



Review

Analytical applications of the electrochemiluminescence of tris(2,2'-bipyridyl)ruthenium(II) coupled to capillary/microchip electrophoresis: A review

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ABSTRACT

A comprehensive review on the development of analytical methods, by coupling electrochemiluminescence (ECL) detection with capillary electrophoresis (CE) and microchip electrophoresis (ME), is presented. After the description of the basic mechanism of ECL, the addition mode of luminescence reagent in CE–ECL system has been discussed. The analytical applications of the CE–ECL technique in terms of different analytes are also given. Due to the importance of ME as a separation method for the present and future, the ME detection methods based on ECL are considered in a relatively detailed way. Finally, possible trends for CE/ME–ECL in the near future are discussed.

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Ming Su received the M.S. degrees in analytical chemistry from He Bei University, He Bei province, China, in 2008. His current research interest focused on the development of new methods for bioanalysis and drug analysis, especially on the coupling of capillary electrophoresis with electrochemiluminescence for simultaneous detection of multi-analytes.



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Abbreviations: ECL, electrochemiluminescence; CE, capillary electrophoresis; ME, microchip electrophoresis; DPA, 9,10-diphenylanthracene; PAHs, polycyclic aromatic hydrocarbons; NPs, nanoparticles; $\text{Ru}(\text{bpy})_3^{2+}$, tris(2,2'-bipyridyl)ruthenium(II); FIA, flow-injection analysis; HPLC, high performance liquid chromatography; (TAS, micro total analysis system; TPA, tripropylamine; LOD, limit of detection; PMT, photomultiplier tube; ITO, indium-tin oxide; DC, direct current; pHPP, p-hydroxyphenylpyruvic acid; RSDs, relative standard deviations; DMEA, N,N-dimethyl ethanolamine; HSA, human serum albumin; EOF, electro-osmotic flow; AEMP, 2-(2-aminoethyl)-1-methylpyrrolidine; ILs, ionic liquids; EMIMBF₄, 1-ethyl-3-methylimidazolium tetrafluoroborate; PDMS, poly(dimethylsiloxane); MEKC, micellar electrokinetic chromatography; BV, benzyl viologen.

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Songqin Liu received the Ph.D. degree from the University of Nanjing in 2003. He has been a professor in the Department of Chemistry since 2005. His major areas of research include the fabrication and development of biosensors for the sensitively recognition of glycoprotein molecules in blood. To establish the new methods and novel techniques for the detecting of glycoproteins, reveal the relationship between these glycoproteins or the level of these glycoprotein express and the illness or the curative effects, and develop novel clinical diagnosis approach with high selectively and sensitivity.

1. Introduction

Electrogenerated chemiluminescence (also called electrochemiluminescence and abbreviated ECL) is the process where species generated at electrode undergo high-energy electron-transfer reactions to form excited states and emit light [1]. Since the ECL was first observed by Bard on 9,10-diphenylanthracene (DPA) and related compounds [2,3], a number of ECL luminophores were synthesized and their ECL properties were investigated. These ECL luminophores could be classified into three categories: (a) inorganic matter, which mainly contain organometallic complexes and clusters [4–7]; (b) organic substances, such as polycyclic aromatic hydrocarbons (PAHs) [8], polymers [9,10], vitamin [11], and acridinium esters [12]; (c) semiconductor nanoparticles (NPs, also known as nanocrystals, quantum dots) [13,14]. Among these luminophores, the compound tris(2,2'-bipyridyl)ruthenium(II) (abbreviated as $\text{Ru}(\text{bpy})_3^{2+}$) and its derivatives have received considerable attention, profiting from their high emission quantum yield ($\sim 4.2\%$ for $\Phi_{\text{em}}(\text{H}_2\text{O})$) [15,16], high chemical stability in aqueous and non-aqueous solvents, long excited-state lifetime (~ 600 ns), very low background signal, good ECL efficiency [17–20]. Since the first report of ECL of $\text{Ru}(\text{bpy})_3^{2+}$ by Tokel and Bard [21], the overall number of publications has increased exponentially over the past years. Nowadays, the $\text{Ru}(\text{bpy})_3^{2+}$ ECL technique has become a very powerful analytical tool and has been widely used in the areas of immunoassay [22,23], clinic diagnosis [24,25], food and water testing [26,27], biowarfare agent monitoring [28,29], as well as DNA hybridization detection [30,31].

Different separation techniques such as capillary electrophoresis (CE), microchip electrophoresis (ME), and high performance liquid chromatography (HPLC) have been coupled with $\text{Ru}(\text{bpy})_3^{2+}$ ECL for simultaneous detection of multiple analytes [32–34]. The “nanoscale” CE and ME techniques have been rapidly developed and widely used in many fields, such as food security [35–37], environmental monitoring [38–40], clinic diagnosis [41,42], drug screening [43,44] and forensic investigations [45,46], because of their high separation speed and efficiency, small injection volume, only μL or nL level, and low running cost [47–50]. During the past several years, $\text{Ru}(\text{bpy})_3^{2+}$ ECL detection coupled with CE and ME separation has been demonstrated as an attractive system for analysis of complex samples. Compared to laser-induced fluorescence or mass spectrum detection that is commonly employed on CE and ME, ECL detection offers comparable sensitivity, high flexibility and relatively low cost [51–56]. The aim of the present review is to give a critical overview of ECL detection coupled with CE and ME separation. Parts of the relative fields have also been summarized by other reviews [57–60]. Early in 2005, Wang's group presented a detailed overview about CE–ECL systems focusing on the ECL mechanism, CE mode, instrumental design, and ECL efficiency [57]. Two years later, they gave a review again to summarize the advances and key strategies in CE and ME with electrochemical and/or ECL detection during the past two years [58]. Rozhitskii and co-worker reviewed the

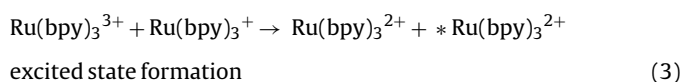
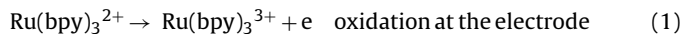
trends of analytical application of CE–ECL, and especially focused on the development of instrument for CE–ECL [59]. Very recently, Chen and co-workers presented a comprehensive survey of progress on CE–ECL during January 2005–September 2010 [60]. They summarized the mechanism involving both co-reactant and inhibitor based ECL, the possible analytes to be detected by CE–ECL, the construction of CE–ECL apparatus and the application of CE–ECL.

In our present work, the analysis system of $\text{Ru}(\text{bpy})_3^{2+}$ ECL detection coupled with CE and ME are reviewed. We mainly focus on the mechanism of $\text{Ru}(\text{bpy})_3^{2+}$ ECL, addition mode of $\text{Ru}(\text{bpy})_3^{2+}$, recent applications of CE–ECL in drug and other substrates analysis, and specifically following with interest on ME–ECL.

2. The mechanism of $\text{Ru}(\text{bpy})_3^{2+}$ ECL

2.1. Ion annihilation ECL

Generally, $\text{Ru}(\text{bpy})_3^{2+}$ ECL is initiated by two main ways: ion annihilation ECL and co-reactant ECL (including oxidative-reduction mode and reductive-oxidation mode) [61]. A mechanism for typical “ion annihilation” is outlined below [62–66]:



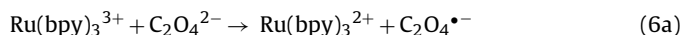
When the potential of working electrode changes quickly, $\text{Ru}(\text{bpy})_3^{2+}$ can be electrochemically oxidized to $\text{Ru}(\text{bpy})_3^{3+}$ (Eq. (1)) or reduced to $\text{Ru}(\text{bpy})_3^+$ (Eq. (2)). Then the newly formed cation $\text{Ru}(\text{bpy})_3^{3+}$ and $\text{Ru}(\text{bpy})_3^+$ annihilates near the electrode surface to form excited state species of $^*\text{Ru}(\text{bpy})_3^{2+}$ (Eq. (3)). Finally, the electrogenerated product of $^*\text{Ru}(\text{bpy})_3^{2+}$ radiates an orange emission centered on 610 nm (Eq. (4)).

2.2. Co-reactant ECL

Co-reactant ECL employs a co-reactant to produce an intermediate on the oxidation or reduction step, which react with ECL luminophore to generate excited states and emit light. In accordance to the activity of intermediate species generated from electrode, the co-reactant ECL can be defined as “oxidative-reduction” or “reductive-oxidation” mode. In oxidative-reduction mode, $\text{Ru}(\text{bpy})_3^{2+}$ ECL occurs by oxidation of $\text{Ru}(\text{bpy})_3^{2+}$ on the electrode, then excited state species of $^*\text{Ru}(\text{bpy})_3^{2+}$ is chemically produced by a strong reducing agent. The common used co-reaction agents in this ECL system are oxalate ($\text{C}_2\text{O}_4^{2-}$) and tripropylamine (TPA). If the highly oxidizing intermediate species are generated by an electrochemical reduction, the corresponding ECL reactions are defined as “reductive-oxidation” ECL [67].

2.2.1. Oxidative-reduction ECL

Oxalate ion ($\text{C}_2\text{O}_4^{2-}$) is the first co-reactant used in ECL system [68]. It produces strong reductant ($\text{CO}_2^{\bullet-}$) upon oxidation in aqueous solution. The mechanism for this co-reactant ECL system is illustrated in following Eqs. (5)–(9) [62,63,65,66,68,69].



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