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**REVIEW**

# Recent Advance in Chemiluminescence Assay and Its Biochemical Applications

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**Abstract:** Chemiluminescence (CL) assay is to measure the optical signal emitted from the CL reagents as a result of transition from the excited state back to the ground state. CL assay has significant advantages such as no external light source, high sensitivity, convenient operation, rapid analysis and easy automation, and has widely applications in clinical diagnosis, drug analysis and environmental monitoring. Recently, the introduction of nanomaterials, biochip, and microfluidic techniques promotes the development of CL assay. In this review, we summarized the recent progress in CL assay with the integration of high-performance liquid chromatography, capillary electrophoresis, quantum dots, microfluidic chips, microarrays, rolling circle amplification, isothermal exponential amplification, and two-stage isothermal amplification for the detection of DNA, small biological molecules, enzymes, proteins, metal ions, and so on. We also gave a summary of its future directions and highlight its potential applications.

**Key Words:** Chemiluminescence; Ultrasensitive detection; Bioanalysis; Review

## 1 Introduction

Chemiluminescence (CL) generates light through a chemical reaction which may induce the transition of an electron from its ground state to an excited electronic state, and the decay of the excited molecules to the electronic ground state may emit photons ranging from ultraviolet, visible to infra-red radiation[1]. Since Schmitz synthesized the CL reagent of luminol and used it to analyze blood in 1902, CL assay has become a powerful tool in biomedical research. The chemiluminescence induced by luminol-$H_2O_2$ reaction and Mn(IV) and the electro-chemiluminescence (ECL) induced by $[Ru(bipy)_3]^{2+}$ are the most common systems for CL assay.

Luminol (3-aminophthalhydrazide, also named 5-amino-2,3-dihydro-1,4-phthalazinedione) is one of the most widely used CL reagents. Albrecht firstly observed the emission of pale blue light in 1928 when adding luminol in alkaline solution (pH = 10–11)[2]. With the addition of oxidants/catalysts such as horseradish peroxidase (HRP), CL intensity could be significantly enhanced[3]. DNAzymes are defined as the DNA sequences with the catalytic function. Upon the complexation with zinc porphyrin and hemin[4–6], the G-quadruplex DNAzymes may catalyze $H_2O_2$-luminol reaction and generate an enhanced CL signal[7]. In addition, DNAzymes have some distinct characteristics including easy synthesis and the capability to minimize nonspecific adsorption. The DNAzymes were widely applied for the detection of enzymes activity[8–10], transcription factors[3], DNAs[11,12], ATP[12], and metal ions[12]. Yu and Zhu used manganese-porphyrin (Mn-PyP) to catalyze the luminol-$H_2O_2$ reaction for the detection of vascular endothelial growth factor (VEGF)[13] and simultaneous measurement of the size and mass concentration of gold nanoparticles (AuNPs)[14], respectively. Mokhtari employed copper hydroxide/copper oxide nanowires to catalyze the luminal-$H_2O_2$ reaction for sensitive detection of cysteine in human plasma[15].

Electro-chemiluminescence (ECL) may produce light from the light-emitting species generated in situ close to the electrode surfaces, which form the excited states as a result of high-energy electron-transfer reactions[16]. ECL has a near

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zero background, as well as the controllable conditions of the temporal and spatial reaction[17]. $[Ru(bipy)_3]^{3+}$ is one of the most widely used ECL reagents. The electro-oxidation of a stable $[Ru(bipy)_3]^{2+}$ complex leads to the formation of a $[Ru(bipy)_3]^{3+}$ which may react with an analyte (or its oxidation product) to generate a $[Ru(bipy)_3]^{2+}$*. The unstable $[Ru(bipy)_3]^{2+}$* returns to the ground state, accompanied by the emission of a photon[18]. The $[Ru(bipy)_3]^{2+}$ may be excited repetitively and has distinct characteristics such as long-term stability, high ECL efficiency, and reversibility of electrochemical reaction[16]. However, $[Ru(bipy)_3]^{3+}$species may oxidize the solvent and exhibit poor stability in aqueous solution. McDermott *et al*[19] prepared two stable $[Ru(bipy)_3]^{3+}$ and used them as the CL reagents to analyze alkaloids, biomolecules and drugs. Dong *et al*[20] employed CdSe quantum dots (QDs) as the coreactants of $[Ru(bipy)_3]^{2+}$ for sensitive detection of DNA. Zhang *et al*[21] integrated bio-conjugated magnetic beads with ECL for prostate-specific antigen (PSA) assay.

Acidic potassium permanganate (Mn(VII)) is another CL reagent[22], but manganese dioxide (Mn(IV)) is seldom used as a CL reagent. In 1998, solid manganese dioxide immobilized in a polyester resin was firstly used as a CL reagent for isoniazid assay based on the inhibition of luminol-$H_2O_2$-hexacyanoferrate (III) reaction by Mn(II) ions generated from the reaction of isoniazid with the manganese dioxide[23]. However, manganese dioxide is insoluble in water, limiting its practical applications. In 2001, Barnett employed Mn(IV) dissolved in orthophosphoric acid and formaldehyde as the CL reagent for the detection of morphine and codeine[24]. They found that the addition of formaldehyde could significantly enhance the CL intensity. Recently, McDermott *et al*[25] integrated high-performance liquid chromatography (HPLC) with CL to analyze glutathione (GSH) and glutathione disulfide (GSSG) in whole blood using manganese (IV) as the CL reagent.

CL assay has distinct advantages including high sensitivity, wide linear range, simple instrumentation, low cost and easy automation without the requirement for an excitation source and optical filters[26]. The limitation of CL assay such as poor specificity and the involvement of pre-treatment of samples may be overcome through the integration with molecular imprinting, biochip and microfluidic techniques, which may significantly simplify the procedures and shorten the analysis time, promoting its applications in clinical diagnosis, drug screening and environmental monitoring. In this review, we summarized recent progress in CL assay with the integration of HPLC, capillary electrophoresis, quantum dot, microfluidic chips, microarrays, rolling circle amplification, isothermal exponential amplification, two-stage isothermal amplification for the detection of DNA, small biological molecules, enzymes, proteins and metal ions. We also summarized its future directions and highlight its potential applications.

## 2 Chemiluminescence assay with integration of other techniques

CL assay faces some challenges in improving specificity and sensitivity, freedom from interference, and multiple assay in complex samples. The integration of CL assay with other techniques, such as HPLC, capillary electrophoresis, quantum dot, microfluidic chips, microarrays, rolling circle amplification, isothermal exponential amplification, two-stage isothermal amplification, may significantly improve its performance and extend its applications.

### 2.1 Chemiluminescence assay without involvement of amplification

#### 2.1.1 Chemiluminescence assay with integration of high-performance liquid chromatography

High-performance liquid chromatography (HPLC) is widely used for the separation and quantification of biological samples. The integration of CL with HPLC combines the advantages of high separation efficiency of HPLC and high sensitivity of CL assay, and may be applied for simultaneous detection of various analytes. McDermott *et al*[19] prepared two stable $[Ru(bipy)_3]^{3+}$ as the post-column CL reagents. One was prepared by dissolving $[Ru(bipy)_3](ClO_4)_2$ in acetonitrile (containing 0.05 M $HClO_4$), and the other was obtained by dissolving $[Ru(bipy)_3]Cl_2 \cdot 6H_2O$ in 95:5 glacial acetic acid-acetic anhydride (containing 0.05 M $H_2SO_4$), followed by oxidation with $PbO_2$. Both of them generated highly reproducible CL signals over long periods of analysis without the need of recalibration.

Ariga *et al*[27] integrated HPLC post-column with CL for the detection of biosynthesized ferric iron (Fe(III)) chelator in the plants, with a detection limit of $7.7 \times 10^{-13}$ M. Mu *et al*[28] coupled HPLC with on-line gold nanoparticle-catalyzed CL for the quantification of catecholamine in rat brain. Conlan and Francis integrated HPLC with CL to detect intracellular GSH and GSSG using acidic potassium permanganate[29] and $[Ru(bipy)_3]^{3+}$ as the CL reagent[25], respectively.

#### 2.1.2 Chemiluminescence assay with integration of capillary electrophoresis

It should be noted that HPLC-CL method suffers from narrow dynamic range. Capillary electrophoresis (CE) has distinct advantages including low sample/solvent consumption, high resolution and rapid speed with a wide dynamic range[30], thus the integration of CE with CL may be applied for high selective and sensitive assay. Notably, microchip capillary electrophoresis (MCE, a miniaturized version of CE) was successfully used for the analysis of inorganic and organic compounds, small biomolecules and biopolymers, with a