Deactivation of the ruthenium excited state by enhanced homogeneous charge transport: Implications for electrochemiluminescent thin film sensors

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ABSTRACT

The electrochemiluminescent (ECL) performance of three ruthenium-based metallopolymer platforms with different homogeneous charge transfer diffusion coefficients (D_{CT}) is reported. Significantly, simultaneous detection of light and current in tandem with steady-state photoluminescence studies demonstrate that *increasing* the rate of Ru^{3+} production via enhanced charge transport results in a *decrease* in ECL intensity of up to 82% when the concentration of the co-reactant, sodium oxalate, is low, i.e., sub-mM. Spectroelectrochemical studies demonstrate that for maximum sensitivity to be obtained, the electroactive properties of the polymeric support matrix need to be considered in tandem with luminophore, analyte and co-reactant concentrations.

1. Introduction

Electrochemiluminescence (ECL) has emerged as a powerful technique for ultrasensitive detection of a broad range of analytes [1–11]. Advances in nanostructured materials such as quantum and carbon nano-dots have played a significant role in establishing ECL as a robust and efficient tool for the detection of both chemical and biological species. However, $[Ru(bpy)_3]^{2+}$ or closely related analogues continue to dominate the field [12,13]. This is primarily due to the high sensitivity, relatively high ECL efficiency and wide dynamic range afforded by transition metal based luminophores [1,4,14,15]. For bio-sensing and diagnostic applications, current research strategies for ruthenium-based platforms focus on optimising the ECL efficiency, i.e., photons emitted per electron passed [16,17]. Important approaches include new co-reactants and altering the electrochemical characteristics of the material itself so as to increase the charge transfer rate to produce more luminophores per unit time. To this end, attempts have been made to improve efficiency by immobilising the ruthenium species within or by tethering to an electroactive polymeric backbone capable of mediating charge transfer between metal centres [18]. Although this approach has exhibited significantly enhanced rates of charge transport in the ground state giving more intense ECL [16,19] a decrease in ECL efficiency and ECL signal is often observed where analyte/co-reactant concentrations are low [16].

In this contribution, we report on the impact of changing the rate of Ru^{3+} generation by changing the structure of the metallopolymer and

its subsequent effect on ECL emission where the co-reactant concentration is low, i.e., sub-millimolar. Steady-state quenching studies provide a unique insight into the limitations of this approach with respect to enhancing ruthenium-based ECL emission. The impact of these findings on the design of future ruthenium-based nanostructured ECL platforms for ultrasensitive detection is highlighted.

2. Materials and methods

The metallopolymers, $[Ru(bpy)_2(PPyBBIM)_{10}]^{2+}$ and $[Ru(bpy)_2(PVP)_{10}]^{2+}$, were prepared as described previously, where bpy is 2,2′-bipyridyl, PPyBBIM is poly[2-(2-pyridyl)-bibenzimidazole], and PVP is poly-4-vinylpyridine [20,21]. Glassy carbon macro working electrodes (3 mm) were modified by applying 15 μL of an ethanolic solution of the metallopolymer or a $[Ru(bpy)_3]^{2+}$/Nafion composite to the electrode surface. The surface coverage was determined by graphical integration of background corrected cyclic voltammograms (scan rate, $\nu < 5\,mV\,s^{-1}$). In all cases the surface coverage ranged from 1.4×10^{-8} to $2.1 \times 10^{-8}\,mol\,cm^{-2}$. Potentials were measured versus a standard Ag/AgCl aqueous reference electrode (3 M KCl). Measurements involving simultaneous detection of light and current utilized a CH instrument model 760B connected to an Oriel 70,680 photomultiplier tube (PMT). An Oriel model IS520 gated intensified CCD coupled to an Oriel model MS125 spectrograph was used to acquire ECL spectra. ECL experiments were carried out using sodium oxalate as co-reactant (pH 6).

Absorbance and photoluminescence were recorded using a Shimadzu UV-240 spectrophotometer and a Perkin-Elmer LS-50 luminescence spectrometer. Spectroelectrochemical experiments were performed in a quartz thin-layer spectroelectrochemical cell (2 mm path length) using a CH Instruments model 660 potentiostat. An ITO modified electrode was used as the working electrode with platinum (Pt) and Ag/AgCl electrodes acting as counter and reference electrodes. Ru^{3+} concentrations at potentials ranging from 0.1–1.0 V vs. Ag/AgCl were determined from the Nernst equation [22]. All photoluminescence spectra were corrected for any differences in ruthenium concentration using the electrochemically measured surface coverage to ensure accurate comparisons of relative signal intensity.

3. Results and discussion

A key issue for any bio-sensing or diagnostic platform is not the overall intensity of signal produced but rather the effective signal-to-noise ratio. Given the inherently low background noise levels associated with ECL detection, the enhancement of ECL intensity or brightness is a clear strategy for improving sensitivity and lowering detection limits. When the co-reactant is freely available, the ECL intensity at a modified electrode is likely to be limited by the rate of charge transfer characterised by the homogeneous charge transfer diffusion coefficient (D_{CT}) [3,16,23]. In the case of ruthenium-based ECL, oxidation of the metal moiety to the 3 + oxidation state at circa 1.0 V followed by reaction with an ECL co-reactant is the precursor to light generation from the Ru^{2+*} excited state luminophore. As the ECL co-reactant is typically present in excess one would expect that increased concentrations of ruthenium metal in the 3 + oxidation state will lead to increased ECL emission. As the amount of ruthenium within a modified layer is fixed, the rate of 3 + regeneration is important since an enhanced rate could lead to brighter ECL, heightened sensitivity and therefore lower limits of detection (LOD).

Incorporation of ruthenium into a conducting polymer matrix or covalent tethering to a conducting polymer backbone have previously demonstrated enhanced rates of charge transfer in metallopolymer systems. Table 1 documents the homogenous charge transfer diffusion coefficient (D_{CT}) for $[Ru(bpy)_2(PVP)_{10}]^{2+}$, $[Ru(bpy)_2(PPyBBIM)_{10}]^{2+}$ and the ruthenium/Nafion composite film. These values were determined from fast scan (100–500 mV s^{-1}) where the response is electrochemically reversible and not influenced by the rate of heterogeneous electron transfer or ohmic drop in conjunction with the Randles-Sevcik equation:

$$i_p = 2.65 \times 10^5 n^{3/2} A D_{CT} v^{1/2} C \tag{1}$$

The D_{CT} for the highly conducting $[Ru(bpy)_2(PPyBBIM)_{10}]^{2+}$ is an order of magnitude larger than the PVP-based $[Ru(bpy)_2(PVP)_{10}]^{2+}$ metallopolymer and the $[Ru(bpy)_3]^{2+}$/Nafion composite. This is consistent with the conducting polymer backbone mediating electron transfer between the metal centres, thereby augmenting the intrinsic electron self-exchange rate [21]. Previous studies using $[Ru(bpy)_2(PPyBBIM)_x]$ metallopolymers have shown that charge compensating ions do not represent the rate-determining step for homogenous

charge transport, and that electron transfer, mediated by the conjugated polymer backbone, limits the overall rate of charge transport [17]. In such cases a reduction in ruthenium oxidation/reduction potentials has also been observed. Fig. 1(a) shows the average comparative oxidative ECL response for $[Ru(bpy)_2(PPyBBIM)_{10}]^{2+}$, $[Ru(bpy)_2(PVP)_{10}]^{2+}$ and $[Ru(bpy)_3]^{2+}$/Nafion films where the ECL emission for each has been normalised to take account of minor differences in the ruthenium surface coverage. At a co-reactant/analyte concentration of 0.5 mM, a decrease in ECL intensity of circa 32% is observed for the highly conducting $[Ru(bpy)_2(PPyBBIM)_{10}]^{2+}$ metallopolymer when compared to the $[Ru(bpy)_2(PVP)_{10}]^{2+}$ metallopolymer, despite the significantly enhanced D_{CT} rates observed for the former. Further analysis shows that when the co-reactant concentration is reduced to 0.2 mM, the observed ECL signal intensity for the $[Ru(bpy)_2(PPyBBIM)_{10}]^{2+}$ metallopolymer is circa 82% lower than the $[Ru(bpy)_2(PVP)_{10}]^{2+}$ metallopolymer, despite containing the equivalent amount of ruthenium. At co-reactant/analyte concentrations of 0.1 mM no discernible ECL signal was observed for the $[Ru(bpy)_2(PPyBBIM)_{10}]^{2+}$ metallopolymer (Fig. 1(a) inset). ECL studies utilising sub mM concentrations of TPA co-reactant also revealed significantly reduced ECL emission for the $[Ru(bpy)_2(PPyBBIM)_{10}]^{2+}$ metallopolymer system with no visible ECL signal being observed at 0.1 mM TPA.

Table 1 displays the calculated ECL efficiency for each polymer matrix at a co-reactant (oxalate) concentration of 0.2 mM. The overall ECL efficiency (ϕ_{ECL}) is defined as the number of photons emitted per faradaic electron passed during the chemiluminescent reaction [24]. It is the product of the efficiency of populating the excited state (ϕ_{EX}) and the quantum yield of emission from that excited state (ϕ_P). Solution phase $[Ru(bpy)_3]^{2+}$ was used as a relative standard for all experiments. The relative efficiency of each material was obtained using the equation:

$$\phi_{ECL} = \phi^0_{ECL} (IQ^0_f / Q_f I^0) \tag{2}$$

where ϕ^0_{ECL} is the ECL efficiency of $Ru(bpy)_3^{2+}$ via annihilation, taken as 5.0% [25,26] I and I^0 are the integrated photomultiplier tube responses for the polymer and $[Ru(bpy)_3]^{2+}$ respectively, and Q_f and Q^0_f are the faradaic charges passed for the sample and standard. It is important to note that as $[Ru(bpy)_3]^{2+}$ itself cannot be immobilised directly as a thin film, the ECL efficiencies of the films are compared to 100 μM $[Ru(bpy)_3]^{2+}$ in solution. At a co-reactant concentration of 0.2 mM the highest ECL efficiency is obtained from the $[Ru(bpy)_2(PVP)_{10}]^{2+}$ film closely followed by the Nafion-$[Ru(bpy)_3]^{2+}$. Previous work has highlighted the capability of the negatively charged Nafion polymer to quench ruthenium-based ECL emission [27]. Quenching however has only been reported for ultra-thin $Ru(bpy)_3^{2+}$ loaded Nafion Langmuir–Blodgett films up to 0.5 μM thick and is considered negligible in this case.

The ECL emission intensity for the conjugated $[Ru(bpy)_2(PPyBBIM)_{10}]^{2+}$ metallopolymer was almost an order of magnitude lower than that obtained for the $[Ru(bpy)_2(PVP)_{10}]^{2+}$ metallopolymer despite displaying faster rates of charge transfer. The dependence of the ECL intensities on the supporting polymer structure observed here has significant consequences for assays where the target itself is a co-reactant, e.g., guanine or certain amino acids, or where a secondary antibody is labelled with a co-reactant.

It has previously been shown that Ru^{3+} acts as an efficient electron acceptor and quenches Ru^{2+*} emission, particularly when confined within a film structure [21]. However, the impact of immobilising the luminophore within an electronically conducting matrix on this process has not been extensively investigated, e.g., the acceptor states of a conducting polymer could enhance quenching [17]. To investigate the importance of this effect for these metallopolymers, spectroelectrochemical investigations were performed. The spectroelectrochemical analysis focussed on the influence of Ru^{3+}

Table 1
Homogeneous charge transfer diffusion coefficients (D_{CT}) and quenching rate data.

Polymer	D_{CT} (cm^2 s^{-1})	ϕ_{ECL}	Quenching sphere radii (nm)
$[Ru(bpy)_2(PVP)_{10}]^{2+}$	$9.2 \pm 1.1 \times 10^{-11}$	0.178	12
Nafion-$[Ru(bpy)_3]^{2+}$	$2.3 \pm 0.8 \times 10^{-11}$	0.146	11
$[Ru(bpy)_2(PPyBBIM)_{10}]^{2+}$	$8.2 \pm 1.2 \times 10^{-10}$	0.033	15
$[Ru(bpy)_3]^{2+}$ solution phase	$8.6 \pm 1.1 \times 10^{-5}$	5.0	N/A[a]

All values based on averaged results from 5 independent measurements.

[a] Applies to static quenching governed by Perrin Model, solution phase quenching adheres to Stern–Volmer quenching dynamics.