摘 要

本论文综述了化学发光（CL）的发展历史及特点、基本原理、化学发光体系、联用技术及分析应用和酚类化合物测定方法的研究进展。基于酚类化合物对硫酸铈(IV)－罗丹明 6G 体系的化学发光具有增强作用，本论文以深入认识硫酸铈(IV)－罗丹明 6G－酚类化合物化学发光体系的规律、机理和分析应用潜力为出发点，通过详细分析化学发光反应的动力学过程、化学发光光谱、荧光谱和紫外－可见吸收光谱，提出了该体系的化学发光机理；用流动注射－化学发光（FI－CL）联用技术系统研究了 32 种酚类化合物的化学发光强度和分子结构之间的关系，总结出酚类化合物对硫酸铈(IV)－罗丹明 6G 体系化学发光的影响规律；为了发展高灵敏度的液相色谱化学发光检测器，在硫酸铈(IV)－罗丹明 6G－酚类化合物化学发光体系的宽响应范围的基础上，建立了一系列酚类化合物的 FIA－CL 和高效液相色谱－化学发光（HPLC－CL）分析法。本论文取得的主要研究成果如下：

1. 基于硫酸铈(IV)和罗丹明 6G 在硫酸介质中可以发生氧化还原反应，产生微弱的化学发光，用 FI－CL 联用技术系统研究了 53 种令人感兴趣的不同分子结构的有机物对硫酸铈(IV)－罗丹明 6G 体系化学发光的影响。结果表明：苯环上的酚羟基是必需基团，且共有 32 种酚类化合物可以增强该体系的化学发光；各种酚类化合物的化学发光强度的大小与其苯环上的取代基种类和位置有关，分别总结出了苯酚、多酚、酚酸、羟基肉桂酸和类黄酮五大类化合物对该体系化学发光的影响规律。通过详细研究化学发光反应的动力学过程、化学发光光谱、荧光谱和紫外－可见吸收光谱，提出硫酸铈(IV)－罗丹明 6G－酚类化合物体系的化学发光机理为：在酸性介质中，硫酸铈(IV)氧化了罗丹明 6G 和酚类化合物，同时自身被还原为铈(III)。硫酸铈(IV)和酚类化合物之间的反应速度比硫酸铈(IV)和罗丹明 6G 之间的反应速度快，因而酚类化合物的存在可以加速激发态的铈(III)的产生。最后，能量从激发态的铈(III)转移给罗丹明 6G，将罗丹明 6G 分子激发，激发态的罗丹明 6G 分子辐射跃迁回到基态，发出波长为 555 nm 的特征光。各种酚类化合物的不同化学发光行为可能与其氧化产物相关，据此提出了硫酸铈(IV)－罗丹明

6G－酚类化合物的化学发光反应存在二个竞争的反应路径。最后，用 FI－CL 分析法研究了该发光体系的分析潜力，分别对 25 种酚类化合物进行了测定实验，其检测限的范围为 0.44－420 ng/ml。

2. 基于豆蔻明对硫酸铈(IV)－罗丹明 6G体系的化学发光的增强作用，建立了一种新颖的测定豆蔻明的FI－CL分析法，其线性范围为 1.0×10^{-8}－8.0×10^{-6} g/ml，检测限为 8.8×10^{-9} g/ml。该法与文献报道的仅有的一种高效液相色谱分离紫外检测法(HPLC－UV)相比，该法检测豆蔻明的线性范围更宽、灵敏度更高，丰富了豆蔻明的测定方法；成功应用于中草药草豆蔻中的豆蔻明含量的测定。

3. 基于葛根素对硫酸铈(IV)－罗丹明 6G体系的化学发光的增强作用，建立了一种新颖的测定葛根素的FI－CL分析法。葛根素浓度（C）的对数值与其净化学发光强度（ΔI）的对数值在 1.3×10^{-9}－8.0×10^{-7} g/ml 范围内呈现良好的线性关系，检测限为 8.4×10^{-10} g/ml，相对标准偏差为 1.86 %。该法的检测限比文献报道的紫外（UV）和电化学（EC）检测法均低了二个多数量级。本实验方法已成功应用于葛根素注射液中的葛根素定量分析，每小时可以测定 120 个样品溶液。

4. 基于黄酮醇可以增强酸性介质中硫酸铈(IV)－罗丹明 6G体系的化学发光，以及此化学发光体系与HPLC流动相的良好兼容性，首次建立了一个同时测定槲皮素、山萘酚和异鼠李素的HPLC－CL新方法。该法采用的流动相为甲醇和 1.0 %乙酸的混合物，在等度洗脱模式下对所测试的三种黄酮醇进行了很好的基线分离。该法测定槲皮素、山萘酚和异鼠李素的线性范围分别为 6.0×10^{-8}－7.0×10^{-5}，6.0×10^{-9}－7.0×10^{-6} 和 3.0×10^{-8}－7.0×10^{-5} g/ml，均达到了三个数量级以上；其检测限分别为 1.6×10^{-8}, 3.5×10^{-9}和 6.5×10^{-9} g/ml，明显优于文献报道的紫外检测法（UV）和蒸发光散射检测法(ELSD)的线性范围及检测限。本实验方法成功用于测定中药制剂心达康胶囊和沙棘颗粒中黄酮醇的含量。

5. 基于硫酸铈(IV)－罗丹明 6G－酚类化合物化学发光体系的宽响应范围，建

立了一种高选择性和高灵敏度的同时测定 20 种酚类化合物的 HPLC－CL 新方法。该方法测定 20 种酚类化合物的检测限范围为 1.5－82.1 ng/ml。对比 DAD 和 CL 的检测限，化学发光法的检测限均低于 DAD 测定的检测限。该化学发光体系与 HPLC 流动相的兼容性良好，为 HPLC 同时测定复杂体系中的多种酚类物质提供了一个简单、快速和高灵敏度的方法。该法检测实际样品时无需预浓缩或任何衍生步骤，已经成功应用于红葡萄酒中的酚类化合物的定量分析。

6. 发现几种对羟基苯甲酸酯在强酸介质中可以增强硫酸铈(IV)－罗丹明 6G 体系的化学发光。在此基础上，首次建立了一个同时测定对羟基苯甲酸甲酯、对羟基苯甲酸乙酯、对羟基苯甲酸丙酯和对羟基苯甲酸丁酯的 HPLC－CL 新方法。本法采用的流动相为甲醇－水(60:40, v/v)，四种酯通过等梯度洗脱在 8.5 分钟内快速完成了基线分离。其线性范围均达到了三个数量级以上，其检测限分别为 1.9、2.7、3.9 和 5.3 ng/ml。由于该法采用等梯度洗脱，检测不同样品时不需要花费过长的时间去平衡分析柱，故该法又具有检测速度快，效率高的优点，有利于实际应用中批量测定复杂基体中的单一或者多种痕量对羟基苯甲酸酯。该方法检测实际样品时无需预浓缩或衍生步骤，已成功应用于化妆品和食品中的多种对羟基苯甲酸酯类防腐剂的定量分析。

关键词： 硫酸铈(IV) 罗丹明 6G 酚类化合物 化学发光 流动注射分析 高效液相色谱 黄酮 对羟基苯甲酸酯

Abstract

The history, characteristics, principle, methodology and analytical application of chemiluminescence (CL) were reviewed. Also, the advance in analytical methods of phenolic compounds (PCs) was summarized. It was found that PCs could enhance the CL of cerium(IV)-rhodamine 6G system in sulfuric acid medium. On this basis, this dissertation focuses on the studies of the CL mechanism and analytical potential of cerium(IV)-rhodamine 6G-phenolic compound CL system. The main results of this study are as follows:

1. The oxidation reaction between cerium(IV) and rhodamine 6G in sulfuric acid medium underwent weak CL. The effects of 53 organic compounds of interest on cerium(IV)-rhodamine 6G CL were investigated by a flow injection (FI) procedure, and 32 PCs were found to enhance CL. PCs mainly include phenols, polyphenols, phenolic acids, hydroxycinnamic acids and flavonoids. The correlation between CL and molecular structure was systematically studied. It was noteworthy that phenolic hydroxyls were the main active groups for the generation of CL. The magnitude of CL was related to the type and position of substituents in the benzene ring. Based on the studies of kinetic process and the spectra of CL, fluorescence and UV-visible absorption, the cerium(IV)-rhodamine 6G-phenolic compound CL mechanism has been proposed to be due to that rhodamine 6G and phenolic compound are oxidized by cerium(IV) in sulfuric acid medium to form the excited-state cerium(III). The reaction rate between cerium(IV) and phenolic compound is faster than that of cerium(IV) with rhodamine 6G. Thus, the presence of phenolic compound can accelerate the generation of the excited-state cerium(III), and then energy is transferred from cerium(III)* to rhodamine 6G to form the excited-state rhodamine 6G, which emits its characteristic radiation at 555 nm. Moreover, there might be two competitive pathways in the present CL system. Finally, the analytical potential of this CL system was explored by a FI procedure, and 25

PCs were detectable at the range of 0.44－420 ng/ml.

2. Based on the CL enhancement by cardamonin of the cerium(IV)-rhodamine 6G system, a FI-CL method has been developed to determine cardamonin. The linear range is from 1.0×10^{-8} to 8.0×10^{-6} g/ml, and the detection limit was 8.8×10^{-9} g/ml cardamonin. In comparison with the results from the previously reported HPLC method, which is the only method using for the determination of cardamonin, wider linear range and lower detection limit were achieved. The applicability of this method was demonstrated by the analysis of cardamonin in *Alpinia katsumadai Hayata*.

3. Based on the CL enhancement by puerarin of the cerium(IV)-rhodamine 6G system, a sensitive and selective FI-CL method for the determination of puerarin was established. Under the optimum conditions, the proposed procedure has a linear range between 1.3×10^{-9} and 8.0×10^{-7} g/ml, with a detection limit of 8.4×10^{-10} g/ml puerarin and a relative standard deviation of 1.86%. In comparison with the results from the previously reported methods with UV and electrochemical (EC) detection, this detection limit was lower more than two orders of magnitude. The method was successfully applied to the determination of puerarin in injection. The sample solutions can be analyzed at a rate of 120 samples h^{-1}.

4. A novel HPLC-CL method has been developed for the simultaneous determination of three flavonols including quercetin, kaempferol and isorhamnetin. The procedure was based on the chemiluminescent enhancement by flavonols of the cerium(IV)-rhodamine 6G system in sulfuric acid medium. The CL reaction was well compatible with the mobile phase of HPLC. The good separation was achieved with an isocratic elution using a mixture of methanol and aqueous 1.0% acetic acid. Under the optimized conditions, a linear working range extends 3 orders of magnitude with relative standard deviations below 4.5%, and detection limits were 1.6×10^{-8}, 3.5×10^{-9}, and 6.5×10^{-9} g/ml for

quercetin, kaempferol and isorhamnetin, respectively. The proposed method has wider linear range and lower detection limit than those of the reported HPLC methods using UV detection and evaporative light scattering detection (ELSD). The proposed method has been successfully applied to the determination of three active flavonols in Shaji granule and Xindakang capsule by a simple extraction procedure.

5. Based on the CL enhancement by PCs of the cerium(IV)-rhodamine 6G system in sulfuric acid medium, A simple, selective and sensitive determination method of 20 PCs has been developed using HPLC with CL detection. The detection limits of 20 PCs were at the range of 1.5-82.1 ng/ml, which are lower than those of DAD. Thus, the proposed HPLC-CL method can be used to determine PCs at trace level in different matrices. Moreover, the CL reaction was well compatible with the mobile phase of HPLC. The method allows for the simultaneous and sensitive detection of PCs in red wine without preconcentration or derivatization step.

6. A new method for the simultaneous determination of parabens including methylparaben, ethylparaben, propylparaben, and butylparaben by HPLC coupled with CL detection was for the first time developed. The procedure was based on the chemiluminescent enhancement by parabens of the cerium(IV)-rhodamine 6G system in the strong sulfuric acid medium. The good separation of parabens was carried out with an isocratic elution using a mixture of methanol and water within 8.5 min. Under the optimized conditions, a linear working range extends 3 orders of magnitude, and the detection limits were 1.9×10^{-9}, 2.7×10^{-9}, 3.9×10^{-9}, and 5.3×10^{-9} g/ml for methylparaben, ethylparaben, propylparaben, and butylparaben, respectively. The CL reaction was well compatible with the mobile phase of HPLC. The proposed method has been successfully applied to the assay of parabens in wash-off cosmetic products and foods with the minimal sample preparation.