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Applications of tris(2,2′-bipyridyl)ruthenium-based electrochemiluminescence luminophores in biological analysis field

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Abstract : This paper reviewed the applications of electrochemiluminescence (ECL) involving tris(2,2′-bipyridyl)ruthenium(II) {$[Ru(bpy)_3]^{2+}$} in biological analysis field and reported the progress of the research in the $[Ru(bpy)_3]^{2+}$-related ECL. $[Ru(bpy)_3]^{2+}$ was widely used in the biological analysis field related to ECL such as food and drug testing, environmental monitoring, medical diagnosis, drug metabolism research, immunoassay, DNA detection, and forensic investigations. The ECL of $[Ru(bpy)_3]^{2+}$ has been coupled with different separation techniques such as high performance liquid chromatography, capillary electrophoresis, flow injection analysis, and microchip electrophoresis for simultaneous detection of multiple analytes. The fabrication of electrodes of new materials and new structure such as film-coated electrodes, carbon electrodes, complex oxides electrodes, and composite-coated electrodes significantly improved the performance of the $[Ru(bpy)_3]^{2+}$-related ECL. Through the modification of biosensor, various substances have been detected efficiently by $[Ru(bpy)_3]^{2+}$-related ECL technique. The utilization of $[Ru(bpy)_3]^{2+}$ derivatives and solid-phase electrodes in ECL detection made considerable progress.

Keywords : Electrochemiluminescence; Tris(2,2′-bipyridyl)ruthenium(II); Biosensor; Trace analysis; Separation techniques.

INTRODUCTION

The electrochemiluminescence (ECL) behavior of tris(2,2′-bipyridyl)ruthenium(II) {$[Ru(bpy)_3]^{2+}$} was first reported by Tokel and Bard in 1972[1]. Thereafter, the ECL properties of $[Ru(bpy)_3]^{2+}$ and its derivatives have been extensively investigated because of its advantages over exiting systems in the area of ECL analysis. These

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advantages include the low background signal[2], high emission quantum yield[3], long excited-state lifetime[4,5], high chemical stability[6,7], and good ECL efficiency[8-10]. In addition, the ECL of $[Ru(bpy)_3]^{2+}$ can be coupled with different separation techniques such as high performance liquid chromatography (HPLC), capillary electrophoresis, flow injection analysis, and microchip electrophoresis for simultaneous detection of multiple analytes. Some works[2,9,11-14] have comprehensively reviewed the nature of ECL of various labels, and several excellent papers[3,7,15-18] specially reviewed the ECL mechanisms and/or application techniques of $[Ru(bpy)_3]^{2+}$. By combining the simplicity of electrochemistry with the intrinsic sensitivity and broad linear range of chemiluminescence method, ECL has become a powerful tool for analytical applications, and the rapidly increasing studies resulted in highly sensitive and selective detection techniques. Among various labels used for ECL systems, $[Ru(bpy)_3]^{2+}$ is the most widely studied label in recent years. Immobilization of $[Ru(bpy)_3]^{2+}$ on a solid-phase electrode has two advantages at least over the solution-phase ECL method the simplicity of detection process and cost-effectiveness[7].

Because of the advantages, $[Ru(bpy)_3]^{2+}$ and its derivatives have been widely or potentially used in the biological analysis field related to ECL such as food and drug testing[10,19-31], environmental monitoring[32-44], medical diagnosis[45-53], drug metabolism research[54-62], immunoassay[15,63-73], DNA detection[15,28,74-88], and forensic investigations[89-94]. The ECL of $[Ru(bpy)_3]^{2+}$ has become a hot topic among researchers interested in ECL detection, and many papers are being published per year. This work intends to give an overview of summarizing the applications of ECL detection involving $[Ru(bpy)_3]^{2+}$ in analytical areas, and thereby reports the progress of the research in the $[Ru(bpy)_3]^{2+}$-related ECL.

APPLICATIONS OF $[Ru(bpy)_3]^{2+}$ AND ITS DERIVATIVES IN ECL

Food and drug testing

The detection of deleterious substances and active ingredients in food and/or drugs is practically important in the food and drug quality control. Many efforts have been done to use the $[Ru(bpy)_3]^{2+}$ ECL detection in food and drug testing.

(A) Detection of residues and pollutants in food

Chen *et al*[19] developed a capillary electrophoresis-ECL detection system to separate and detect acephate and dimethoate. An electrically heated $[Ru(bpy)_3]^{2+}$/multi-wall-carbon-nanotube (MWCNT) paste electrode was equipped in the system, and the temperature of the electrode could be accurately controlled. This nanoparticles modified electrode was fabricated by mixing $[Ru(bpy)_3]^{2+}$ with the MWCNT paste. The results indicated that the increase of temperature of the electrode could help to improve performance. Wang and co-workers[28] presented a direct $[Ru(bpy)_3]^{2+}$ ECL detection of four polyamines, putrescine, cadaverine, spermidine, and spermine, separated by capillary electrophoresis. The calibration curve is linear over a concentration range of two or three orders of magnitude for the polyamines. The analysis time is less than 25 min. Detection limits for putrescine and cadaverine are 1.9×10^{-7} mol L^{-1} and 7.6×10^{-9} mol L^{-1} for spermidine and spermine, respectively. The intraday and interday RSD of the ECL peak intensities are less than 8%.

A flow injection process with inhibited ECL detection was developed for the determination of tetracycline and oxytetracycline[20]. Under the optimized condition, the linear ranges of 2.0×10^{-5}–1.0×10^{-2} and 1.0×10^{-5}–1.0×10^{-2} g L^{-1} were obtained for tetracycline and oxytetracycline, respectively; the limits of detection (LOD) were 4.0×10^{-6} and 3.8×10^{-6} g L^{-1} for tetracycline and oxytetracycline, respectively; the relative standard deviations (RSD) were 0.68% and 1.18% for 5.0×10^{-4} g L^{-1} tetracycline and oxytetracycline, respectively. It was successfully applied to analysis of tetracycline in a Chinese proprietary medicine, tetracyclini and cortisone eye ointment, and the residues of tetracycline in honey products. The determination of glyphosate and several structurally related compounds using HPLC with $[Ru(bpy)_3]^{2+}$ ECL detection was investigated by Ridlena *et al*[22]. These related compounds are iminodiacetic acid (IDA), diethanol amine (DEA), hydroxyethyl glycine (HEG), and glycine. The rank order of the resulting ECL intensities was glycine < DEA < HEG < IDA < glyphosate. The detection limit

for glyphosate is 1.0×10^{-8} mol L^{-1} with a linear working range that extends five orders of magnitude.

Guo *et al*[21] developed an ECL inhibition method combined with molecularly imprinted solid phase extraction for the quantitative determination of phenolphthalein in drug, slimming food, and human plasma. They found that phenolphthalein strongly inhibited the ECL signal of the $[Ru(bpy)_3]^{2+}$/2-(dibutylamino)ethanol system, which was used as the basis of the analysis. Under the optimized conditions, quenched ECL intensity versus the logarithm of the concentration of phenolphthalein was in good linear relationship over a concentration range of 3.18×10^{-7}–1.59×10^{-4} g L^{-1}, and the LOD was 1.0×10^{-7} g L^{-1}.

Yu *et al*[23] presented a commercial sensor which combines immunomagnetic separation with $[Ru(bpy)_3]^{2+}$ ECL detection. The sensor was evaluated for detection of enterohemorrhagic *E. coli* O157 and *Salmonella typhimurium* in foods and fomites. The detection limits are in the range of 100 to 1000 bacteria per mL in pristine buffer for *E. coli* O157 and *S. typhimurium*, respectively, or 1000 to 2000 bacteria per mL in food samples; the total processing and assay time is less than 1 h even in food samples. Long *et al*[29] proposed a method using hyperbranching rolling circle amplification combined with magnetic beads based ECL to offer an isothermal, highly sensitive, and specific assay for the detection of *Listeria monocytogenes*. Through their approach, as low as 1.0×10^{-17} mol L^{-1} synthetic *hly* gene targets and about 2.0×10^{-7} g L^{-1} of genomic DNA from *Listeria monocytogenes* can be detected. A bifunctional nanoarchitecture has been developed by combining the magnetic iron oxide and the luminescent $[Ru(bpy)_3]^{2+}$ encapsulated in silica[30]. The luminescent $[Ru(bpy)_3]^{2+}$ serves as a luminescent marker, while magnetic Fe_3O_4 nanoparticles allow external operation by a magnetic field. Zhang et al. explored the feasibility of applying the as-prepared nanostructure to fabricating an ECL sensor for the detection of the typical $[Ru(bpy)_3]^{2+}$ co-reactant tripropylamine and some practically important polyamines. A linear range from 6.9×10^{-8} to 7.3×10^{-4} mol L^{-1} with a remarkable LOD of 6.5×10^{-9} mol L^{-1} for tripropylamine was obtained, and the ECL RSD was 0.5% during continuous potential scanning for 16 cycles.

The work of Zhan *et al*[24] describes a microfluidics-based sensing system, which provides a convenient and sensitive method for detecting the electrochemical reactions not directly participating in an ECL reaction. The system was applied to detection of benzyl viologen in solution. This technique presents the prospect of broadening application range of ECL detection.

(B) Detection of drug ingredients

The mode of coupling ECL detection with capillary electrophoresis is rather common among the proposed analytical systems. Wang and co-workers[10] described the determination of benzhexol hydrochloride by capillary zone electrophoresis with an end-column ECL detector. The detection was based on the $[Ru(bpy)_3]^{2+}$ ECL reaction with the analyte. A linear calibration curve of three-orders of magnitude was obtained from 1.0×10^{-8} to 1.0×10^{-5} mol L^{-1}. The LOD of benzhexol hydrochloride was 6.7×10^{-9} mol L^{-1}. Chiang *et al*[25] reported a $[Ru(bpy)_3]^{3+}$-based ECL detector for capillary electrophoresis. The detector was operated in the wall-jet configuration, and an indium/tin oxide (ITO)-coated glass plate was used for the end-column detection. The ITO electrode potential was controlled using a DC battery, without decoupling the detector from the capillary electrophoresis field. In the presence of tertiary or some secondary amines, ECL emission was produced through reduction of in situ generated $[Ru(bpy)_3]^{3+}$ by analytes at the ITO surface. A detection limit of 1.0×10^{-3} mol L^{-1} proline with a theoretical plate number of 4000 was obtained. Wang *et al*[26] established a sensitive method for the determination of chlophenamine maleate in Vitamin C Yinqiao tablets using capillary electrophoresis coupled with $[Ru(bpy)_3]^{2+}$ ECL detection. Under the optimum conditions, chlophenamine maleate in the tablets could be separated and detected in 3 min. The linear concentration of chlophenamine maleate ranged from 5.0×10^{-7} to 1.0×10^{-4} mol L^{-1}; the LOD was 5.1×10^{-8} mol L^{-1}. Capillary zone electrophoresis coupled with $[Ru(bpy)_3]^{2+}$-based end-column ECL has been utilized to detect bisoprolol in drugs and tablets after its separation from metoprolol[27]. Tetrahydrofuran was used as an additive in the running buffer to obtain the absolute ECL peak of bisoprolol, which reacts as a co-reactant in the $[Ru(bpy)_3]^{2+}$ ECL system. Under the optimized experimental conditions, bisoprolol was separated

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successfully and efficiently from metoprolol and other co-existed materials in tablets and urine samples. The ECL intensity of the system is linear with the concentration of bisoprolol from 1.5×10^{-6} to 3.0×10^{-4} mol L^{-1} with LOD of 3.0×10^{-7} mol L^{-1}. The RSD of the ECL intensity is 2.58% for the detection of 1.5×10^{-5} mol L^{-1} bisoprolol. Xiang *et al*[31] described a sensitive determination method for atropine based on end-column ECL of $[Ru(bpy)_3]^{2+}$ detection. Favorable ECL intensity of atropine was achieved in a solution consisting 5×10^{-3} mol L^{-1} $[Ru(bpy)_3]^{2+}$ and 0.05 mol L^{-1} phosphate at applied voltage of 1.20 V. The standard curve was linear between 1×10^{-6} and 2×10^{-5} mol L^{-1} for atropine, and LOD of 5×10^{-8} mol L^{-1} was achieved.

Environmental monitoring

By coupling with proper separation techniques, ECL approach can be used to detect various trace amounts of substances of interest existing in the atmosphere, water, and soil.

Oter *et al*[32] investigated the photophysical and optical oxygen sensing properties of $[Ru(bpy)_3]^{2+}$ chloride in the sol-gel matrix modified by ionic liquid. Effects of the ionic liquid to the oxygen sensitivity and to surface characteristics were examined. The response and regeneration times were 5 and 10 s after exposure to 100% O_2 and 100% N_2, respectively.

Three aminocarboxylic acids, ethylenediaminetetraacetic acid (EDTA), nitrilotriacetic acid (NTA), and 2-hydroxyethylethylene diaminetriacetic acid (HEDTA), have been investigated as potential co-reactants for promoting $[Ru(bpy)_3]^{2+}$ ECL behaviour[33]. The detection limits of $[Ru(bpy)_3]^{2+}$ using NTA, EDTA, and HEDTA as co-reactants are 1.0×10^{-15}, 6.0×10^{-14}, and 6.8×10^{-13} mol L^{-1}, respectively. The results indicate that NTA has a much higher efficiency than the generally used co-reactant, tripropylamine, to excite $[Ru(bpy)_3]^{2+}$-labeled ECL under their own optimal conditions. NTA could be widely used in many fields because it is less toxic, corrosive, and volatile than tripropylamine.

Fang's group[34] developed a solid-state ECL sensor through the electrodeposition of $[Ru(bpy)_3]^{2+}$/AuNPs/chitosan composite film onto electrode. This solid-state electrode was used in ECL to detect tripropylamine with LOD of 5×10^{-10} mol L^{-1}. Wang and co-workers[35] also prepared a solid-state ECL sensor based on a composite film consisting of partial sulfonation of polystyrene (PSP) and carbon nanotube. Because of the involving of carbon nanotubes, the ECL intensity was increased to over 3-fold the value of the pure PSP film. The availability was verified of this sensor by the high sensitivity in detecting 2-(dibutylamino)ethanol and tripropylamine.

Pittman *et al*[36] proposed an ultrasensitive detection of 2,4,6-trinitrotoluene (TNT) in soil or water using enhanced ECL. The ECL intensity is linearly proportional to the analyte TNT concentration from 1.0×10^{-10} to 1.0×10^{-6} g g^{-1}. The LOD is $\leq (1.0 \pm 0.1) \times 10^{-13}$ g g^{-1}, and the absolute detection limit in mass is about 1.0×10^{-13} g.

Chiu *et al*[37] presented a capillary electrophoresis method coupled with ECL detection, which was used to detect glyphosate (GLY) and its major metabolite aminomethylphosphonic acid (AMPA). An ITO-coated electrode was used in the $[Ru(bpy)_3]^{2+}$ ECL detection system. Linear correlation between ECL intensity and analyte concentration was obtained in the ranges 1.69×10^{-4}–1.69×10^{-2} and 5.55×10^{-3}–1.11×10^{-1} g L^{-1} for GLY and AMPA, respectively. The LOD for GLY and AMPA in water were 6.0×10^{-5} g L^{-1} and 4.04×10^{-3} g L^{-1}, respectively. This method was applied to analysis of GLY in soybeans with LOD of 6.0×10^{-7} g g^{-1}.

Lin *et al*[38] established an ECL-based method for detection of nicotine in phosphate buffer solution. At the optimized experimental conditions, a linear range of ECL current vs. nicotine concentration up to 1.0×10^{-4} mol L^{-1} and a lower LOD of 1.2×10^{-9} mol L^{-1} were observed. The RSD of 20 analyses for nicotine concentration of 5.0×10^{-6} mol L^{-1} was 1.4%, and recovery rate of 94% was observed in a real sample analysis without any complications/disturbance in measurement. Zhu *et al*[39] studied the quenching of the $[Ru(bpy)_3]^{2+}$ ECL by sodium diphenylamine-4-sulfonate (SDS). The quenching behaviors can be observed with a 100-fold excess of SDS over $[Ru(bpy)_3]^{2+}$. The mechanism of the quenching involves the energy transfer from the excited-state of $[Ru(bpy)_3]^{2+}$ to the dimer of SDS, which was formed after the electrochemical oxidation of SDS on the electrode surface.

Lin *et al*[40] developed a MWCNT/Nafion com-

posite film-modified electrode, which was applied to the determination of some carbamates including pirimicarb, methomyl, aldicarb, and carbofuran in the nature water. When these carbamates existed, this modified electrode was found to be able to give significantly enhanced ECL intensity of $[Ru(bpy)_3]^{2+}$. Chen *et al*[41] studied the luminescence electrooxidation of monohydric alcohols and polyhydric alcohols in $[Ru(bpy)_3]^{2+}$ alkaline solutions on a glassy carbon electrode. As the potential vs. Ag|AgCl of this electrode reached +1.30 V, $[Ru(bpy)_3]^{2+}$ was oxidized to $[Ru(bpy)_3]^{3+}$. The luminescence intensities of monohydric alcohols decreased as alkyl chain length of the molecules increased. With the increase in the number of hydroxyl groups in a molecule, luminescence intensity increased for polyhydric alcohols.

In the work of Xu *et al*[42], ethylenediaminetetraacetate (EDTA) was chosen for the investigation of the effect of metal ions on $[Ru(bpy)_3]^{2+}$ ECL. The results showed that if the metal ions (not involving Al^{3+} and Y^{3+}) are preferentially bound to oxygen atoms, they have no effect on the ECL intensity. However, if the metal ions are preferentially bound to nitrogen atoms, they retard oxidation of EDTA and decrease the ECL intensity. Another work of Xu *et al*[43] indicated that the ECL of the $[Ru(bpy)_3]^{2+}/(S_2O_8)^{2-}$ system in purely aqueous solution at a carbon paste electrode is strong enough to be seen with the naked eye for $[Ru(bpy)_3]^{2+}$ concentrations higher than 1.0×10^{-3} mol L^{-1}. The double-log plot of the emitted light intensity vs. $[Ru(bpy)_3]^{2+}$ concentration is linear over the region 1.0×10^{-3}–1.0×10^{-7} mol L^{-1}. The ECL intensity increases linearly with the $(S_2O_8)^{2-}$ concentration from 1.0×10^{-6} mol L^{-1} up to 3.0×10^{-4} mol L^{-1} and drops off sharply at concentrations higher than 1.0×10^{-3} mol L^{-1}.

Tsukagoshia *et al*[44] found that emetine dithiocarbamate metal complex prepared from emetine, carbon disulfide, and metal (II) can generate a large chemiluminescence intensity of $[Ru(bpy)_3]^{2+}$ ECL. Liquid chromatography equipped with a chemiluminescence detector was developed for analyzing a model sample containing trace level of Cu(II) and Co(II) ions. The Cu(II) and Co(II) complexes were determined over the range 1–300 × 10^{-9} mol L^{-1} and 3–500 × 10^{-8} mol L^{-1}, respectively.

Medical diagnosis

Skotty *et al*[45] accomplished the determination of dansyl amino acids and oxalate by HPLC with ECL detection using $[Ru(bpy)_3]^{2+}$ as luminophore. In this method, different concentrations of $[Ru(bpy)_3]^{2+}$ are dissolved in the mobile phase and the HPLC column flushed with the mobile phase until the column is saturated with $[Ru(bpy)_3]^{2+}$. The separated analytes along with $[Ru(bpy)_3]^{2+}$ pass through an optical-electrochemical flow cell which has a dual platinum electrode, where $[Ru(bpy)_3]^{2+}$ is oxidized to $[Ru(bpy)_3]^{3+}$, and then $[Ru(bpy)_3]^{3+}$ reacts with the analytes to emit light.

Gan *et al*[46] developed a renewable and ultrasensitive ECL immunosenor based on magnetic $[Ru(bpy)_3]^{2+}/SiO_2$-Au~$[Ru(bpy)_3]^{2+}$-Ab2 sandwich-type nano-immunocomplexes for the detection of tumor markers. The immunosensor showed a linear range of 5.0×10^{-8}–1.0×10^{-4} g L^{-1} for detecting alpha-fetoprotein (AFP) with LOD of 2.0×10^{-8} g L^{-1}. In an article of the same research group, a renewable and magnetic ECL immunosensor for AFP detection was described[49], where the RuL-MWCNTs-doped Au composite was used as labels {RuL = $[Ru(bpy)_3]^{2+}$ complex}. The sensor exhibited high sensitivity and wide liner for detection AFP in the content from 1.0×10^{-8} to 5.0×10^{-5} g L^{-1} and the LOD was 3.0×10^{-9} g L^{-1}.

Ding *et al*[47] reported a polymerase chain reaction-free ECL approach that uses ECL nanoprobes for the determination of cancer cells with high sensitivity. The ECL nanoprobe consists of gold nanoparticles, linker DNA, and $[Ru(bpy)_3]^{2+}$-labeled signal DNA. The intensity of ECL signals is proportional to the cell concentration in the range of 5–100 cells/mL, and the LOD is 5 cells/mL.

Zhan *et al*[48] presented an ECL detection of rotavirus by reverse transcription-polymerase chain reaction based on a magnetic primer. Rotavirus in fecal specimens was detected within 1.5 h, and the detection limit was 2.0×10^{-7} g L^{-1} of rotavirus. The intensity of ECL signals was linearly dependent on the concentration from 2.0×10^{-7} to 4.0×10^{-4} g L^{-1}. The method shows good specificity for the detection of rotavirus.

Bertolinoa *et al*[50] proposed an integrated solution to DNA hybridisation monitoring based on a monolithic silicon platform. The fabrication process was developed to pattern a gold initiation electrode directly

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onto a PIN photodiode detector. Duplex DNA was prehybridised to consist of thiolated oligonucleotide and ruthenium-labelled complementary oligonucleotide and then was assembled on the gold initiation electrode. The results indicate that DNA assembled on the surface containing sufficient ruthenium may generate a measurable signal.

In the work of Huang *et al*[51], the combined FI-CE (FI = flow injection and CE = capillary electrophoresis) system was exploited by coupling to an ECL detection system. The baseline separation of proline, valine, and phenylalanine elucidated the high efficiency of the system; a high throughput of 50 h^{-1} was achieved under optimized conditions. Detection limits of 1.2, 50, and 25 × 10^{-6} mol L^{-1} for proline, valine, and phenylalanine were observed, respectively. Martin *et al*[52] fabricated ECL biosensors using $[Ru(bpy)_3]^{2+}$ as luminophore. $[Ru(bpy)_3]^{2+}$ and dehydrogenase enzymes were immobilized in cation exchange polymers. The electrode modified by the cation exchange polymers was used in a flow injection analysis system as an ECL detector. This concept for ECL biosensor is applicable for detecting enzymes depending on NAD^+ or $NADP^+$.

Wang and co-workers[53] prepared $[Ru(bpy)_3]^{2+}$-doped silica nanoparticles via a water-in-oil microemulsion approach and studied the ECL of the nanoparticles with covalently coated biomacromolecules. By covalent cross-linking with glutaraldehyde, the γ-(aminopropyl) triethoxysilane-pretreated nanoparticles were coupled with different concentrations of bovine serum albumin (BSA), hemoglobin, and myoglobin, respectively. ECL from these biomacromolecule-coated nanoparticles decreased with the increase of the loading of biomacromolecules.

Drug metabolism research

It is very important to accurately predict which new drugs will be associated with a significant incidence of idiosyncratic drug reactions[95], which helps to reduce attrition rates of drugs. Predicting toxicity at the earliest stages of drug development becomes a critical issue. The application of $[Ru(bpy)_3]^{2+}$-related ECL in drug metabolism research has received considerable attention.

Greenwood *et al*[54] have made a portable μ-TAS drug screening device, which utilizes both the chemiluminescence and the electrogenerated chemiluminescence resulted from the $[Ru(bpy)_3]^{2+}$-involved reactions. Wang's group[55] developed a new technique for investigating drug-protein binding, which employs capillary electrophoresis coupled with ECL detection after equilibrium dialysis. Pridinol, procyclidine, and its analogue trihexyphenidyl, were successfully separated by capillary zone electrophoresis with the end-column $[Ru(bpy)_3]^{2+}$ ECL detection. Forbes *et al*[56] described an on-line $[Ru(bpy)_3]^{3+}$ ECL detection of β-blockers. The β-blockers were separated with capillary electrophoresis. The LOD for oxprenolol is 6.0 × 10^{-4} g L^{-1}.

Fang's group[57] proposed a method for selective immobilization of $[Ru(bpy)_3]^{2+}$ onto the surface of a target electrode. The immobilization was based on the electrodeposition of $[Ru(bpy)_3]^{2+}$-doped silica nanoparticles on chitosan composite film. The ECL sensor based on this modified electrode exhibits excellent reproducibility, stability, and sensitivity for the detection of tri-n-propylamine. A linear range from 1.0 × 10^{-10} to 1.0 × 10^{-6} mol L^{-1} and LOD of 5.0 × 10^{-11} mol L^{-1} was observed.

Ding *et al*[58] presented a solid-state ECL detector involving an electrophoretic microchip, which was used to detect tramadol, lidocaine, and ofloxacin. This ECL detector is based on an electrode modified by $[Ru(bpy)_3]^{2+}$–Zirconia–Nafion composite. These three pharmaceuticals were baseline separated without using any additives. The LODs were 2.5 × 10^{-5}, 5.0 × 10^{-6}, and 1.0 × 10^{-5} mol L^{-1} for tramadol, lidocaine, and ofloxacin, respectively. The injected dose of the samples was in the magnitude of picoliter, and the linear ranges were 5.0 × 10^{-5} - 2.5 × 10^{-3}, 1.0 × 10^{-5} - 1.0 × 10^{-3}, and 1.0 × 10^{-5} - 2.5 × 10^{-3} mol L^{-1} for tramadol, lidocaine, and ofloxacin, respectively.

In the work of Zhao *et al*[59], ECL of $[Ru(bpy)_2(PVP)_{10}]^{2+}$ was used to investigate differences in metabolite-mediated toxicity of tamoxifen in rodents vs. humans. The detector arrays feature thin-film spots containing $[Ru(bpy)_2(PVP)_{10}]^{2+}$, DNA, and liver microsomes.

Shibano *et al*[60] proposed a method for screening metabolizing enzyme inhibitors of cytochrome P450 2D6 using column-switching HPLC with $[Ru(bpy)_3]^{2+}$ ECL, which can be applied to the evaluation of the genetic diversity concerning the ability of cytochrome P450 2D6

to metabolize drug *in vitro*.

Synergistic use of ECL arrays (labeled with Ruthenium metallopolymer, RuPVP) and enzyme/DNA nanoreactors with liquid chromatography-mass spectrometry (LC-MS) was shown to be able to provide efficient screening of toxic reactive metabolites that correlates with animal liver toxicity data[61]. ECL arrays provide rapid screening, and the biocolloid reactor LC-MS approach provides valuable follow-up information about chemical structures and formation rates for ECL hits. Metabolic toxicity screening using ECL arrays coupled with enzyme-DNA biocolloid reactors and LC-MS holds great promise for new drug and chemicals development[62].

Immunoassay

Blackburn *et al*[63] reported the immunoassays based on the use of $[Ru(bpy)_3]^{2+}$-NHS (NHS = N-hydroxysuccinimide) ester. One of the bipyridyl ligands of $[Ru(bpy)_3]^{2+}$ reacted with reactive groups to form activated species, so that the modification of structure of $[Ru(bpy)_3]^{2+}$ was realized. The activated species were able to react with proteins, haptens, and nucleic acids. Through the detection of polymerase chain reaction products, this method was applicable for the determination of the HIV-1 *gag* gene. They illustrated ECL in nonseparation immunoassays for digoxin and thyrotropin and in separation immunoassays for carcinoembryonic antigen and α-fetoprotein.

Miao *et al*[64] also used modified $[Ru(bpy)_3]^{2+}$ as a molecular label in ECL determination of DNA and C-reactive protein. The capture strands of DNA were modified by thiol, and then were self-assembled onto a gold electrode because they were complementary to the $[Ru(bpy)_3]^{2+}$-labeled targets. The ECL signals resulted from tri-n-propylamine radicals were proportional to the targets. Similarly, biotinylated anti-C-reactive protein species were immobilized onto Au(111) substrate precovered with a layer of avidin. The ECL peak intensity was linearly proportional to the analyte C-reactive protein concentration over the range $1\text{-}24 \times 10^{-3}$ g L^{-1}. Miao *et al*[65] introduced an ECL method of adding multiple $[Ru(bpy)_3]^{2+}$ to a single antibody by encapsulating a hydrophobic $[Ru(bpy)_3]^{2+}$ compound in polymer (polystyrene) microspheres. Each microsphere contained approximately 10^9 water-insoluble $Ru(bpy)_3[B(C_6F_5)_4]_2$ molecules. The ECL intensity was observed to be proportional to the analyte C-reactive protein concentration over the range of 1.0×10^{-5}-1.0×10^{-2} g L^{-1}. Zhan *et al*[66] prepared liposomes (~100 nm diameter) containing $[Ru(bpy)_3]^{2+}$, and these ECL tags were used in a sandwich-type immunoassay of human C-reactive protein. The detection limit is 1.0×10^{-4} g L^{-1} for human C-reactive protein with good linearity of ECL intensity versus antigen concentration over the range 1.0×10^{-4}-1.0×10^{-2} g L^{-1}.

Zhang *et al*[67] developed a highly sensitive ECL immunoassay for the determination of anti-digoxin antibody and digoxin hapten. $Ru(bpy)_2$(dcbpy)NHS (dcbpy = 2,2′-bipyridine-4,4′-dicarboxylic acid) was used as the ECL label, and BSA was used as a carrier protein. The anti-digoxin antibody concentration from 7.6×10^{-5} to 7.6×10^{-3} g L^{-1} was determined by direct homogeneous format. Digoxin hapten was determined throughout the range 4.0×10^{-7} - 1.0×10^{-4} g L^{-1} with LOD of 1.0×10^{-7} g L^{-1} by competitive format. The RSD for 6.0×10^{-6} g L^{-1} was 4.3%.

Egashira *et al*[68] developed a method to detect the presence of hemagglutinin molecules by combining ECL with an immunoliposome encapsulating a Ru complex. Under optimum conditions, hemagglutinin molecules of influenza virus were determined in a concentration range from 3×10^{-10} to 4×10^{-8} g L^{-1}. This level of sensitivity suggests that a detectable lower concentration could be 1.2×10^{-14} mol L^{-1}.

Fang's group[69] reported a thrombin aptasensor. The labels were $[Ru(bpy)_3]^{2+}$-doped silica nanoparticles. The aptamers were assembled on the surface of an Au electrode through Au-S binding. Hybridization of complementary DNA with the labels resulted in an ECL signal in the presence of tripropylamine at positive potentials. With the addition of thrombin, the nanoparticle-labeled DNA was replaced, so the intensity of the ECL signals was decreased. The decrease was proportional to the thrombin concentration from 10^{-14} mol L^{-1} to 10^{-11} mol L^{-1} and the detection limit was down to 10^{-15} mol L^{-1}. Analogous work was done to detect thrombin with two different aptamers, which recognize different positions of thrombin[70]. The aptamers feature sandwich type; one was immobilized onto the gold electrode for capturing thrombin onto the electrode, and the other was used for detection. The increase of the ECL signal

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generated by $[Ru(bpy)_3]^{2+}$-doped silica nanoparticles was observed in dependent manner on the concentration of thrombin added. BSA and bovine hemoglobin had almost negligible responses, so the selectivity is improved.

The paper of Wu *et al*[71] described an on-chip microarray platform based on an ECL resonance energy transfer strategy for rapid assay of cancer cell surface biomarkers. Carcinoembryonic antigen, α-fetoprotein, and prostate-specific antigen as models were detected on this microfluidic device. This multiplexed competitive immunoassay was also successfully used for cell counting. Sardesai *et al*[72] reported an ECL immunosensor for detection of protein cancer biomarkers, where a sandwich-type ECL sensor was fabricated. Prostate-specific antigen in cell lysates and human serum samples was successfully detected. Zhang *et al*[73] also reported a sandwich-type ECL immunosensor for carcinoembryonic antigen on a gold nanoparticles-modified glassy carbon electrode. The concentrations of carcinoembryonic antigen were obtained in the range from 10^{-9} g L^{-1} to 10^{-5} g L^{-1} with LOD of 8×10^{-10} g L^{-1}.

DNA detection

Fang's group[74] developed a controllable solid-state ECL film based on efficient and stable quenching of ECL of $[Ru(bpy)_3]^{2+}$ by oxidizing ferrocene on the electrode. The biosensing system employed the $[Ru(bpy)_3]^{2+}$-AuNPs-modified (AuNPs = Au nanoparticles) Au electrode to emit ECL and the ferrocene-labeled molecular beacon to control the ECL intensity. The ECL intensity was correlated to the conformation of the ferrocene-labeled molecular beacon, which could be combined with its target biomolecule via the reaction of DNA hybridization or aptamer-protein combination. Therefore, the system can be applied to recognize the relevant biomolecules selectively. Li *et al*[75] also reported the use of AuNPs-modified Au electrode in ECL detection of DNA hybridization, and a ruthenium complex served as the ECL label. The LOD of target ssDNA on the AuNPs-modified electrode (6.7×10^{-12} mol L^{-1}) is much lower than that on a bare gold electrode (1.2×10^{-10} mol L^{-1}).

Fang's group[76] described a sensitive ECL detection of DNA hybridization, where the $[Ru(bpy)_3]^{2+}$-doped silica nanoparticles were used as DNA tags. High-concentration of molecules of the ECL-producing agent were sequestered by the porous nanoparticles, which led to the greatly enhanced luminescence. The assay allows detection of the target DNA with a concentration as low as 1.0×10^{-13} mol L^{-1}. The ECL intensity is linearly related to the DNA concentration in range of $2.0 \times 10^{-13} - 2.0 \times 10^{-9}$ mol L^{-1} of the complementary sequence.

Xie *et al*[77] prepared a redox active and ECL threading bis-intercalator consisting of two N,N'-bis(3-propyl-imidazole)-1,4,5,8-naphthalene diimides (PIND) linked by a $[Ru(dmbpy)_2]^{2+}$ (dmbpy = 4,4′-dimethyl-2,2′-bipyridine) complex (PIND-Ru-PIND). The DNA biosensor based on the PIND-Ru-PIND allows detection in the concentration range of $7.0 \times 10^{-13} - 4.0 \times 10^{-10}$ mol L^{-1} with a detection limit of 4.0×10^{-13} mol L^{-1}.

Zhang *et al*[78] reported an ECL biosensor for the detection of target ssDNA, which uses hairpin DNA as the recognition element and ruthenium complex as the signal producing compound. In the absence of target ssDNA, a strong ECL signal could be generated; in the presence of target ssDNA, the ECL signal decreased. LOD of 9×10^{-11} mol L^{-1} complementary target ssDNA was achieved.

Miao *et al*[79] developed an ECL detection method by employing polystyrene beads as a carrier of the $Ru(bpy)_3$-$[B(C_6F_5)_4]_2$ labels. The polystyrene beads containing a large number of water insoluble $Ru(bpy)_3$-$[B(C_6F_5)_4]_2$ species were immobilized on the target ssDNA. Probe ssDNA was attached to the surface of magnetic beads and hybridized with the target ssDNA. The integrated ECL intensity was linearly proportional to the concentration of target DNA in a range of 1.0×10^{-15} mol L^{-1} to 1.0×10^{-8} mol L^{-1}. The principle described in this paper could be also applied to many other ECL analyses, such as immunoassays.

Fang's group[80] described the detection of T4 DNA ligase using a solid-state ECL biosensing switch based on ferrocene-labeled molecular beacon. The system includes an ECL substrate modified by the complex of AuNPs and $[Ru(bpy)_3]^{2+}$ and an ECL intensity switch of molecular beacon labeled by ferrocene.

Bio bar code assay based on oligonucleotide-modified AuNPs provides a polymerase chain reaction

(PCR)-free method for quantitive detection of nucleic acid targets[81]. Zhu *et al* reported a novel PCR-free ECL-based bio bar code assay for the detection of genetically modified organism in raw materials with high speed and sensitivity. In the work of Kurita *et al*[82], cytosine methylation in DNA was determined by an enzyme linked immunosorbent assay with ECL detection and employed for the DNA methylation assay of a long and real genomic sample. Methyl-cytosine was measured quantitatively in the concentration range of 10^{-12} to 10^{-10} mol L^{-1}. This PCR-free method exhibits sufficiently high sensitivity to achieve real DNA measurements.

Lee *et al*[83] reported a detection method for DNA hybridization based on the $[Ru(bpy)_3]^{2+}$-labeled ECL, where a DNA-binding intercalator was used to reduce $[Ru(bpy)_3]^{3+}$. The double-stranded DNA intercalated with doxorubicin, daunorubicin, or 4,6-diamidino-2-phenylindole (DAPI) shows a good ECL with $[Ru(bpy)_3]^{2+}$; the DAPI-intercalated $[Ru(bpy)_3]^{2+}$-labeled ECL provides a good specificity of single point mutations for hepatitis disease.

Duan *et al*[84] presented an ECL biobarcode method based on cysteamine-gold nanoparticle conjugates. The assay exhibits excellent selectivity for single-mismatched DNA detection with limit of 10^{-13} mol L^{-1}.

So *et al*[85] described detection of bioactivated genotoxicity employing ultrathin films of DNA, model metabolic enzymes, and ECL-generating metallopolymer $[Ru(bpy)_2PVP_{10}]^{2+}$ on pyrolytic graphite electrodes. DNA damage in the sensor films was measured using a simple apparatus involving a standard voltammetry cell coupled with an optical fiber and photomultiplier tube. The ECL and square wave voltammetry signals increased with the enzyme reaction time and provided relative enzyme turnover rates for DNA damage suitable for toxicity screening applications. Hvastkovs *et al*[86] reported similar ECL arrays suitable for genotoxicity screening, where array spots containing DNA, various human cytochromes P450, and $[Ru(bpy)_2PVP_{10}]^{2+}$ were exposed to H_2O_2 to activate the enzymes.

Wang and co-workers[87] described a method to detect procyclidine in human urine by coupling $[Ru(bpy)_3]^{2+}$-labeled ECL and capillary electrophoresis. The LOD of procyclidine is 1.0×10^{-9} mol L^{-1} in an on-capillary stacking mode. Extraction recovery was nearly 90% for application in urine.

Li *et al*[88] developed a detection method of DNA hybridization based on a single-wall carbon-nanotubes ECL probe. A derivative of $[Ru(bpy)_3]^{2+}$ was loaded on the probe. The linear detection range of perfect-matched target ssDNA is from 2.4×10^{-14} to 1.7×10^{-12} mol L^{-1}, and LOD is 9.0×10^{-15} mol L^{-1}.

$[Ru(bpy)_3]^{2+}$-related ECL has become a common method for DNA detection and immunoassays. The application of the technique in these two areas was also presented in the review of Fan *et al*[96]. The use of nanomaterials modified electrodes in ECL detection could lead to an improvement in sensitivity and selectivity; this improvement is essential for detection of biomolecules such as DNA or proteins. The use of carbon nanotube electrodes for biomolecular analysis using ECL detection merits extensive investigation[97].

Forensic investigations

The ability of $[Ru(bpy)_3]^{2+}$ ECL to detect drugs or narcotics enables it to be used in forensic investigations. By coupling with flow-analysis methodologies such as flow injection analysis, HPLC, capillary electrophoresis, and microfluidic devices, various controlled drugs can be determined[89].

Wang and co-workers[90] established a detection system coupling capillary electrophoresis with $[Ru(bpy)_3]^{2+}$ ECL, which was used to determine contamination of banknotes with controlled drugs. The standard curves were linear in the range of 7.5×10^{-8} to 1.0×10^{-5} mol L^{-1} for heroin and 2.5×10^{-7} to 1.0×10^{-4} mol L^{-1} for cocaine, and LODs of 5.0×10^{-8} mol L^{-1} for heroin and 6.0×10^{-8} mol L^{-1} for cocaine were achieved, respectively. By using the system of Li *et al*[91], simultaneous determination of psychotropic drugs in human urine was accomplished. The capillary electrophoresis-coupled $[Ru(bpy)_3]^{2+}$ ECL system displayed a linear range from 5.0×10^{-6} to 8.0×10^{-4} g L^{-1} for amitriptyline, doxepin, and chlorpromazine. Their LODs were 8.0×10^{-7} g L^{-1}, 1.0×10^{-6} g L^{-1}, and 1.5×10^{-6} g L^{-1}, respectively. Liu *et al*[92] also presented a method, for determination of enoxacin and ofloxacin, based on capillary electrophoresis coupling with $[Ru(bpy)_3]^{2+}$ ECL. The LODs are 9.0×10^{-9} and 1.6×10^{-8} mol L^{-1} for enoxacin and ofloxacin, respectively. The quantitation limits for enoxacin and

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ofloxacin are 3.2×10^{-7} and 5.4×10^{-7} mol L^{-1} in human urine samples and 4.1×10^{-7} and 6.9×10^{-7} mol L^{-1} in human serum samples, respectively.

A carbon paste electrode was modified with zeolite Y, and $[Ru(bpy)_3]^{2+}$ was immobilized in the modified electrode, through which Zhuang *et al*[93] realized sensitive determination of heroin. The ECL sensor showed a linear response to flow injection of heroin in the range of 2.0×10^{-6} - 8.0×10^{-5} mol L^{-1} with LOD of 1.1×10^{-6} mol L^{-1}.

Based on the increasing effect of tramadol on $[Ru(bpy)_3]^{2+}$-labeled ECL, an ECL method for the determination of tramadol hydrochloride at a MWCNT/chitosan-$[Ru(bpy)_3]^{2+}$ composite film-modified graphite electrode was described[94]. The ECL signal was proportional to tramadol hydrochloride concentration from 5.0×10^{-6} to 6.0×10^{-4} mol L^{-1}, and the LOD was 2.0×10^{-6} mol L^{-1}.

SUMMARY

$[Ru(bpy)_3]^{2+}$ and its derivatives as ECL labels have the advantages of sensitivity, compatibility, and transformability. The $[Ru(bpy)_3]^{2+}$-related ECL has become a hot area of research in recent years. Many efforts have been done in order to improve electrode performance, leading to the fabrication of electrodes of new materials and new structure such as film-coated electrodes, carbon electrodes, complex oxides electrodes, and composite-coated electrodes. The nanomaterials-modified solid-state electrodes have attracted much attention because of the increased sensitivity, selectivity, stability, and applicability. Through the modification of biosensor, various substances have been detected efficiently by $[Ru(bpy)_3]^{2+}$-related ECL technique. The extensive applications of $[Ru(bpy)_3]^{2+}$-related ECL in combination with capillary electrophoresis[10,25-28,37,51,55,56,87,91-93], HPLC[21,45,60], flow injection analysis[19,24,51,52,94], and microchip electrophoresis[58,71] make itself as a versatile, cost-effective, and reliable detection tool. The synthesis and utilization of new Ru(II) complexes with high ECL activity will further broaden the prospects for the use of $[Ru(bpy)_3]^{2+}$ ECL detection[98,99].

In comparison with the vast amount of reports on the combination of $[Ru(bpy)_3]^{2+}$ ECL with capillary electrophoresis and flow injection analysis, the applications of ECL detection coupling $[Ru(bpy)_3]^{2+}$ with microchip electrophoresis are still insufficient. Microchips exhibit considerable advantages such as compactness, operation convenience, reduced reagent requirements, and flexibility, so the combination of the microchip and $[Ru(bpy)_3]^{2+}$-labeled solid-state ECL sensors will be worth of further investigation[100].

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