Trends in Analytical Chemistry 51 (2013) 107–116

Contents lists available at ScienceDirect

Trends in Analytical Chemistry

journal homepage: www.elsevier.com/locate/trac

Review

Electrochemiluminescence quenching of tris(2,2′-bipyridyl)ruthenium

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ARTICLE INFO

Keywords:
Capillary electrophoresis
Co-reactant
Electrochemiluminescence
First review
Flow-injection analysis
Quenching
Ruthenium
$Ru(bpy)_3^{2+}$
Separation
Tris(2,2′-bipyridyl)ruthenium

ABSTRACT

Electrochemiluminescence (ECL) of tris(2,2′-bipyridyl)ruthenium [$Ru(bpy)_3^{2+}$] has received considerable attention over broad applications due to its remarkably high sensitivity and extremely wide dynamic range.

This review presents the state of ECL quenching of $Ru(bpy)_3^{2+}$. After a brief introduction of the ECL of $Ru(bpy)_3^{2+}$, we discuss in detail ECL-quenching co-reactants, the ECL-quenching mechanism and applications of ECL quenching coupled with capillary electrophoresis and flow-injection analysis.

Contents

1. Introduction

Electrochemiluminescence [1,2] (ECL) is a means to emit measurable luminescent signals by converting electrochemical energy into radiative energy via an electrochemical reaction. Luminescent signals can be obtained from the excited states of an ECL luminophore generated at electrode surfaces during the electrochemical reaction. Since the initial discovery of ECL emission reported by Hercules and Bard et al. in the mid-1960s [3–6], lots of papers, patents, and book chapters have been published on ECL, ranging from the very fundamental to the very applied, due to the remarkably high sensitivity, very low background signal and good temporal and spatial control.

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0165-9936/$ - see front matter © 2013 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.trac.2013.06.012

There are many ECL luminophores, such as tris(2,2′-bipyridyl) ruthenium(II) ($Ru(bpy)_3^{2+}$), luminol, lucigenin, acridinium esters, 9,10-diphenylanthracene (DPA), peroxyoxalate (PO), semiconductor nanocrystals, semiconductor nanoparticles, and metal nanoclusters. Among these, ECL luminophore, $Ru(bpy)_3^{2+}$, has received considerable attention because $Ru(bpy)_3^{2+}$ has several advantages in different analytical areas, such as strong luminescence and good solubility in a variety of aqueous and non-aqueous solvents, inherent sensitivity, good ECL efficiency, wide linear range. Also $Ru(bpy)_3^{2+}$ can be regenerated during the ECL reaction.

Since $Ru(bpy)_3^{2+}$-based ECL was first elucidated in the 1970s [7], solution-phase ECL has been extensively studied and there have also been some excellent reviews and monographs [1,2,8–23]. However, ECL itself can hardly be used for multi-analyte detection due to its poor selectivity. This drawback can be overcome by coupling with separation techniques, such as capillary electrophoresis (CE) and flow-injection analysis (FIA).

On the whole, two types of ECL are covered: enhanced and quenched. More attention has been paid to enhanced ECL [24]. Quenched $Ru(bpy)_3^{2+}$ ECL is also an active area of research. It is well known that some substances are weak co-reactants in enhanced ECL but they can strongly quench ECL. Based on this, quenched ECL can be more sensitive than enhanced ECL in determining these substances. In recent years, a growing number of researchers did a lot of research in ECL quenching and proposed several quenching mechanisms. However, no review has focused on quenched ECL, so we present the state of ECL quenching of $Ru(bpy)_3^{2+}$. This review mainly focuses on ECL-quenching co-reactants, the ECL-quenching mechanism and application of ECL quenching coupled with CE and FIA since 1999.

2. ECL quenching co-reactants

In quenched ECL, to obtain good sensitivity, strong co-reactants are usually needed to enhance the background of $Ru(bpy)_3^{2+}$ ECL. Co-reactants are species that, upon electrochemical oxidation or reduction, produce intermediates that react with other compounds to produce excited states capable of emitting light [25,26]. Oxalate, amino acids, tri-n-propylamine and NADH are typical "oxidative-reductive" co-reactants [2,22]. $S_2O_8^{2-}$ and H_2O_2 are typical "reductive-oxidative" co-reactants. Co-reactants of $Ru(bpy)_3^{2+}$ were reviewed in detail by Yuan, et al. [8]. According to the previous literature, oxalate ($C_2O_4^{2-}$), TPA(tri-*n*-propylamine) and DBAE [2-(dibutylamino)ethanol] are the dominant strong co-reactants in quenched ECL.

2.1. ($C_2O_4^{2-}$)

In 1977, Bard's group [26] studied the electrochemical oxidation of oxalate at a platinum electrode in acetonitrile solutions. This was the first account of a co-reactant ECL system reported in the literature. In 1981, Bard and co-workers [25] first reported the determination of $Ru(bpy)_3^{2+}$ ECL using oxalate as co-reactant at a platinum electrode in aqueous solution. In 2002, adrenaline was found to inhibit strongly the ECL of the $Ru(bpy)_3^{2+}$/TPA and $Ru(bpy)_3^{2+}/(C_2O_4^{2-})$ systems [27]. The linear range of detection of adrenaline for $Ru(bpy)_3^{2+}$/TPA and $Ru(bpy)_3^{2+}/C_2O_4^{2-}$ was from 2×10^{-8}–1×10^{-4} M and 5×10^{-7}–1×10^{-5} M respectively, which demonstrated that $Ru(bpy)_3^{2+}$/TPA was obviously superior to $Ru(bpy)_3^{2+}/C_2O_4^{2-}$. Later, Cui et al. [28] developed a ECL-quenching method for the detection of 30 phenols and anilines, and the limits of detection (LODs) for these compounds were found to be 10^{-8}–10^{-9} M for $Ru(bpy)_3^{2+}$/TPA system and 10^{-6}–10^{-7} M for $Ru(bpy)_3^{2+}/C_2O_4^{2-}$ system, respectively. In comparison with $Ru(bpy)_3^{2+}$/TPA system, the inhibitive signals of the compounds in $Ru(bpy)_3^{2+}/C_2O_4^{2-}$ were obviously weaker than those in $Ru(bpy)_3^{2+}$/TPA.

The $Ru(bpy)_3^{2+}/C_2O_4^{2-}$ system is a typical example for the ECL of $Ru(bpy)_3^{2+}$ in the presence of a reductant. The proposed mechanism was summarized by Bard et al. [25,26,29–31]. First, $Ru(bpy)_3^{2+}$ is oxidized at a platinum or carbon electrode:

$$Ru(bpy)_3^{2+} - e \rightarrow Ru(bpy_3^{3+})$$

The following reactions then occur in the diffusion layer near the electrode surface:

$$C_2O_4^{\cdot-} - e \rightarrow C_2O_4^{\cdot-}$$

$$Ru(bpy)_3^{3+} + C_2O_4^{2-} \rightarrow Ru(bpy)_3^{2+} + C_2O_4^{\cdot-}$$

$$C_2O_4^{\cdot-} \rightarrow CO_2 + CO_2^{\cdot-}$$

The intermediate radical anion, $CO_2^{\cdot-}$, is a strong reducing agent and produces the excited state, $Ru(bpy)_3^{2+*}$, in an electron-transfer reaction with the $Ru(bpy)_3^{3+}$:

$$Ru(bpy)_3^{3+} + CO_2^{\cdot-} \rightarrow Ru(bpy)_3^{2+*} + CO_2$$

Alternatively, $Ru(bpy)_3^{2+*}$ may be produced by the following sequence:

$$CO_2^{\cdot-} + Ru(bpy)_3^{2+} \rightarrow CO_2 + Ru(bpy)_3^{+}$$

$$Ru(bpy)_3^{+} + Ru(bpy)_3^{3+} \rightarrow Ru(bpy)_3^{2+} + Ru(bpy)_3^{2+*}$$

$$Ru(bpy)_2^{2+*} \rightarrow Ru(bpy)_3^{2+} + h\nu$$

2.2. TPA

The ECL of $Ru(bpy)_3^{2+}$ with aliphatic amines has been studied for more than two decades [32–37]. It was reported that, for the aliphatic amines, the LODs can be ordered tertiary < secondary < primary. For example, the LODs of tripropylamine, dipropylamine and propylamine are 0.28 pM, 24 pM and 370 pM, respectively [32]. Due to its high ECL efficiency, TPA is usually adopted in analytical ECL applications.

In 1990, Leland and Powell first reported the ECL of $Ru(bpy)_3^{2+}$ with TPA as a co-reactant [33]. In 1999, McCall et al. [38,39] first reported that phenols, hydroquinones, catechols and benzoquinones could quench $Ru(bpy)_3^{2+}$/TPA ECL. They found that the inhibition of ECL happened only when ratios of inhibitor(quencher) to luminophore $Ru(bpy)_3^{2+}$ were higher than 100. Later, Richter et al. found that, when the phenol quenchers were covalently bound to an oligonucleotide, only about 50% of the ECL intensity was quenched. This lacked the quenching efficiency required for quantifying DNA through hybridization [40]. To overcome this drawback, Cao et al. [41] reported that $Ru(bpy)_3^{2+}$/TPA could be quenched by ferrocene and proposed a potential application to quantitative DNA detection. When the ferrocene was covalently bound to an oligonucleotide, the quenching efficiency was virtually complete, indicating the potential application of this method for application to sequence specific DNA detection. Spehar et al. [42] also reported that, upon hybridization, the 3′ end of an oligonucleotide was labeled with $Ru(bpy)_3^{2+}$ and the 5′ end of a complementary strand with Cy5. The ECL signal of the $Ru(bpy)_3^{2+}$-labeled oligonucleotide was strongly quenched by the Cy5-labeled complementary strand, and the quenching efficiency was calculated to be 78%.

Cui's group observed the inhibition of $Ru(bpy)_3^{2+}$/TPA by adrenaline [27], noradrenaline and dopamine [43], gallic acid [44], phenols and anilines [28]. And it was reported that inhibited ECL began to appear when the ratios were 1×10^{-4}–1×10^{-3} (estimated from