Luminiscent Metal Complexes for Diagnostic Applications
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Chapter 1

Introduction.
1.1 Transition metal complexes as luminescent probes.

The determination of the presence and quantity of certain compounds is of tremendous importance for several applications such as bio-medical assays, in particular for diagnostic purposes. Traditional approaches utilize radioactive labels and fluorescent reagents, since these can exhibit high sensitivities and robustness.\textsuperscript{1,2} More recently, probes based on luminescent compounds, emitting in the low-energy visible region of the electromagnetic spectrum, have also been developed.\textsuperscript{3-5} The large interest on luminescent probes is driven by the possibility to replace isotope assays, hence preventing problems with radioactive waste storage.

In general, the signalling unit (probe) is excited by light. The resulting emission is then called photoluminescence (PL). Alternatively, the formation of the excited species may be obtained by a strongly exergonic redox reaction between two compounds, which leads to chemiluminescence (CL). In this case, the energy needed for the excited state formation derives from bond cleavage (as with luminol, lucigenin, peroxyoxalate, dioxetanes) or from an electron transfer reaction (as with $[\text{Ru(bpy)}_3]^{2+}$, bpy = 2,2'-bipyridine).\textsuperscript{6,7} When one or both of the active species are generated at an electrode, the CL reaction is called electrochemiluminescence or electrogenerated chemiluminescence (ECL), Figure 1.1.\textsuperscript{7-10}

![Figure 1.1 Schematic representation of an ECL reaction.](image)

Bio-medical assays based on ECL show several advantages over those based on PL and CL.\textsuperscript{7,11} One of them is the possibility to avoid probe excitation with light. Therefore, the background signal in PL-assays, originating from fluorescence of biological molecules and
scattered light, is absent when ECL is performed. This results in higher sensitivities. The triggering of the ECL reaction by application of a voltage at the electrode is also an important advantage, as it allows a more facile temporal and spatial control of the signal generation as compared to CL, where the reaction is initialized by mixing the reactant solutions. However, some disadvantages are also present. Because the ECL reaction is bimolecular, slow diffusion of the active species and their possible deactivation before encountering may reduce the ECL efficiency. Furthermore, accessibility and vicinity of the reacting species to the electrode surface is a fundamental requirement in order to allow efficient redox reactions.

![Diagram](image)

**Figure 1.2** Scheme depicting the advantage of time-gated detection using luminescent labels with a long-lived excited state.

In designing new luminescent probes several specific properties must be considered. Obviously, the probe must exhibit high luminescence efficiency that enables high detection sensitivity. Furthermore, for assays where the excited probe is obtained upon irradiation, a lifetime longer than a few hundreds of nanoseconds is often required in order to allow time-resolved measurements and avoid detection of background signal (usually of a few nanoseconds duration). Figure 1.2 shows how, using luminescent labels with long excited state lifetimes, sensitivity can be improved by time-gated detection. For ECL-based assays, low redox potentials of the reagents and stability of their redox forms are mandatory. Thermal and photochemical stability, good solubility in water, and synthetic accessibility are other important requirements for the probes.

A promising class of compounds for sensor and probe technologies are luminescent transition metal complexes, in particular those of the group 7 and 8 metals (Ru(II), Os(II), Re(I), Rh(III) and Ir(III)) and the lanthanides. Due to their long excited state lifetimes
(hundreds of nanoseconds to tens of microseconds) transition metal complexes are more appealing than organic fluorophores (that usually have decay times of a few nanoseconds), while luminescence quantum yields, although mostly modest compared to organic fluorophores, are adequate for a variety of applications. Furthermore, for many metal complexes the emission has a large Stokes shift, that allows a reduced background signal at the detection wavelength, and is less affected by oxygen quenching, compared to traditional fluorescent dyes and lanthanides.

This Thesis focuses mainly on the use of transition metal complexes as luminescent probes for PL or ECL applications. The discussion sections of the experimental chapters will refer frequently to several types of photophysical and redox processes which can occur in transition metal complexes. The remainder of this chapter will give information about these processes, focusing on the parts relevant for this Thesis. The subjects that will be dealt with consecutively are as follows: first of all, the basic photophysical and redox properties of transition metal complexes, followed by an outline of the Marcus model for electron transfer reactions. Since the majority of the complexes described in this Thesis belong to the ruthenium(II) polypyridyl family, a separate section is devoted to relevant physicochemical properties of the parent complex, \([\text{Ru(bpy)}_3]^{2+}\), and to strategies toward increasing the excited state lifetime of the complexes. Because osmium(II) polypyridyl fragments compose homo- and heterodinuclear complexes described in the last two chapters, Section 1.6 outlines the main differences between osmium(II) and ruthenium (II) polypyridyl complexes, followed by description of the principles governing energy transfer in dinuclear systems. The subsequent section gives a background information on electrochemiluminescence. The chapter is concluded with a section on the scope of this Thesis.

1.2 Photophysical processes in transition metal complexes

In this Section the main photophysical processes occurring in photoexcited transition metal complexes and their major differences as compared to organic molecules are outlined.

Upon irradiation with light quanta, a molecule may be promoted from the ground to an electronically excited state with a probability that is largest for transitions that retain the spin state multiplicity (\textit{spin-allowed transition}).\textsuperscript{12,13} For closed-shell molecules, such as organic molecules and the transition metal complexes discussed in this Thesis, the ground state has singlet multiplicity, hence the most favoured are the transitions populating singlet excited states. In metal complexes the presence of a heavy (5d metal) atom may induce spin-orbit coupling to such extent that absorption bands due to \textit{spin-forbidden transitions} gain intensity and may be observed (see for example the osmium complexes).\textsuperscript{14-16}

After photoexcitation, the molecule in a high excited state decays rapidly (on a femto- to picosecond time scale), by non-radiative pathways, to the lowest state of the same spin
multiplicity (internal conversion, IC) or to a state of different spin multiplicity (intersystem crossing, ISC).\textsuperscript{12,13,17} Figure 1.3 shows an overview of the various photophysical processes that can occur in molecules (simplified Jablonski diagram). Due to the fast decay of the upper states, for most molecules the lowest singlet and triplet states are the only important levels for their emission properties and bimolecular interactions, such as photoinduced energy and electron transfer processes and photochemical reactions.

When a molecule is in the lowest singlet excited state, it may undergo radiative (fluorescence) or non-radiative (internal conversion) decay to the ground state, or intersystem crossing to the lowest triplet excited state (Figure 1.3). In turn, the triplet state can decay to the ground level via non-radiative (ISC) or radiative (phosphorescence) transitions (Figure 1.3).

![Jablonski diagram](image)

**Figure 1.3** Jablonski diagrams of the electronic states and various photochemical processes of a typical organic compound. The processes are indicated by the corresponding rate constants, i.e. internal conversion ($k_{ic}$), intersystem crossing ($k_{isc}$), fluorescence ($k_{fl}$), phosphorescence ($k_{ph}$) and bimolecular quenching ($k_q$).

In organic molecules, the intersystem crossing $S_1 \rightarrow T_1$ is strongly spin-forbidden and internal conversion and fluorescence occur with large rate constants. The singlet excited state has therefore a short lifetime (pico- to nanosecond time scale) and fluorescence is observed. Contrary to this, spin-forbidden phosphorescence and $T_1 \rightarrow S_0$ ISC have small rate constants and, hence, the triplet state is long-lived (millisecond to second timescale). Quenching
processes with another molecule (such as molecular oxygen, \( ^3\text{O}_2 \)) can strongly compete with the radiative decay, therefore phosphorescence is usually not observed in aerated solutions at room temperature.

For transition metal complexes, the presence of the heavy (metal) atom increases the efficiency of the intersystem crossing from the lowest singlet to the lowest triplet state to nearly 100%, in the case of second- and third-period transition metal ions. Fluorescence is therefore usually not observed in these cases and light emission occurs only from the lowest triplet state.\(^{10,16}\) Since the radiative and non-radiative \( T_1 \rightarrow S_0 \) transitions are more allowed, the lowest triplet state is shorter lived (nano- to millisecond time scale) compared to organic molecules and less susceptible to environmental quenching; emission is therefore observed also in aerated solution at room temperature. Due to the spin-orbit coupling, the spin character of the lowest excited state is not a pure triplet but a mixed triplet and singlet spin state. Emission of metal complexes is therefore usually referred to as \textit{luminescence}.

Another important difference between metal complexes and organic molecules lies in the nature of the excited states. Apart from intraligand electronic transitions from \( \pi \) (bonding) or \( n \) (non-bonding) orbitals to \( \pi^* \) (anti-bonding) orbitals of a ligand, metal complexes also exhibit transitions that involve only the metal or both the metal and the ligands. The energy of these transitions, and therefore the spectroscopic properties, can be conveniently tuned upon judicious choice of the metal ion and the chelating ligands.

For complexes that consist of metal ions and ligands with limited delocalization of their frontier orbitals, the spectroscopic properties can be described in terms of localized molecular orbital configurations, thus the excited states can be classified according to the localized molecular orbitals involved in the electronic transition.\(^{10,16-18}\) Using this criterion, three main types of excited states may be then distinguished (Figure 1.4): (1) metal-centered (MC), also known as “d-d” or “ligand field”, excited states; (2) intraligand (IL), also called “ligand-centered”, excited states; (3) charge transfer (CT) excited states, such as metal-to-ligand (MLCT) or ligand-to-metal (LMCT) excited states (Figure 1.4). In some cases different excited states may be isoenergetic and intermix. This simplified picture is then no longer applicable. The same applies for complexes with extensively delocalized \( \pi\)-bonding between the metal and the ligands, where the electronic transitions possess limited CT character.
Introduction

Figure 1.4. MO diagram depicting various possible electronic transitions between different frontier orbitals in octahedral metal complexes ML₃ (M = metal, L = chelating ligand).

*Metal-to-ligand charge transfer* states are particularly important for the discussions in this Thesis, as they are the lowest-energy excited states for the metal complexes herein studied and are responsible for their photoluminescence and electrochemiluminescence processes. MLCT transitions in octahedral d⁶ metal complexes occur from occupied πₘ(t₂g) metal based to empty π* ligand based MOs (Figure 1.4) and formally correspond to the oxidation of the metal and reduction of the ligand in the excited state.¹⁰⁻¹⁸ Their energies are therefore related to the redox properties of the metal complex. Indeed, for complexes with easily reducible ligands such as α-diimines, MLCT levels are significantly stabilized. MLCT absorption bands are usually intense (ε ~ 10⁴ mol⁻¹ dm³ cm⁻¹) and emission occurs with lifetime in the order of nano- to microseconds time scale. Because MLCT transitions generally involve a change in the dipole moment of the complex, on going from the ground to the excited states, their corresponding absorption and emission bands are usually solvatochromic, i.e. they shift on changing solvent polarity.
The lifetime ($\tau$) of the excited state and the emission quantum yield ($\Phi_{em}$) are important experimental parameters for the investigation of the spectroscopic properties of the molecules, and can be expressed as (1.1) and (1.2), respectively\textsuperscript{12,13}

$$\frac{1}{\tau} = k_r + k_{nr} \quad (1.1)$$

$$\Phi_{em} = k_r \tau \quad (1.2)$$

where $k_r$ and $k_{nr}$ are the radiative and non-radiative decay rate constants, respectively. For molecules that undergo bimolecular quenching (with rate constant $k_q$), the excited state lifetime decreases with increasing concentration of the quencher ([Q]), Eq. 1.3

$$\frac{1}{\tau} = k_r + k_{nr} + k_q[Q] \quad (1.3)$$

### 1.3 Redox properties of transition metal complexes

The specific redox properties exhibited by transition metal complexes are another important aspect that makes them suitable for diverse applications where electron transfer reactions are involved. This Section will mainly refer to ruthenium(II) and osmium(II) polypyridyl complexes, that are the topic of this Thesis.

Similarly to electronic transitions to excited states, for most of the ruthenium(II) and osmium(II) polypyridyl complexes the electrochemical processes can be thought of as occurring at localized orbitals.\textsuperscript{10,19} For those metal complexes with lowest MLCT excited state, the oxidation process involves subtraction of an electron from a $\pi_M(t_{2g})$ orbital and can be considered as occurring at the metal center, e.g. Eq.1.4,

$$[\text{Ru}^{II}(bpy)_3]^2^+ \rightarrow [\text{Ru}^{III}(bpy)_3]^{3^+} + e^- \quad (1.4)$$

while the reduction process, involving addition of an electron to the lowest unoccupied $\pi^*$ orbital, can be considered as a reduction of the ligand (Eq.1.5)

$$[\text{Ru}^{II}(bpy)_3]^{2^+} + e^- \rightarrow [\text{Ru}^{II}(bpy)_2(bpy^*)]^+ \quad (1.5)$$

For most of the ruthenium(II) and osmium(II) polypyridyl complexes the oxidized and reduced redox forms are rather stable, and the redox potentials can be conveniently tuned by properly chosen ligands. Strong $\sigma$-donor ligands stabilize the oxidized metal, and therefore oxidation potential become less positive. Strong $\pi^*$ accepting ligands more readily
accommodate electron density, and the electrochemical reductions occur at less negative potentials.

When a complex (R*) is in the CT excited state, it is both a stronger oxidant and a stronger reductant than the parent species (R) in the ground level. The oxidation and reduction potentials of the excited complex can be evaluated from the zero-zero excitation energy ($E^{00}(R^*)$) and the ground state redox potentials ($E^{0}_{\text{ox}}(R^+/R)$ and $E^{0}_{\text{red}}(R/R^*)$, respectively), Eqs. 1.6 and 1.7 respectively.

$$E^{0}(R^+/R^*) \approx E^{0}_{\text{ox}}(R^+/R) - E^{00}(R^*) \quad (1.6)$$

$$E^{0}(R/R^*) \approx E^{0}_{\text{red}}(R/R^*) + E^{00}(R^*) \quad (1.7)$$

These equations are valid in the approximation that (i) the vibrational partitioning functions of the ground and excited states are not very different (therefore the enthalpy associated to the electronic excitation can be approximated to the zero-zero excitation energy $\Delta H(M^*/M) \approx N AE^{\infty}(R^*)$, $N_A$ is the Avogadro number) and (ii) the entropic contribution can be neglected (that is strictly true for complexes undergoing electronic excitation without changes in size, shape and solvation). 10,19

Because the excited state has a stronger redox power, electron transfer reactions thermodynamically forbidden for a complex in its ground state can become thermodynamically allowed in the CT excited state (Figure 1.5A)

![Energy scheme for excited state electron transfer](image)

**Figure 1.5** Energetic schemes for a photoinduced electron transfer reaction (A) and an electrochemiluminescence reaction (B).
1.4 Photoinduced electron transfer and electrochemiluminescent processes

Transition metal complexes are often employed in electron transfer reactions such as photoinduced electron transfer (PET)\(^{20,21}\) processes (Figure 1.5A) and generation of light by redox reactions (such as electrochemiluminescence, ECL)\(^{7,8}\) (Figure 1.5B), due to the stability of their reduced and oxidized forms and low redox potentials.

In a photoinduced electron transfer process, the excitation energy of a donor (D) is transferred to an acceptor (A) with formation of two redox species (e.g. D\(^+\) and A\(^-\)), Figure 1.5A. This is allowed when the energy of the molecule in the excited state (D\(^*\)) exceeds the energy of the two redox products. The driving force ($\Delta G_{PET}^0$) can be calculated as \(^{13,17,19}\)

$$\Delta G_{PET}^0 = E_0^0(D^*/D) - E_0^0red(A/A') + E_{Coul}(D-A) - E_{Coul}(D^+-A^-)$$  \hspace{1cm} (1.8)

where $E_0^0(D^*/D^*)$ is the oxidation potential of D\(^*\) and is given by Eq. 1.6, $E_0^0red(A/A')$ is the standard reduction potential of A and $E_{Coul}(D-A)$ and $E_{Coul}(D^+-A^-)$ are Coulombic interaction energies required to respectively bring the reactants and the products together, to the most probable separation distance $r_{DA}$. For two oppositely charged ions, $E_{Coul}$ can be calculated using the Coulombic expression (Eq. 1.9)\(^8,9\)

$$E_{Coul}(r_{DA}) = -N_A e_0^2 / 4\pi \varepsilon_0 \varepsilon r_{DA}$$  \hspace{1cm} (1.9)

where $N_A$ is the Avogadro constant, $e_0$ the electron charge, $\varepsilon_0$ the permittivity of vacuum, and $\varepsilon$ the dielectric constant.

Considering ECL (Figure 1.5B), the electron transfer between two electrogenerated species A\(^+\) and B\(^-\) yields formation of a molecule in the excited state (e.g. A\(^*\)). It can be then regarded as counterpart to the photoinduced electron transfer process and occurs when the energy associated to the charge separated components A\(^+\) and B\(^-\) exceeds the energy of A in the excited states (A\(^*\)). The free energy ($\Delta G_{ECL}^0$) of this reaction can be calculated as (1.10)\(^9,19\)

$$\Delta G_{ECL}^0 = E_0^0red(B/B^-) - E_0^0(A^+/A^*) + E_{Coul}(A^+-B^-) - E_{Coul}(A-B)$$  \hspace{1cm} (1.10)

The kinetics for photoinduced electron transfer as well as for ECL can be described on the grounds of the classical model for an electron transfer reaction in a unimolecular system developed by Marcus, Hush and Sutin (hereinafter indicated as Marcus model).\(^9,13,17\) The treatment of the theory is beyond the purpose of this chapter and only very general and brief concepts are here described.

According to this model, the electron transfer rate constant ($k_{et}$) can be expressed by (1.11)
\[ k_{e1} = \nu_N \kappa \exp(-\Delta G^*/RT) \]  

(1.11)

The terms in (1.11) can be best discussed referring to two curves representing the potential energies of reactants and products as function of reaction coordinates, that include internal coordinates (bond lengths and angles of the reacting molecules, “inner coordinates”) and coordinates specifying the arrangement of the solvent molecules surrounding the reactants and products (“outer coordinates”). The \( \Delta G^* \) is the energy (free activation energy) necessary to reach the configuration at which electron transfer between the reactants can occur. Thus, it corresponds to the energetic difference between the crossing point of the two potential energy curves and the minimum of the reactant energy curve (Figure 1.6). \( \kappa \) is the transmission coefficient (also called the electronic factor) of the reaction, that is the probability that the reactants convert into products when they reach the crossing point, and \( \nu_N \) is the nuclear frequency factor of the reaction.

Figure 1.6. Potential energy curves of reactants and products for a unimolecular electron transfer reaction.

The free activation energy \( \Delta G^* \) is determined by the combined effects of the degree of distortion between products and reactants (horizontal displacement), and the driving force (\( \Delta G^0 \)) of the reaction. Marcus theory expresses this combined dependence as a parabolic function of \( \Delta G^0 \) (Eq.1.12).

\[ \Delta G^* = \left( \lambda/4 \right) \left[ 1 + \Delta G^0/\lambda \right]^2 \]  

(1.12)

where \( \lambda \) is the so-called reorganization energy, that corresponds to the vertical separation, at equilibrium geometry, between reactant and product curves for a hypothetical isoergonic
reaction, and can be expressed as sum of two contributions, $\lambda_0$ and $\lambda_n$, corresponding to reorganization of inner and outer nuclear modes, respectively.

The most important result arising from Eq. 1.12 is that it predicts a parabolic dependence of $\ln k_{cl}$ on $\Delta G^0$. For small negative values of $\Delta G^0$, the activation free energy $\Delta G^* \text{ decreases}$ (and $\ln k_{cl}$ increases) with increasingly negative values of $\Delta G^0$. In this region of driving force values (called “normal region”) the configurations of coordinates at the intersection point represent a compromise between the stable configuration of the reactants and that of the products (Figure 1.7). When $\Delta G^0$ becomes so negative that it equals $\lambda$, $\Delta G^*$ vanishes and $\ln k_{cl}$ reaches the maximum value, (“activationless region”) Figure 1.7. For more negative values of $\Delta G^0$, $\Delta G^*$ increases (and $\ln k_{cl}$ decreases) with increasingly negative values of driving force. For these values $\Delta G^0$, the configuration of coordinates at the crossing point becomes increasingly different from both the stable configuration of the reactants and that of the products (“inverted region”), Figure 1.7.

Thus, reactions strongly thermodynamically allowed may result not kinetically favoured. In ECL reactions, for instance, ground state formation $(A + B)$ by charge recombination between the electrogenerated $A^+$ and $B^-$ species may be not kinetically favoured, due to its corresponding large driving force, leading to formation of the excited $A^*$ species with high efficiency, due to the smaller driving force ($\Delta G^0_{ECL}$). A more extended description of the processes competing in ECL reactions can be found in Section 1.8.

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**Figure 1.7** Potential energy curves for reactants and products in the three regimes of the Marcus model.
1.5 Ru(II) polypyridyl complexes

One of the most extensively investigated luminescent metal complexes is \([\text{Ru(bpy)}_3]^{2+}\). This represents the prototype of several thousands of derivatives, some of them also described in this Thesis. The combination of chemical stability, different redox states, rich excited state properties, high emission quantum yield and long excited state lifetime have attracted a lot of attention for both pure and applied chemistry. \([\text{Ru(bpy)}_3]^{2+}\) and its derivatives play an important role in diverse fields, such as photoenergy conversion, biomedical applications and electronic devices (e.g. light emitting diodes).

The lowest-energy absorption band in the spectrum of \([\text{Ru(bpy)}_3]^{2+}\) (Figure 1.8) lies at 450 nm (ε = 14600 M⁻¹ cm⁻¹) and is attributed to dₓ(Ru)→π*(bpy) (MLCT) transitions. In addition, the absorption spectrum shows two intense IL bands at 185 (not shown) and 285 nm. The less intense band at 240 nm belongs to a higher MLCT transition, while the shoulder at 344 nm is due to MC transitions. At room temperature, the emission from the \(^3\text{MLCT}\) state appears as a structureless band with maximum at 607 nm (Figure 1.8), and exhibits high quantum yield (Φₑₘ = 0.042) and long decay time (τ ≈ 0.6 μs). At 77 K in rigid matrix, it becomes vibronically structured and shifts to higher energy (Figure 1.8) due to the lack of stabilization of the excited state by reorganization of solvent molecules. As recently reported, the \(^3\text{MLCT}\) state of \([\text{Ru(bpy)}_3]^{2+}\) is populated from the upper excited states within 300 fs after photoexcitation, and intersystem crossing from \(^1\text{MLCT}\) to \(^3\text{MLCT}\) occurs with unitary
efficiency.\textsuperscript{44,45} Although usually considered as photochemically inert, deactivation of the lowest $^3$MLCT by thermal population of the $^3$MC state may occur, giving rise to photodecomposition by ligand displacement. However, the thermal stability can be improved by increasing the energy gap between $^3$MLCT and $^3$MC states, for instance by increasing the $\sigma$-donor character of the ligand.

\begin{center}
\begin{tikzpicture}
\node (A) at (0,0) {\(1\) (MLCT)[Ru(bpy)$_3$]$^{2+}$};
\node (B) at (1,1) {\(3\) (MLCT)[Ru(bpy)$_3$]$^{2+}$};
\node (C) at (-1,1) {$[\text{Ru(bpy)}_3]^{3+}$};
\node (D) at (1,-1) {$[\text{Ru(bpy)}_3]^{2+}$};
\node (E) at (-1,-1) {$[\text{Ru(bpy)}_3]^+$};
\draw[->] (A) -- (B) node[midway,above] {$E_1 = 2.73$ eV};
\draw[->] (B) -- (C) node[midway,above] {$E_3 = 2.12$ eV};
\draw[->] (B) -- (D) node[midway,above] {$\tau = 0.6$ $\mu$s};
\draw[->] (B) -- (E) node[midway,above] {$\phi_{em} = 0.042$};
\node (F) at (0,2) {$\lambda_{max} = 450$ nm};
\node (G) at (0,1) {$\epsilon_{max} = 14600$ M$^{-1}$.cm$^{-1}$};
\node (H) at (0,0) {$E_1 = 2.73$ eV};
\node (I) at (0,-1) {$E_3 = 2.12$ eV};
\node (J) at (0,-2) {$\tau = 0.6$ $\mu$s};
\node (K) at (0,-3) {$\phi_{em} = 0.042$};
\end{tikzpicture}
\end{center}

\textbf{Figure 1.9} Schematic representation of the ground and excited state properties of [Ru(bpy)$_3$]$^{2+}$. From Ref.\textsuperscript{10}

The spectroscopic and redox properties of [Ru(bpy)$_3$]$^{2+}$, in the ground and excited states, are summarized in Figure 1.9. The oxidation and reduction potentials are $+1.26$ V and $-1.28$ V (vs NHE) in the ground state and drop to $-0.86$ V and $+0.84$ V, respectively, in the lowest excited $^3$MLCT state.\textsuperscript{10,46}

Although the $^3$MLCT is rather long-lived and its emission occurs with a high quantum yield, longer excited state lifetimes (a few microseconds) and higher emission intensities are of great interest for several analytical application (see Section 1.1). Therefore, several studies have been performed in order to prolong $\tau$ and increase $\phi_{em}$. Some of these approaches, more closely related to the subject of this Thesis, are hereafter reviewed.

Besides the interesting spectroscopic properties, one of the biggest advantages of utilizing ruthenium(II) polypyridyl complexes lies in the fact that their chelation chemistry, to form strong and stable complexes, is less demanding than, for instance, that of the lanthanide complexes.
1.5.1 Ruthenium(II) polypyridyl complexes with rigid and delocalized ligands

The excited state lifetime and the luminescence intensity of $^3$MLCT emitting complexes are largely determined by the non-radiative decay, since the spin-forbidden radiative process has small rate constant. Therefore, approaches aimed to reduce $k_{nr}$ are of high importance in order to have more competitive radiative decay.

Several examples reported in the literature have proved that an increase of rigidity and/or $\pi$ delocalization within the accepting ligand reduces the nuclear configuration distortion ($\Delta Q_e$) in the excited state as compared to the ground state, leading to a decreased overlap between the iso-energetic vibrational wavefunctions of the ground and excited states, and finally to a reduced $k_{nr}$. An example is given by $[(\text{dm-bpy})_2\text{Ru(\mu-bbpe)Ru(dm-bpy)}_2]^{4+}$ (dm-bpy = 4,4'-dimethyl-2,2'-bipyridine, bbpe = trans-1,2-bis(4'-methyl-2,2'-bipyridyl-4-yl)-ethene, Figure 1.10) that shows longer excited state lifetime (1.3 $\mu$s in acetonitrile at 298 K) compared to $[\text{Ru(dm-bpy)}_3]^{2+}$ (950 ns, under the same experimental conditions) even though its energy gap is higher (15600 cm$^{-1}$) as compared to $[\text{Ru(dm-bpy)}_3]^{2+}$ (13300 cm$^{-1}$). This effect was ascribed to the extended $\pi$ conjugation of the accepting bbpe ligand, that delocalizing the electron on a larger framework than dm-bpy reduces $\Delta Q_e$ and therefore $k_{nr}$.

Similarly, a combination of rigidity and delocalization accounts for the enhanced lifetimes of $[\text{Ru(bpy)}_2(\text{dpp'})]^{2+}$ (dpp' = 4',7'-phenanthrolino-5',6:5,6-pyrazine) and $[\text{Ru(bpy)}_2(\text{dpq'})]^{2+}$ (dpq' = dipyrdo[2,3-a:3',2'-c]phenazine) ($\tau = 1214$ and 327 ns, respectively) compared respectively to $[\text{Ru(bpy)}_2(\text{dpp})]^{2+}$ (dpp = 2,3-bis(2'-pyridyl)pyrazine) and $[\text{Ru(bpy)}_2(\text{dpq})]^{2+}$ (dpq = 2,3-bis(2'-pyridyl)quinoxaline) ($\tau = 226$ and 71 ns, respectively). The structures of the ligands are depicted in Figure 1.10.
Among the rigid and flat ligands, the most studied is dppz (dppz = dipyridophenazine[3,2-a:2',3'-c]phenazine, Figure 1.10). Its ruthenium complexes [Ru(L)\(_2\)(dppz)]\(^{2+}\) (L = bpy, phen; phen = 1,10-phenanthroline) have been extensively used as probes for DNA structure. The probes are described as molecular “light switch”. The luminescence, quenched in aqueous solutions, becomes intense upon addition of double-helical DNA. This is due to the intercalation of the dppz ligand into the hydrophobic helix of the DNA, that therefore protects the dppz from interacting with water, responsible for the emission quenching.

![Energy levels of the Ru-pyrene complex shown in the inset.](image)

**Figure 1.11** Energy levels of the Ru-pyrene complex shown in the inset.

### 1.5.2 Equilibria between isoenergetic states.

The covalent linkage of [Ru(bpy)\(_3\)]\(^{2+}\) to a chromophore possessing a long-lived \(^3\)IL state, isoenergetic with the lowest \(^3\)MLCT of the metal complex, proved to be in some cases a successful approach for increasing the \(^3\)MLCT state lifetime, as result of a thermal equilibrium between the two states.\(^{50,63}\) In order to observe thermal equilibrium, a necessary condition is that the metal complex and the chromophore are at short distance.

In this regard, one of the most investigated chromophores is the pyrene molecule.\(^{63,64}\) An example of system containing a ruthenium complex and a pyrene is given in Figure 1.11. The excitation into Ru(\(d_z\)) \(\rightarrow\) bpy(\(\pi^*\)) \(^1\)MLCT or \(^1\)IL(pyrene) absorption bands gives rise to emission from the \(^3\)MLCT state (with band shape and \(k_c\) similar to those of [Ru(bpy)\(_3\)]\(^{2+}\)), with decay time of 5.3 \(\mu s\).\(^{63}\) It was suggested that the long lifetime is due to the fast internal conversion between the isoenergetic \(^3\)MLCT and \(^3\)IL(pyrene) states, that redistributes population between them, leading to a longer-lived \(^3\)MLCT excited state. However, if the \(^3\)IL
excited state of the chromophore contributes significantly to the equilibrated states, the emission quantum yield may be largely decreased, due to the low efficiency of the emission in the organic molecule. \(^{64}\)

1.5.3 Dendritic structures

One of the most important excited state quenchers for the ruthenium complexes is molecular oxygen, which is often present in solution, in particular in biological assays. Therefore, means to reduce dioxygen quenching are of great importance.

![Molecular structure of the ruthenium-dendrimer described in the text. From Ref.\(^{65}\).](image)

Figure 1.12 Molecular structure of the ruthenium-dendrimer described in the text. From Ref.\(^{65}\).

It was shown that the binding of extended dendritic branches to the bipyridine ligands in \([\text{Ru(bpy)}_3]^{2+}\) has the effect of increasing the emission intensity and the excited state lifetime in aerated solution. \(^{65,66}\) A five-fold increase of the lifetime could be obtained with branches reaching up to 54 peripheral units (Figure 1.12) leading to a \(k_q\) reduced by one order of magnitude \((\tau = 1.01 \text{ µs and } k_q = 0.22 \times 10^9 \text{ s}^{-1})\) as compared to the "naked" \([\text{Ru(bpy)}_3]^{2+}\) in the same experimental conditions \((\tau = 172 \text{ ns and } k_q = 2.5 \times 10^9 \text{ s}^{-1})\).\(^{65}\)

The dendritic branches exhibit a shielding effect towards the excited metal core due to different factors, such as lower solubility of molecular oxygen in the interior of the
dendrimer, hindrance of suitable orbital overlap for energy transfer by exchange mechanism, and decrease of the diffusion rate constant of the substituted ruthenium complex.

This approach could offer several advantages since the combination of an emitting core and branches of different polarity and hydrophobicity can lead to compounds soluble in water as well as in apolar solvents. Furthermore, the branches could contain receptor groups able to carry specific drugs as radioactive labels, in order to perform more than one function. Examples are also known of dendrimers containing metal complexes both as core and as peripheral groups, able to absorb light and tunnel the energy to the central metal ("antenna effect"). Several studies in this area are in progress.

1.6 Os(II) polypyridyl complexes

Most of what written for the ruthenium(II) polypyridyl metal complexes is also valid for their corresponding osmium(II) compounds. An important feature of Os(II) α-diimine complexes is that metal-nitrogen distances are similar to those of the analogous Ru(II) complexes. Thus, their physicochemical properties are similar. However, some important differences must be noted: (1) the oxidation potential of the osmium complexes is generally less positive than that of the analogous ruthenium complexes, due to the higher energy of the 5d valence orbitals of the osmium; therefore, (2) their MLCT states lie at lower energy. (3) Because osmium is a third-row transition metal, the crystal field splitting is larger for its complexes and the lowest $^3\text{MC}$ state lies at higher energy. Osmium(II) complexes are therefore more stable towards photosubstitution reactions that occur via thermal population of the $^3\text{MC}$ state. (4) Furthermore, the larger heavy-atom effect in the osmium complexes induces a mixing between singlet and triplet states, therefore spin-forbidden $^3\text{MLCT}$ absorption transitions may be fairly intense, as observed for [Os(bpy)$_3$]$^{2+}$, while they are in general not observed for the corresponding ruthenium derivatives. Another important difference is that osmium compounds are in general more difficult to synthesize, as the osmium is chemically more inert than ruthenium.

1.7 Energy transfer in heteronuclear complexes

Due to the low energies of their $^3\text{MLCT}$ states, luminescent Os(II) polypyridyl complexes are often used as accepting unit (A) for the study of energy transfer processes from chromophores (donor, D) with excited state at higher energy, such as the analogous ruthenium polypyridyl complexes.
The free energy change, $\Delta G^0$, for the energy transfer reaction can be expressed by the difference between the zero-zero spectroscopic energies of the donor and the acceptor excited state.\(^{73,74}\)

$$E_{calc} = E_{00}^D - E_{00}^A$$

**Figure 1.13.** Schematic representation of Förster- and Dexter-type energy transfer mechanisms.

The energy transfer processes can occur by two mechanisms: Förster-and Dexter-type. Förster-type (also called “coulombic”, “dipole-dipole” or “resonance”) energy transfer mechanism takes place through the interaction of the oscillating dipole of the excited donor ($^*D$) with that of the acceptor ($A$).\(^{12,13,17}\) Because the electrons involved in the energy transfer are not exchanged between the two chromophores (Figure 1.13A), this mechanism does not require overlap of the orbitals of D and A (“through-space mechanism”), therefore this interaction can take place at long distances (up to the order of 100Å) and decays inversely with the sixth power of the interchromophore separation. The rate Förster-type energy transfer can be expressed in terms of spectroscopic quantities by Eq. 1.13\(^{77}\)

$$k_{Coul} = 8.8 \times 10^{-25} K^2 \left( \frac{\Phi}{n^4 \tau r^6} \right) J_F$$  \hspace{1cm} (1.13)

where $\Phi$ and $\tau$ are the luminescence efficiency and lifetime, respectively, of the donor $^*D$ and $J_F$ is the overlap integral, according to Förster theory, between the luminescence spectrum of the donor and the absorption spectrum of the acceptor. $k_{Coul}$ depends on the radiative decay rate constant (in the terms $\Phi$ and $\tau$) of $^*D$ and on the oscillator strength ($\varepsilon$) of $A$ (in the overlap integral), thus no change of spin multiplicity is “allowed” and the most favoured process is singlet-singlet energy transfer.\(^{12}\)
The Dexter-type (also called "exchange") mechanism may be regarded as a double electron transfer process where the excited electron in *D is transferred to the lowest unoccupied molecular orbital (LUMO) of A and, simultaneously, an electron from the highest occupied molecular orbital (HOMO) of A is transferred to the HOMO of D (Figure 1.13B). This mechanism requires overlap of the orbitals involved ("through-bond mechanism"), therefore it occurs only at short distances, up to 20Å. The rate constant for Dexter-type energy transfer can be expressed as (1.14)\(^7\)

\[
k_{\text{Coul}} = (4\pi^2H^2 / h) J_D
\]

where \( H \) is the intercomponent electronic interaction energy between D and A and exhibits exponential dependence on their separation distance, and \( J_D \) is the overlap integral, according to Dexter theory, between the luminescence spectrum of the donor and the absorption spectrum of the acceptor.\(^7,73,77,79\) In the exchange mechanism only the spin of donor and acceptor as whole system should remain the same, therefore processes not allowed by Coulombic mechanism can occur by exchange mechanism, such as the triplet-triplet energy transfer.\(^12\)

For Ru-Os complexes covalently linked by a bridging ligand (BL), both through-bond and through-space energy transfer mechanisms can operate. Very often they occur competitively and their relative contribution can be difficult to evaluate.

In transition metal complexes, the only excited state of appreciable lifetime is generally the lowest triplet excited state, so that coulombic energy transfer is not expected to be frequent in these systems.\(^80\) However, for complexes containing ruthenium and osmium units covalently linked and exhibiting small electronic coupling, such as dinuclear compounds containing bridging ligands with saturated spacers, the Förster-type mechanism may be the most efficient. Dexter-type energy transfer is not favoured for metal centres that show weak electronic interaction. Furthermore, for Ru and Os dinuclear complexes Förster-type mechanism is more spin allowed, due to the partial singlet character of the \( ^3\text{MLCT} \) levels. By contrast, a large electronic coupling between ruthenium and osmium units favours the Dexter mechanism. The experimental values observed for the rate of the energy transfer process then become larger than the estimated values calculated using the Förster equation (Eq.1.13).\(^21\)

An evaluation of the energy transfer rate constant can be obtained from experimental parameters following Eqs. 1.15a-b

\[
k_{\text{en}} = 1/\tau^\theta (\tau^\theta/\tau-1)
\]

\[
k_{\text{en}} = 1/\tau - 1/\tau^\theta
\]
where $I^0$ and $\phi$ are, respectively, the luminescence intensity and the excited state lifetime of the donor in the absence of energy transfer (the Ru(II)-based component), and $I$ and $\tau$ are its luminescence intensity and lifetime after quenching. Evidence for the energy transfer as the sole quenching mechanism can be obtained by sensitisation experiments or by risetime measurements. For dinuclear ruthenium and osmium metal complexes, however, the measurements may be complicated by the lack of selective excitation of the Ru-based chromophoric unit, due to the similar absorption properties of the Os-component. A substantial fraction of Os-based excited states are already formed when energy transfer from the ruthenium unit occurs. Furthermore, it is interesting to notice that intramolecular energy transfer processes are almost temperature independent because of the small reorganizational energy involved in these reactions.

1.8 Electrochemiluminescence (ECL)

In Sections 1.1 and 1.4 we have seen that light can be obtained from an excited species $(A^*)$ formed by electron transfer between a reductant $(B^-)$ and an oxidant $(A^+)$ leading to the so-called chemiluminescence (CL), or electrochemiluminescence (ECL) if the reacting species are generated at the electrode surface. When the excited molecules $A^*$ are obtained without bond cleavage of the reactants, the ECL reaction is also called electroluminescence (EL). Since the electrochemiluminescence is the subject of part of this Thesis (Chapter 3 and 4), the process itself and its applications in immunoassays are herein more extensively described.

1.8.1 Energetics and kinetics

The ECL reaction may involve two electrogenerated redox species $R^+$ and $R^-$ deriving from the same molecule $R$ (ion-annihilation mechanism), Eq. 1.16.

$$R^+ + R^- \rightarrow R + R^* \rightarrow R + h\nu \quad (1.16)$$

In a schematic description, the reaction between $R^+$ and $R^-$ can be thought of as undergoing two possible pathways: (1) formation of the ground state (Eq.1.17a) and (2) formation of an excited state, with population of the singlet ("S-route", Eq.1.17b) or triplet ("T-route", Eq.1.17c) state.

$$R^+ + R^- \rightarrow R + R \quad (1.17a)$$

$$R^+ + R^- \rightarrow R + ^1R^* \quad (1.17b)$$
\[ \text{R}^+ + \text{R}^- \rightarrow \text{R} + \text{^3R}^* \]  

(1.17c)

Following the formulations in Section 1.4, the free energy \( \Delta G^0 \) for Eq. 1.17a can be expressed by Eq. 1.18a

\[ \Delta G^0 \approx E_{\text{red}}(\text{R}/\text{R}) - E_{\text{ox}}(\text{R}/\text{R}) + E_{\text{Coul}}(\text{R}^\text{+}/\text{R}^-) - E_{\text{Coul}}(\text{R}-\text{R}) \]  

(1.18a)

Thus the free energy associated to Eqs. 1.18b-c can be respectively expressed as

\[ \Delta G_1^0 \approx \Delta G^0 + E_{1}^{00} \]  

(1.18b)

\[ \Delta G_3^0 \approx \Delta G^0 + E_{3}^{00} \]  

(1.18c)

where \( E_{1}^{00} \) and \( E_{3}^{00} \) are the zero-zero spectroscopic energies of the singlet and triplet excited states (Figure 1.14), that can be experimentally determined from the lowest energy absorption and highest energy emission at 77 K.

**Figure 1.14.** Energetic scheme for the ECL reaction.

When \( -\Delta G^0 > E_{1}^{00} \), the formation of singlet as well as the triplet excited states are energetically allowed. Population of the singlet excited state can also occur via triple-triplet annihilation (Eq. 1.19).^{8,9}
\[ {^3R^* + ^3R^* \rightarrow R + ^1R^*} \]  

(1.19)

The relative probabilities for the reaction pathways (1.17a-c) depend on their relative free energies, as described in Section 1.4 following Marcus model. When the ground state formation (Eq. 1.17a) is highly exergonic, \( \Delta G^0 \) lies in the inverted Marcus region, resulting in a small \( k_{el} \). By contrary, excited state formation is less thermodynamically favoured, being less exergonic. However, since its driving force (\( \Delta G_{1}^0 \) or \( \Delta G_{2}^0 \)) lies in the activationless or normal Marcus regions, it can be kinetically more allowed, and therefore excited state is formed more preferably than the ground state.

The ECL efficiency (\( \phi_{el} \)) defined as ratio between photons emitted and electrons transferred between reductant and oxidant species, depends on the yield of the (emitting) excited state generation (\( \phi_{es} \)) and emission quantum yield (\( \phi_{em} \)) of the emitting species (R*), Eq.1.20

\[ \phi_{el} = \phi_{es} \times \phi_{em} \]  

(1.20)

Experimentally, \( \phi_{el} \) can be evaluated from the total ECL intensity, \( I \) (Einstein / s), integrated over a finite period of time, divided by the integrated cathodic or anodic current over the same time period that is equal to the total cathodic (\( Q_c \)) or anodic (\( Q_a \)) charge [Coulomb], respectively. (Eq.1.21)

\[ \phi_{el} = \frac{\int I \, dt}{\int i_{c,a} \, dt} = \frac{\int I \, dt}{Q_{c,a}} \]  

(1.21)

1.8.2 Ion-annihilation mechanism in Ru(II) polypyridyl complexes.

Since the discovery in 1972 \(^{88}\) of the ECL of \([\text{Ru(bpy)}_3]^{2+}\) several studies were performed on the ECL of transition metal complexes, \(^{89-92}\) in particular ruthenium compounds. \(^{85,86,89,93-96}\) The high interest in the ECL reactions of \([\text{Ru(bpy)}_3]^{2+}\) and its derivatives is due to their higher \( \phi_{el} \) compared to organic molecules. \(^{9,97}\) Furthermore, ruthenium polypyridine complexes can be reversibly oxidized and reduced, and their redox forms are stable enough to enable bimolecular reactions. Due to the reversibility of their redox reactions and stability of their redox product, these compounds can also undergo several ECL cycles.

Generation of the oxidized \([\text{Ru(bpy)}_3]^{3+}\) and reduced \([\text{Ru(bpy)}_3]^{1+}\), by stepping the electrode potential at the oxidation and first reduction potentials of \([\text{Ru(bpy)}_3]^{2+}\), gives rise to ECL (Eq. 1.22) with luminescence spectrum closely resembling the \(^3\text{MLCT}\) emission observed for \([\text{Ru(bpy)}_3]^{2+}\) by photoexcitation. \(^{88}\)
\[
[Ru(bpy)_3]^{3+} + [Ru(bpy)_3]^+ \rightarrow [Ru(bpy)_3]^{2+} + [Ru(bpy)_3]^{2+*}
\]

(1.22)

The free energy \( \Delta G^0 \) for the ground state formation is 2.6 eV\(^{88} \) while \( E^{00} \) and \( E^{00} \) are 2.73 and 2.12 eV,\(^{72} \) respectively. Therefore, direct formation of the emitting \(^{1}\)MLCT is energetically allowed and, given the experimental error, even the higher \(^{3}\)MLCT can be populated, while ground state formation is not observed. The resulting \( \phi_{\text{rel}} \) is 0.05 (in acetonitrile at 25 °C).\(^{98} \) This value is generally used as ECL standard efficiency for the ECL reaction via ion-annihilation of transition metal complexes. Since \( \phi_{\text{em}} \) of \([Ru(bpy)_3]^{2+}\) is 0.062 in deaerated acetonitrile, \(^{99} \phi_{\text{es}} (= \phi_{\text{cl}} / \phi_{\text{em}}) \) is very high compared to the \( \phi_{\text{es}} \) values of organic compounds (< 50%).\(^{87} \) The high \( \phi_{\text{es}} \) in \([Ru(bpy)_3]^{2+}\) is due to the possibility to form the emitting excited state \(^{3}\)MLCT directly by electron transfer, while for several organic systems where the emitting (singlet) excited state is populated by triplet-triplet annihilation mechanism (Eq.1.19). Moreover, intersystem crossing from \(^{1}\)MLCT state to the emitting \(^{3}\)MLCT level occurs in \([Ru(bpy)_3]^{2+}\) with unitary efficiency (see Section 1.5), thereby further increasing \(^{3}\)MLCT population.

1.8.3 ECL of Ru(II) polypyridyl complexes with tri-n-propylamine

In diagnostics applications, the ECL reaction is generally performed in aqueous solutions. Due to the reduction of water observed at negative potentials, the ECL reaction of \([Ru(bpy)_3]^{2+}\) must be performed by a mechanism that does not require reduction of the ruthenium complex to \([Ru(bpy)_3]^+\) (occurring at -1.28 V vs NHE).\(^{46} \) Among the several species (such as oxalate,\(^{100} \) amines,\(^{101,102} \) amino acids\(^{7,11} \)) able to undergo ECL reaction with \([Ru(bpy)_3]^{2+}\), the most frequently used in diagnostics is tri-n-propylamine (TPrA),\(^{102} \) due to its highest ECL efficiency, \( \phi_{\text{cl}} \) (Figure 1.15).\(^{7,11} \)

The ECL reaction for \([Ru(bpy)_3]^{2+}\) and TPrA system occurs by oxidative-reduction mechanism (Eqs. 1.23–1.27 and Figure 1.16).\(^{102} \)

\[
[Ru(bpy)_3]^{2+} \rightarrow [Ru(bpy)_3]^{3+} + e^- 
\]

(1.23)

\[
\text{TPrA} \rightarrow \text{Pr}_2\text{N}^+\text{CH}_2\text{Et} + e^- 
\]

(1.24)

\[
\text{Pr}_2\text{N}^+\text{CH}_2\text{Et} \rightarrow \text{Pr}_2\text{NC}^+\text{HET} + \text{H}^+ 
\]

(1.25)

\[
[Ru(bpy)_3]^{3+} + \text{Pr}_2\text{NC}^+\text{HET} \rightarrow [Ru(bpy)_3]^{2+} + \text{Pr}_2\text{N}^+ = \text{CHEt} 
\]

(1.26)

\[
[*(Ru(bpy)_3)]^{2+} \rightarrow [Ru(bpy)_3]^{2+} + h\nu 
\]

(1.27)
Upon application of a potential at the anode, \([\text{Ru(bpy)}_3]^{2+}\) is oxidized to \([\text{Ru(bpy)}_3]^{3+}\) (Eq. 1.23) and, simultaneously, TPrA is oxidized to a radical cation \(\text{Pr}_2\text{N}^{\ast+}\text{CH}_2\text{Et}\) (TPrA\(^{\ast+}\)) (Eq. 1.24). \(\text{Pr}_2\text{N}^{\ast+}\text{CH}_2\text{Et}\) is very reactive and converts to the reductant active in the ECL reaction. The exact nature of this reductant is not yet clear. Leland and Powell\(^{102}\) suggested that by loss of an \(\alpha\)-proton \(\text{Pr}_2\text{N}^{\ast+}\text{CH}_2\text{Et}\) forms the radical species \(\text{Pr}_2\text{NC}^{\ast}\text{CH}_2\text{Et}\) (TPrA\(^{\ast+}\), Eq.1.25) that reduces \([\text{Ru(bpy)}_3]^{3+}\) to the excited \([\text{Ru(bpy)}_3]^{2+\ast}\) and forms \(\text{Pr}_2\text{N}^{\text{+}}\text{=CH}_2\text{Et}\) (Eq.1.26). The excited \([\text{Ru(bpy)}_3]^{2+\ast}\) decays by light emission and regenerates the ground state complex (Eq. 1.27). \([\text{Ru(bpy)}_3]^{2+}\) can therefore undergo multiple reactions, while TPrA is irreversibly consumed.

**Figure 1.15** Scheme of the ECL reaction using \([\text{Ru(bpy)}_3]^{2+}\) and TPA, as sacrificial reductant.

The oxidation potential of \(\text{Pr}_2\text{NC}^{\ast}\text{CH}_2\text{Et}\) (1.28) is \(E_{\text{ox}}^0 \sim +0.90\) V (vs NHE)\(^{103,104}\), therefore the energy (\(\Delta G^0\)) possessed by \(\text{Pr}_2\text{NC}^{\ast}\text{CH}_2\text{Et}\) and \([\text{Ru(bpy)}_3]^{3+}\) is sufficient to produce the \(^3\text{MLCT}\) state of the ruthenium complex.

\[
\text{Pr}_2\text{NC}^{\ast}\text{CH}_2\text{Et} \rightarrow \text{Pr}_2\text{N}^{\text{+}}\text{=CH}_2\text{Et} + \text{e}^- \tag{1.28}
\]
The mechanism represented by Eqs 1.23-1.27 is simplified, the ruthenium complex and the TPrA undergoing several side-reactions. Furthermore, due to its high reactivity, Pr$_2$NCH$_2$Et tends to deactivate very easily, therefore a large excess of TPrA must be used. The concentration of TPrA in immunoassays therefore usually exceeds 7 orders of magnitude that of the ruthenium complex!

Due to the complexity of the mechanism of the reaction between TPrA and [Ru(bpy)$_3$]$^{2+}$, the determination of the ECL absolute efficiency ($\Phi_{\text{cell}}$) from the experimental parameters (anodic current and ECL intensity signal) is prevented. Furthermore, the control of the several factors influencing the reaction is rather difficult. The pH of the buffer solution determines the rate for the radical Pr$_2$N$^+$CH$_2$Et formation by loss of a proton from the radical cation Pr$_2$N$^+$CH$_2$Et, thus higher ECL intensities are observed in slightly basic conditions (pH=8-9). The electrode surface also strongly affects the ECL reaction. Hydrophobic electrodes, such as platinum and gold, increase the anodic current and ECL intensity as compared to carbon glassy electrode. This is probably due to a more facile approach of the active (hydrophobic) molecules TPrA and [Ru(bpy)$_3$]$^{2+}$ to their surface. On the other hand, platinum and gold electrodes undergo passivation in aqueous solutions, due to platinum and gold oxides formation, largely reducing the anodic current and ECL intensity. Another important factor, that strongly influences the ECL performance, in particular in diagnostics applications, is the presence of surfactant in the buffer solutions. Surfactants are usually used to improve liquid flow through the tubes of the automatized instruments, avoid formation of bubbles and improve removal of the analytes from the ECL cell after each measurement. It was recently proved that, in the presence of surfactant, the ECL intensity arising from the reaction between [Ru(bpy)$_3$]$^{2+}$ and TPrA largely increases (up to five-fold the intensity recorded in the absence of surfactant). This influence cannot only be due to the effects outlined above. The role played by the detergent is not yet completely clear. Beside the increased solubility of the reacting species in aqueous solutions, it probably facilitates the generation of the reacting species at the electrode upon formation of an hydrophobic layer on its surface.

Many aspects of the ECL reaction between metal complexes and TPrA are still not known, therefore several studies aimed at a deeper understanding of the process are still needed. A description of the principle of the immunoassays based on ECL detection can be found in Chapter 2.
1.9 Scope of this Thesis.

The aim of the studies described in this Thesis was the design and investigation of luminescent metal complexes for diagnostics applications.

Population of the excited state can be achieved by chemical reaction between two electrogenerated species of sufficient energy (electrochemiluminescence, ECL) or by photoexcitation. As already outlined in Section 1.1, ECL assays have the advantage of having higher sensitivity due to the possibility to avoid excitation light.

Some effort has been devoted to the ECL investigation of mononuclear ruthenium complexes with substituted bipyridine ligands (Chapter 3). Due to the strong interest in ECL assays with high sensitivity, systems containing several ECL active centers can be a powerful tool to increase the signal intensity. Multinuclear ruthenium complexes were synthesized and their ECL behavior investigated (Chapter 4).

In the search for luminescent probes with long-lived excited state, ruthenium-dendrimers with the metal complex in the core were prepared and investigated (Chapter 5). In order to elongate the excited state lifetime, a possible approach is indeed the protection of the luminescent core from dioxygen or other species able to quench the emitting excited state.

Another interesting possibility is the design of metal complexes with a large aromatic (appended or chelating) ligand possessing long-lived triplet excited state of suitable energy. In particular, a good matching between the energy of $^3\text{MLCT}$ (triplet metal-to-ligand charge-transfer) state of the metal centre, that is often the lowest excited state in ruthenium and osmium polypyridyl complexes, and the $^3\text{IL}$ (triplet intraligand) state of the large aromatic ligand could lead to an equilibrium between the energetically close states, resulting in longer emission decay time. The spectroscopic properties of three dinuclear metal complexes with a bridging ligand possessing a long-lived $^3\text{IL}$ state at low energy were investigated (Chapter 6 and 7).
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