenous electron transfer rate constant (k_s) between reduced GOx and the oxidized Co(III) complexes and its corresponding redox potential (E°).

Compound	E° (mV) vs Ag/AgCl	$\log k_s$ ($\text{M}^{-1} \text{s}^{-1}$)	$\log K_{12}$
$[\text{Co}(2,2'\text{-bipyridine})_3](\text{BF}_4)_2$	86	n.m.	8.40
$[\text{Co}(1,10\text{-phenanthroline})_3](\text{BF}_4)_2$	145	3.243	9.40
$[\text{Co}(5\text{-Chloro-1,10-phenanthroline})_3](\text{BF}_4)_2$	274	4.281	11.59
$[\text{Os}(2,2'\text{-bipyridine})_3]^{2+}$	655	6.301	18.13 ^a
$[\text{Os}(1,10\text{-phenanthroline})_3]^{2+}$	675	6.707	18.38 ^a
$[\text{Os}(5\text{-Chloro-1,10-phenanthroline})_3]^{2+}$	770	6.322	20.00 ^a
$[\text{Ru}(2,2'\text{-bipyridine})_3]^{2+}$	1090	6.322	25.33 ^a
$[\text{Ru}(1,10\text{-phenanthroline})_3]^{2+}$	1156	6.243	26.54 ^a

^a From reference ⁵⁰

Synthesis of the complexes [CoL₃](BF₄)₂.

These metal complexes were synthesized adding dropwise 5 ml of 3 equivalents previously dissolved in methanol to 5 ml 0.02 M of Co(BF₄)•6H₂O in methanol solution. The reaction mixture was stirred for 2 hours. After this time the solvent was removed on a rotary evaporator at 60°C until a yellow powder was observed. The product was filtered and washed with ethylic ether.

Synthesis of the complex [CoL₂](NO₃)₂. 1 mmol of Co(NO₃)₂•6H₂O and 3 mmol of the ligand were dissolved separately in methanol. Then the ligand solution was added dropwise to the metallic salt solution. The reaction mixture was stirred for 2 hours. The solvent was removed by slow evaporation until a yellow powder was observed. The product was filtered and washed with ethylic ether.

[Co(1,10-phenanthroline)₃](BF₄)₂. Elemental analysis for CoC₃₆H₂₄N₆B₂F₈, Calc.: %C, 55.9; %H, 3.1; %N, 10.9. Λ_M (H₂O): 268 Ω^{-1} cm² mol⁻¹. μ_{eff} 4.07 BM (3 unpaired electron) Found: %C, 55.4; %H, 2.7; %N, 10.9. Mayor IR bands (cm⁻¹): $\nu(\text{C-H})$ 3068 3024; $\nu(\text{C=C}) + \nu(\text{C=N})$ 1627, 1604, 1582; $\delta(\text{C-H})$ 846, 724 out of plane; ionic BF₄⁻ $\nu(\text{B-F})$ broad center at 1060. Raman band (cm⁻¹): $\nu(M-N)$ 274, 226. NIR diffuse reflectance data; ${}^4T_{1g}(F) \rightarrow {}^4T_{2g}(F) = 11260$, ${}^4T_{1g}(F) \rightarrow {}^4A_{2g}(F) = 21770$ and ${}^4T_{1g}(F) \rightarrow {}^4T_{1g}(P) = 26800$ cm⁻¹

[Co(5-methyl-1,10-phenanthroline)₃](BF₄)₂•3H₂O. Elemental analysis for CoC₃₄H₃₆N₆O₃B₂F₈, Calc.: %C, 55.5; %H, 4.9; %N, 10.4. Λ_M (H₂O): 258 Ω^{-1} cm² mol⁻¹. μ_{eff} 4.06 BM (4 unpaired electron) Found: %C, 50.9; %H, 4.5; %N, 10.6. Mayor IR bands (cm⁻¹): $\nu(\text{C-H})$ 2921, 2867; $\nu(\text{C-H})$ 3054, 3096; $\nu(\text{C=C}) + \nu(\text{C=N})$ 1626, 1602, 1585; $\delta(\text{C-H})$ 897, 876, 804, 813, 734, 724 out of plane; ionic BF₄⁻ $\nu(\text{B-F})$ 1062. Raman band (cm⁻¹): $\nu(M-N)$ 273, 229. NIR diffuse reflectance data; ${}^4T_{1g}(F) \rightarrow {}^4T_{2g}(F) = 11260$, ${}^4T_{1g}(F) \rightarrow {}^4A_{2g}(F) = 22060$ and ${}^4T_{1g}(F) \rightarrow {}^4T_{1g}(P) = 27000$ cm⁻¹

[Co(4-methyl-1,10-phenanthroline)₃](BF₄)₂•6H₂O. Elemental analysis for CoC₃₉H₄₂N₆O₆B₂F₈, Calc.: %C, 50.7; %H, 4.6; %N, 9.1. Λ_M (H₂O): 268.1 Ω^{-1} cm² mol⁻¹. μ_{eff} 4.07 BM (3 unpaired electron) Found: %C, 51.0; %H, 3.8; %N, 9.5. Mayor IR bands (cm⁻¹): $\nu(\text{C-H})$ 2918, 2865; $\nu(\text{C-H})$ 3065; $\nu(\text{C=C}) + \nu(\text{C=N})$ 1623, 1604, 1590, 1575; $\delta(\text{C-H})$ 861, 847, 835, 786, 727 out of plane; ionic BF₄⁻ $\nu(\text{B-F})$ 1055. Raman band (cm⁻¹): $\nu(M-N)$ 268, 225. NIR diffuse reflectance data; ${}^4T_{1g}(F) \rightarrow {}^4T_{2g}(F) = 11540$, ${}^4T_{1g}(F) \rightarrow {}^4A_{2g}(F) = 22900$ and ${}^4T_{1g}(F) \rightarrow {}^4T_{1g}(P) = 28000$ cm⁻¹

[Co(5,6-dimethyl-1,10-1,10-phenanthroline)₃](BF₄)₂•8H₂O. Elemental analysis for CoC₄₂H₅₂N₆O₈B₂F₈, Calc.: %C, 50.4; %H, 5.2; %N, 8.4. Λ_M (H₂O): 168.1 Ω^{-1} cm² mol⁻¹. μ_{eff} 4.17 BM (3 unpaired electron) Found: %C, 50.7; %H, 4.2; %N, 8.8. Mayor IR bands (cm⁻¹): $\nu(\text{C-H})$ 2985, 2931, 2874; $\nu(\text{C-H})$ 3093; $\nu(\text{C=C}) + \nu(\text{C=N})$ 1605, 1585; $\delta(\text{C-H})$ 883, 825, 814, 735, 693 out of plane; ionic BF₄⁻ $\nu(\text{B-F})$ 1057. Raman band (cm⁻¹): $\nu(M-N)$ 281, 236. NIR diffuse reflectance data; ${}^4T_{1g}(F) \rightarrow {}^4T_{2g}(F) = 11310$, ${}^4T_{1g}(F) \rightarrow {}^4A_{2g}(F) = 22950$ and ${}^4T_{1g}(F) \rightarrow {}^4T_{1g}(P) = 26800$ cm⁻¹

[Co(5-chloro-1,10-phenanthroline)₃](BF₄)₂•4H₂O Elemental analysis for CoC₃₆H₂₉N₆O₄B₂F₈ Cl₃, Calc.: %C, 45.6; %H, 3.1; %N, 8.9. Λ_M (H₂O): 168.1 Ω^{-1} cm² mol⁻¹. μ_{eff} 4.27 BM (3 unpaired electron) Found: %C, 44.9; %H, 2.7; %N, 9.1. Mayor IR bands (cm⁻¹): $\nu(\text{C-H})$ 3043; $\nu(\text{C=C}) + \nu(\text{C=N})$ 1614, 1604, 1581; $\delta(\text{C-H})$ 891, 876, 825, 809, 781, 733, out of plane; ionic BF₄⁻ $\nu(\text{B-F})$ 1060. Raman band (cm⁻¹): $\nu(M-N)$ 266, 232. NIR diffuse reflectance data; ${}^4T_{1g}(F) \rightarrow {}^4T_{2g}(F) = 10290$, ${}^4T_{1g}(F) \rightarrow {}^4A_{2g}(F) = 22540$ and ${}^4T_{1g}(F) \rightarrow {}^4T_{1g}(P) = 27600$ cm⁻¹

[Co(5-nitro-1,10-phenanthroline)₃](BF₄)₂•7H₂O. Elemental analysis for CoC₃₀H₃₅N₉O₁₂B₂F₈, Calc.: %C, 41.8; %H, 3.4; %N, 12.2. Λ_M (H₂O): 168.1 Ω^{-1} cm² mol⁻¹. μ_{eff} 4.01 BM (3 unpaired electron) Found: %C, 41.3; %H, 2.7; %N, 12.6. Mayor IR bands (cm⁻¹): $\nu(\text{C-H})$ 3084; $\nu(\text{C=C}) + \nu(\text{C=N})$ 1626, 1610, 1587; $\delta(\text{C-H})$ 837, 824, 814, 750, 733, 722 out of plane; $\nu(\text{NO}_2)_{\text{sy}}$ 1517, $\nu(\text{N=O})$ 1537; $\nu(\text{NO}_2)_{\text{as}}$ 1357, 1332; ionic BF₄⁻ $\nu(\text{B-F})$ 1061. Raman band (cm⁻¹): $\nu(M-N)$ 266, 249. NIR diffuse reflectance data; ${}^4T_{1g}(F) \rightarrow {}^4T_{2g}(F) = 10700$, ${}^4T_{1g}(F) \rightarrow {}^4A_{2g}(F) = 22750$ and ${}^4T_{1g}(F) \rightarrow {}^4T_{1g}(P) = 27800$ cm⁻¹

[Co(5-amine-1,10-phenanthroline)₃](BF₄)₂•3H₂O Elemental analysis for CoC₃₆H₃₃N₉O₃B₂F₈, Calc.: %C, 49.6; %H, 3.8; %N, 14.4. Λ_M (H₂O): 168.1 Ω^{-1} cm² mol⁻¹. μ_{eff} 4.2 BM (3 unpaired electron) Found: %C, 50.1; %H, 2.9; %N, 14.1. Mayor IR bands (cm⁻¹): $\nu(\text{C-H})$ 3048; $\nu(\text{N-H})$ 3442, 3390; $\nu(\text{C=C}) + \nu(\text{C=N})$ 1640, 1616, 1596, 1517; $\delta(\text{C-H})$ 853, 823, 803, 731, 723 out of plane; ionic BF₄⁻ $\nu(\text{B-F})$ 1056. Raman band (cm⁻¹): $\nu(M-N)$ 274 and 226. NIR diffuse reflectance data; ${}^4T_{1g}(F) \rightarrow {}^4T_{2g}(F) = 11000$, ${}^4T_{1g}(F) \rightarrow {}^4A_{2g}(F) = 21000$ and ${}^4T_{1g}(F) \rightarrow {}^4T_{1g}(P) = 26000$ cm⁻¹

[Co(2,2'-bipyridine)₃](BF₄)₂•7H₂O Elemental analysis for CoC₃₀H₃₆N₆O₆B₂F₈, Calc.: %C, 44.5; %H, 4.5; %N, 10.4. Λ_M (H₂O): 168.1 Ω^{-1} cm² mol⁻¹. μ_{eff} 4.03 BM (3 unpaired electron) Found: %C, 44.4; %H, 4.0; %N, 10.5. Mayor IR bands (cm⁻¹): $\nu(\text{C-H})$ 3098, 3072, 3053, 3027; $\nu(\text{C=C}) + \nu(\text{C=N})$ 1604, 1596, 1574, 1566; $\delta(\text{C-H})$ 774, 736 out of plane; ionic BF₄⁻ $\nu(\text{B-F})$ 1058. Raman band (cm⁻¹): $\nu(M-N)$ 236. NIR diffuse reflectance data; ${}^4T_{1g}(F) \rightarrow {}^4T_{2g}(F) = 11780$, and ${}^4T_{1g}(F) \rightarrow {}^4T_{1g}(P) = 24600$ cm⁻¹

[Co(2,2':6',2''-terpyridine)₂](NO₃)₂•4H₂O Elemental analysis for CoC₃₀H₃₀N₈O₁₀, Calc.: %C, 49.9; %H, 4.2; %N, 15.5. Λ_M (H₂O): 168.1 Ω^{-1} cm² mol⁻¹. μ_{eff} 4.01 BM (1 unpaired electron) Found: %C, 50.1; %H, 4.0; %N, 15.1. Mayor IR bands (cm⁻¹): $\nu(\text{C-H})$ 3076, 3062, 3040; $\nu(\text{C=C}) + \nu(\text{C=N})$ 1600, 1574, 1159; $\delta(\text{C-H})$ 829, 773, 732 out of plane; ionic NO₃⁻ 1333. Raman band (cm⁻¹): $\nu(M-N)$ 240

Theoretical calculations

Density functional theory [76-78] as implemented in Gaussian 09 [79] was used for all calculations for single ligand molecules. Full geometry optimization without symmetry constraints was performed using the three-parameter B3LYP [80-82] density functional and the 6-311+G(d,p) [83] basis set. Optimized geometries of local minima were verified by the number of imaginary frequencies (which should be zero). Previous studies indicate that DFT results are very good describing

stabilities and equilibrium geometries when using hybrid functionals which include partially the Hartree-Fock exchange energy and are consistent with those obtained using the Möller–Plesset perturbational theory at second order and basis sets of medium quality, such as 6-31G(d,p), and cc-pVDZ [84].

Physical measurements

Elemental analyses were carried out using a Fisons Instruments Analyzer model EA 1108 using a sulfanilamide standard. IR spectra were acquired with a Nexus Thermo Nicolet spectrophotometer in the spectral range 4000 a 400 cm^{-1} on KBr disks. Raman spectroscopy measurements were obtained with a Thermo Scientific DXR Raman microscope, with a 780 nm laser (4 mW power out) in the range from 100 to 3500 cm^{-1} . Electronic spectra were recorded on Thermo Evolution Array spectrophotometer (200–1100 nm). Solid state UV–Vis–NIR spectra were recorded (40000–4000 cm^{-1}) on a Cary-5E (Varian) spectrophotometer. Conductivity measurements were performed using an YSI conductimeter model 302, with parallel plates ($\phi=1\text{cm}^{-1}$). Magnetic susceptibility measurements were recorded at room temperature on a Johnson-Matthey DG8 5HJ balance, using the Gouy method.

Electrochemical studies

Electrochemical measurements were carried out with a Potentiostat/Galvanostat Biologic SP-50, at sample concentrations of 1×10^{-3} M in 0.1 M phosphate buffer pH 7.2. A typical three electrode array was employed. A glassy carbon disk ($\phi = 3$ mm) was used as working electrode. A Platinum served as counter-electrode and, a commercial Ag/AgCl electrode was used as reference. Prior each experiment the working electrode was polished with α Alumina (0.3 μm), rinsed and placed on an ultrasonic bath. The solutions were also bubbled with nitrogen for 10 minutes before starting the electrochemical measurements. Cyclic voltammetry was initiated from open circuit potential in positive and negative direction using a scan rate from 10 to 1000 mVs^{-1} . One step chronoamperometry experiments were obtained by stepping the potential from open circuit potential (E_1) to value E_2 where the electrochemical process is limited by diffusion, with a perturbation time of 1s. Current interrupt method was used for ohmic drop (IR) compensation.

For the homogenous electron transfer rate constant (redox mediation) experiments the sample concentration of metal complexes was 1×10^{-3} M in 0.1M phosphate buffer pH 7.2+ 0.05 M of Glucose (prepared from a 1M stock solution stored overnight) and 3.04 μM of Glucose Oxidase (EC 1.1.3.4 type II, from *Aspergillus Niger* molecular weight 186000 Oriental Yeast Co., LTD). Enzyme concentration was calculated from homogenous electron transfer rate constant $k_s = 2.01 \times 10^5$ ($\text{M}^{-1} \text{s}^{-1}$) experiments using Ferrocene carboxylic acid as reference, according to the literature [73]. This was also confirmed with a spectrophotometric method reported before [85].

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