联吡啶-钌体系电位分辨的电致化学发光

摘　　要

本论文在综述了电致化学发光（ECL）尤其是$Ru(bpy)_3^{2+}$ ECL的性质、机理和分析应用研究现状和最新进展的基础上，以探索$Ru(bpy)_3^{2+}$ ECL体系的多通道发光、深入认识其发光机理和进一步拓展其分析应用范围为出发点，在静态条件下，研究了$Ru(bpy)_3^{2+}/C_2O_4^{2-}$ ECL体系在惰性金属（铂、金）电极、碳（石墨充蜡、玻碳）电极和几种聚合物修饰电极上的电位分辨电致化学发光（prECL）和伏安行为，观察到两条发光通道，提出了相应的反应机理；在流动注射条件下，系统研究了 30 种多酚、酚酸、苯胺类化合物和 20 种氨基酸在各种反应条件下对$Ru(bpy)_3^{2+}$/TPrA、$Ru(bpy)_3^{2+}/C_2O_4^{2-}$和$Ru(phen)_3^{2+}$/TPrA体系ECL的影响，发现许多化合物的ECL增强和抑制作用及其相关性，提出了ECL增强和抑制作用机理，在此基础上建立了一系列化合物流动注射（FI）- ECL分析法。本论文取得的主要研究成果如下：

运用prECL 研究方法分别在 1.22V和 1.41V于金电极上、1.22 V和 1.40 V于铂电极上得到两个发光通道。对影响该体系两个ECL峰的峰电位、峰形和强度的几个因素，即电极表面不同预处理方式（直接抛光、阳极极化和阴极极化的电极以及S吸附电极）、体系中$C_2O_4^{2-}$浓度、pH值、溶解氧和溶解二氧化碳等条件进行了细致的研究。而预氧化处理的金电极表面用X射线光电子能谱技术（XPS）进行了表征。通过比较$i-E$和$I-E$曲线，提出了第 2 条发光通道的可能机理，即在较正电位下，由于铂、金电极表面受含氧物种的修饰，电极表面吸附的氧阻碍了$CO_2^{-\cdot}$进一步电氧化生成CO_2，从而导致直接电氧化生成的$CO_2^{-\cdot}$同$Ru(bpy)_3^{2+}$ 或$Ru(bpy)_3^{3+}$反应，形成第 2 个ECL峰。

利用循环伏安（CV）、方波循环阶跃（CSW）及恒电位脉冲 3 种不同手段对两种碳电极（玻碳和石墨充蜡电极）的伏安和ECL行为进行了比较研究。在CV实验中，观察到预极化处理后的两种碳电极上同样具有双发光通道特性。在 100 mV/s 扫速下，$C_2O_4^{2-}$离子在石墨充蜡电极上的检出限可达到 1×10^{-9} mol/L。在水相含$C_2O_4^{2-}$的溶液中，当方波循环阶跃实验在合适电位间进行时，在两种电极上均可获得较高ECL信号。而在计时电流实验中，当电极电位从+0.15 V阶跃到+1.85

V时，ECL信号在石墨充蜡电极上仅需要 389 ms 就可达到最大值，而在玻碳电极上却需要 837 ms。实验中，廉价的石墨充蜡电极表现出比玻碳电极更强的电极响应速度和灵敏度，并且在水相中性质非常稳定。

采用聚吡咯啉酮（PVP）、聚 4-乙烯吡啶（P4VP）均聚物、聚苯乙烯（PS）-*b*-P4VP-*b*-PS三嵌段共聚物，以及PS-*b*-P4VP-*b*-PS/Nafion 夹层复合聚合物固载$Ru(bpy)_3^{2+}$制得不同修饰电极。通过对以上不同聚合物修饰电极的CV、prECL行为和寿命进行比较，评估了不同聚合物修饰电极的性能。综合修饰电极表现的ECL强度及其稳定性两个因素，发现PS-*b*-P4VP-*b*-PS/Nafion复合膜修饰电极性质性能最优。该修饰电极既具有Nafion的强选择性和强疏水性，又具有PS-*b*-P4VP-*b*-PS膜的纳米孔道性和荷电性。另外，研究了$Ru(bpy)_3^{2+}$直接吸附修饰的石墨充蜡电极的prECL行为，从其CV图可以看出，当$Ru(bpy)_3^{2+}$直接吸附修饰电极时，对$C_2O_4^{2-}$离子的电化学响应有明显的催化作用。

研究了肾上腺素对$Ru(bpy)_3^{2+}$/三丙胺（TPrA）和$Ru(bpy)_3^{2+}/C_2O_4^{2-}$体系的ECL的抑制作用。发现在pH 8.0 的磷酸缓冲溶液中，当$Ru(bpy)_3^{2+}$ECL体系的电极电位为 1.05 V时，肾上腺素表现出对$Ru(bpy)_3^{2+}$/TPrA体系ECL强的抑制作用，由此建立了测定肾上腺素的FI-ECL抑制法。测定肾上腺素的线性范围可达 2×10^{-8} - 1×10^{-4} mol/L，检出限为 7.0×10^{-9} mol/L (S/N=3)。应用此FI-ECL方法我们对肾上腺素注射液中肾上腺素进行了成功测定。在详细研究$Ru(bpy)_3^{2+}$/TPrA/肾上腺素体系的电致化学发光光谱、UV-vis光谱和CV图的基础上，分析了肾上腺素的抑制机理为激发态的$Ru(bpy)_3^{2+*}$同肾上腺素的邻苯醌类电氧化产物间发生能量转移引起的。

对FI-ECL间接法在$Ru(bpy)_3^{2+}$/TPrA和$Ru(phen)_3^{2+}$/TPrA两种ECL体系中分别测定去甲肾上腺素或多巴胺的结果进行了比较研究。实验中，观察到$Ru(bpy)_3^{2+}$/TPrA和$Ru(phen)_3^{2+}$/TPrA两种ECL体系在流动溶液中同样产生prECL现象，$Ru(bpy)_3^{2+}$/TPrA体系在 0.9 V和 1.05 V，$Ru(phen)_3^{2+}$/TPrA体系在 1.01 V和 1.25 V分别出现两个发光通道。$Ru(phen)_3^{2+}$/TPrA 体系中测定结果比$Ru(bpy)_3^{2+}$/TPrA体系好。在$Ru(phen)_3^{2+}$/TPrA 体系中，去甲肾上腺素和多巴胺的线性范围分别为 2×10^{-8} ~ 2×10^{-5} mol/L和 4×10^{-8} ~ 2×10^{-5} mol/L，检出限分别为 7.1×10^{-9} mol/L和 1.5×10^{-8} mol/L。在此基础上成功的测定了重酒石酸去甲肾上腺

素、盐酸多巴胺注射液中去甲肾上腺素和多巴胺的含量。最后，讨论了抑制现象产生的原因。

基于没食子酸对$Ru(bpy)_3^{2+}$/TPrA体系的抑制作用建立了其FI-ECL间接分析法。没食子酸浓度为 2×10^{-8} ~ 2×10^{-5} mol/L时，在$Ru(bpy)_3^{2+}$/TPrA 体系中，其浓度（C）的对数值与对净发光强度抑制值（ΔI）的对数值呈现良好的线性关系，其检出限可达 9.1×10^{-9} mol/L，相对标准偏差为 1.0%。应用此FI-ECL抑制法对中成药-健民咽喉片的丙酮萃取液中的没食子酸进行测定，方法结果同对照实验吻合得很好。

系统研究了 20 种天然氨基酸，在 6 个pH值下，于PBS、$NaHCO_3$-Na_2CO_3和NaOH三种不同介质中对$Ru(bpy)_3^{2+}$/TPrA体系ECL的影响。发现当缓冲溶液pH值从 8 递增至 13 时，酪氨酸、色氨酸、组氨酸、胱氨酸和甲硫氨酸对ECL影响呈现从抑制到增强的变化规律；而脯氨酸和羟脯氨酸在所有pH条件下均表现为增强ECL效应。利用酪氨酸和色氨酸在pH 8 的条件下，对$Ru(bpy)_3^{2+}$/TPrA体系ECL强烈的抑制作用，建立了分别测定酪氨酸和色氨酸的FI-ECL新方法。最后我们根据实验结果对抑制和增强现象产生机理进行了分析推导，认为酪氨酸、色氨酸等的抑制、增强现象同这些具有特殊官能团的氨基酸的阳极电氧化产物和发光活性物质$Ru(bpy)_3^{2+*}$间的能量转移有关。

应用FI-ECL方法，研究了 30 种多酚类和苯胺类化合物对$Ru(bpy)_3^{2+}$/TPrA、$Ru(bpy)_3^{2+}$/$C_2O_4^{2-}$和$Ru(phen)_3^{2+}$/TPrA体系ECL的影响。发现大多数化合物对ECL体系的影响同pH相关，在低pH时具有ECL抑制作用，而在高pH时具有ECL增强作用。另外，ECL抑制和增强作用的大小也与有机物的结构明显相关，其大小顺序为：间位 > 邻位 > 对位。初步探讨了多种酚和苯胺类化合物在$Ru(bpy)_3^{2+}$/$C_2O_4^{2-}$，$Ru(bpy)_3^{2+}$/TPrA两种ECL体系的抑制作用的分析潜力。在$Ru(bpy)_3^{2+}$/TPrA体系中，各种化合物的检出限低至 10^{-8} ~ 10^{-9} mol/L。最后，针对加入酚酸化合物前后ECL发光光谱图以及UV-vis光谱图的变化，对抑制作用、增强作用产生的机理进行了综合探讨。认为是由各种化合物的电氧化产物或发光反应中间态自由基同发光体之间的作用造成的。

Potential-resolved Electrochemiluminescence of $Ru(bpy)_3^{2+}$

Abstract

The state and arts on characteristics, mechanisms and analytical application of the electrochemiluminescence (ECL), especially $Ru(bpy)_3^{2+}$ ECL were reviewed. In this dissertation, in order to explore multi-channel ECL, gain an inside view of the ECL mechanism and expand the analytical application of $Ru(bpy)_3^{2+}$ ECL, the potential-resolved ECL (prECL) and cyclic voltammetric (CV) behaviors of the typical $Ru(bpy)_3^{2+}/C_2O_4^{2-}$ ECL system in static state on various electrodes, including gold (Au), platinum (Pt), paraffin-impregnated graphite electrode (PIGE), glassy carbon electrode (GCE), and modified electrodes with various polymers, were investigated. The two ECL pathways were resolved and the corresponding mechanisms were proposed. In flow-injection (FI)-ECL system, the effects of 30 polyphenols and anilines and 20 amino acids on the ECL intensity of $Ru(bpy)_3^{2+}/C_2O_4^{2-}$, $Ru(bpy)_3^{2+}$/TPrA, and $Ru(phen)_3^{2+}$/TPrA systems were explored. The ECL enhancement and inhibition and their relationship were found, and the corresponding mechanisms were suggested. On this basis, the FI-ECL methods were established for the determination of numerous compounds. The main results of this study are as follows:

On Au and Pt electrodes with prECL method, two anodic ECL pathways were resolved at $Ru(bpy)_3^{2+}/C_2O_4^{2-}$ system, corresponding to two ECL peaks observed at 1.22 V (*vs.*SCE) (EP1) and 1.41 V (*vs.*SCE) (EP2) on Au electrode, at 1.22 V (EP1) and 1.40 V (EP2) on Pt electrode, respectively. The effects of $C_2O_4^{2-}$ concentration, medium pH, dissolved O_2, dissolved CO_2 and electrode pretreatment were studied. The surface state of the pre-oxidized Au electrode was also characterized by X-ray Photoelectron Spectroscopy (XPS) technique. Moreover, comparative studies on *i-E* and *I–E* curves were carried out and a possible mechanism involving the direct electro-oxidation pathway was proposed on the electrodes for the EP2 of $Ru(bpy)_3^{2+}/C_2O_4^{2-}$ system. It was postulated that the $CO_2^{-\cdot}$ was formed by electrooxidation at higher potentials because the surface absorbed oxygen on the Au electrode blocked

the further oxidation of $CO_2^{-\cdot}$ to CO_2. The observation of EP2 and its ECL mechanism involving the direct reaction of $C_2O_4^{2-}$ to $CO_2^{-\cdot}$ have not been reported in previous work.

On a PIGE and a GCE, the ECL and amperometric behavior of $Ru(bpy)_3^{2+}$ system was studied by CV, cyclic square wave (CSW) and chronoamperometric techniques. Under conventional CV conditions, the similar prECL behavior were observed at 1.18 V and 1.37 V for a PIGE, and at 1.20 V and 1.44 V for a GCE. A detection limit as low as 1×10^{-9} mol/L of $C_2O_4^{2-}$ was obtained on the PIGE by the CV method at 100 mV/s. The strong ECL signals were found on both electrodes in either an oxalate-containing aqueous solution or an organic solution when a CSW between two suitable potentials was used. Chronoamperometry was also applied by using a potential step from 0.15 to 1.85 V. It took 389 ms on a PIGE and 837 ms on a GCE to reach each maximal ECL intensity. It could be concluded that the low-cost PIGE exhibited better ECL-responses and sensitivity than the GCE, and presented excellent stability in aqueous solution.

Four $Ru(bpy)_3^{2+}$-immobilized polymer modified electrodes were fabricated by adsorbing different polymer on electrode surface, such as PVP homopolymer, P4VP homopolymer, PS-*b*-P4VP-*b*-PS block copolymer and PS-*b*-P4VP-*b*-PS/Nafion complex. The performance of different polymer modified electrode was evaluated based on the ECL and amperometric behavior and their lifetime. The experimental results showed that the PS-*b*-P4VP-*b*-PS/Nafion complex membrane modified electrode behavior was superior to other polymers in sensitivity and stability. The complex membrane posses not only the high selectivity and hydrophobicity of Nafion, but also the property of nanochannel, and specific charge of PS-*b*-P4VP-*b*-PS block copolymer.

The effect of adrenaline on ECL from $(bpy)_3^{2+}/C_2O_4^{2-}$or $Ru(bpy)_3^{2+}$/TPrA system was studied. Adrenaline was found to strongly inhibit ECL from $Ru(bpy)_3^{2+}$/ tripropylamine system when working electrode Pt was maintained at 1.05 V (vs. Ag/AgCl) in pH 8.0 phosphate buffer solution. Subsequently, a flow injection procedure with inhibited ECL detection was developed for determination of

adrenaline. The method exhibited a good reproducibility, sensitivity, and stability with a detection limit of 7.0×10^{-9} mol/L and dynamic concentration range of 2×10^{-8}-1×10^{-4} mol/L. The relative standard deviation is 2.18 % (n=11). It was successfully applied to the determination of adrenaline in pharmaceutical samples. Moreover, ECL emission spectra, UV-vis absorption spectra and CV of $Ru(bpy)_3^{2+}$/TPrA/ adrenaline were investigated. The inhibition mechanism has been proposed as the interaction of electrogenerated $Ru(bpy)_3^{2+*}$ and o-benzoquinone derivative such as adrenochrome and adrenalinequinone at the electrode surface.

Potential-dependent ECL in FI-ECL system was observed for both $Ru(bpy)_3^{2+}$/TPrA and $Ru(phen)_3^{2+}$/TPrA systems. Two ECL peaks were visible for $Ru(bpy)_3^{2+}$/TPrA system at 0.90 V and 1.05 V, and for $Ru(phen)_3^{2+}$/TPrA at 1.01 V and 1.25 V (*vs.* Ag/AgCl) in pH 8.0 phosphate buffer solutions. Sensitive ECL inhibition was observed in the presence of noradrenaline and dopamine for both of these systems. Moreover, for the detection of noradrenaline and dopamine, $Ru(phen)_3^{2+}$/TPrA system was better than $Ru(bpy)_3^{2+}$/TPrA system. Therefore, an FI-ECL inhibition method for determination of noradrenaline and dopamine has been developed based on $Ru(phen)_3^{2+}$/TPrA system. Under optimal conditions, the linear ranges for noradrenaline and dopamine were 2×10^{-8} ~ 2×10^{-5} mol/L and 4×10^{-8} ~ 2×10^{-5} mol/L, respectively, and detection limits were 7.1×10^{-9} mol/L and 1.5×10^{-8} mol/L, respectively. The $Ru(phen)_3^{2+}$/TPrA system was applied for determination of commercial pharmaceutical injection samples with satisfied results.

A FI –ECL method has been developed for determination of gallic acid based on an inhibition effect on $Ru(bpy)_3^{2+}$/TPrA ECL system in pH 8.0 phosphate buffer solution. The method is simple and convenient with a determination limit of 9.0×10^{-9} mol/L and dynamic concentration range of 2×10^{-8} - 2×10^{-5} mol/L. The relative standard deviation was 1.0 % for 1.0×10^{-6} mol/L gallic acid (n=11). It was successfully applied to the determination of gallic acid in Chinese proprietary medicine - Jianming Yanhou Pian.

The enhancement and inhibition of 20 natural amino acids on $Ru(bpy)_3^{2+}$/TPrA ECL system in three media such as PBS, $NaHCO_3$-Na_2CO_3 and NaOH were

systemically studied. It was found that the effects of tryptophan, tyrosine, cystine histidine and methionine transferred from the inhibition to the enhancement when the pH value increased from 8 to 13. However, the proline and hydroproline only exhibited the enhancement effect on the ECL of the $Ru(bpy)_3^{2+}$/TPrA system in all investigated pH. Based on the inhibition of the tyrosine and tryptophan in neutral media, a new FI-ECL method has been developed for the determination of them. The method is simple, rapid and sensitive with a detection limit of 2.7×10^{-8} mol/L and 1.3×10^{-8} mol/L for tyrosine and tryptophan. Finally, the mechanisms of both inhibition and enhancement of tryptophan, tyrosine, cystine histidine and methionine were proposed based on the results of ECL spectra, UV-vis spectra and variation of reaction condition investigation. It was suggested that the reaction between the exited state $Ru(bpy)_3^{2+*}$ and the different electro-oxidation products formed in different pH of the amino acids with specific structure was responsible for the enhancement and inhibition.

The effects of 30 polyphenols and analines on typical Ru complex ECL system were studied carefully. It was found that the ECL inhibition and enhancement were dependent on the pH of the solution from 8 to 13. At lower pH, the most of compounds showed the ECL inhibiting signal, whereas, at higher pH they exhibited ECL enhancing signal. Moreover, the ECL inhibition and enhancement were also related to the position of substituting group of the compounds. Both of the ECL inhibition and enhancement decreased as following order: *meta-* > *ortho-* > *para-*. The potential of analytical application was explored by use of the inhibited ECL. The results demonstrated that numerous compounds were detectable with the detection limits in the range of 10^{-8}-10^{-9} mol/L for $Ru(bpy)_3^{2+}$/TPrA system and 10^{-6}-10^{-7} mol/L for $Ru(bpy)_3^{2+}/C_2O_4^{2}$ system, respectively. The oxidation potential of the aromatic compounds, the ECL spectra, the UV-vis spectra, the direct ECL of $Ru(bpy)_3^{2+}$/aromatic compound were measured. The mechanism of inhibition and enhancement was proposed due to the reaction between the electro-oxidation products of these aromatic compounds and the exited state $Ru(bpy)_3^{2+*}$ or the free radical intermediate of the ECL reaction.