Available online at www.sciencedirect.com
SCIENCE DIRECT
Journal of Photochemistry and Photobiology A: Chemistry 181 (2006) 238–245
Journal of Photochemistry and Photobiology A:Chemistry
www.elsevier.com/locate/jphotochem

Chemiluminescence of cerium(IV)–rhodamine 6G–phenolic compound system

Hua Cui *, Qunlin Zhang, Aung Myint, Xuewu Ge, Lijuan Liu

Department of Chemistry, University of Science and Technology of China, Hefei, Anhui 230026, PR China

Received 27 June 2005; received in revised form 29 September 2005; accepted 1 December 2005
Available online 4 January 2006

Abstract

The oxidation reaction between cerium(IV) and rhodamine 6G in sulfuric acid medium underwent weak chemiluminescence (CL). The effects of 53 organic compounds of interest on cerium(IV)–rhodamine 6G chemiluminescence were investigated by a flow injection procedure, and 32 phenolic compounds were found to enhance CL. Phenolic compounds 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. The maximal emission wavelength of CL spectra for all tested phenolic compounds was at about 555 nm, and luminophors were assigned to rhodamine 6G. Based on the studies of kinetic process and the spectra of CL, fluorescence and UV–vis absorption, a CL mechanism has been proposed to be due to that rhodamine 6G and phenolic compound are oxidized by cerium(IV) to form the excited-state cerium(III), which transfers energy to rhodamine 6G, resulting in light emission. However, on the other hand, if the oxidation products of some phenolic compounds such as benzoquinone or ketone could significantly quench the emissive rhodamine 6G via energy transfer, no light emission was observed for such compounds as hydroquinone and catechol.

Keywords: Chemiluminescence; Cerium(IV); Rhodamine 6G; Phenolic compound

1. Introduction

There are some reports in the literature regarding the use of cerium(IV)–rhodamine chemiluminescence (CL) system for the determination of glutathione [1], captoril [2,3], chlorpromazine hydrochloride [4], flurosemide [5], phenothiazines [6], folic acid [7], analgin [8], tiopronin [9], hydrochlorothiazide [10], ceph-based β-lactam antibiotics [11], phentolamine [12], ascorbic acid [13] and DNA [14,15]. Most of these analytes are N- or S-containing compounds, but phenolic compounds (PCs) have not been systematically studied. In the most cases, including glutathione [1], captoril [2,3], chlorpromazine hydrochloride [4], flurosemide [5], phenothiazines [6], folic acid [7], analgin [8], tiopronin [9], hydrochlorothiazide [10], ceph-based β-lactam antibiotics [11] and phentolamine [12], rhodamine was used as a sensitizer, and the possible mechanism can be expressed as Scheme 1. In Scheme 1 SP represents glutathione, captoril, chlorpromazine hydrochloride, flurosemide, phenothiazines, folic acid, analgin, tiopronin, hydrochlorothiazide, ceph-based β-lactam antibiotics and phentolamine; SP_{OX}, the oxidized form of SP; Rho, rhodamine 6G or B.

For ascorbic acid [13], the oxidation product of rhodamine B was served as luminophor. The pathways can be described as Scheme 2. In Scheme 2 Rho B is rhodamine B; H_2A, the ascorbic acid; $[^{\bullet}\text{Rho B}^{-}]^{*}_{ox}$, the rhodamine B luminophor with negative oxygen ion.

For DNA [14], the oxidation product of the complex between rhodamine B and DNA was luminophor. The reaction scheme is (Scheme 3).

PCs occur as secondary metabolites in all plants [16] and embrace a considerable range of substances possessing

Abbreviations: CL, chemiluminescence; FI, flow injection; PC(s), phenolic compound(s)

* Corresponding author. Tel.: +86 551 3606645; fax: +86 551 3601592.
E-mail address: hcui@ustc.edu.cn (H. Cui).

1010-6030/$ – see front matter © 2006 Elsevier B.V. All rights reserved.
doi:10.1016/j.jphotochem.2005.12.003

$$Ce(IV) + SP \longrightarrow Ce(III)^{*} + SP_{ox}$$
$$Ce(III)^{*} + Rho \longrightarrow Ce(III) + Rho^{*}$$
$$Rho^{*} \longrightarrow Rho + h\nu \quad (\lambda = 555\ nm)$$

Scheme 1. The mechanism of the cerium(IV)–rhodamine–SP CL reaction.

$$Rho\ B \xrightarrow[H_2SO_4]{Ce(IV)} [{}^{\bullet}Rho\ B^{-}]^{*}_{OX} \longrightarrow [{}^{\bullet}Rho\ B^{-}]_{OX} + h\nu \quad (\lambda = 420\ nm)$$
$$H_2A \xrightarrow[H_2SO_4]{Ce(IV)} HA^{\bullet}$$
$$[{}^{\bullet}Rho\ B^{-}]_{OX} + HA^{\bullet} \xrightarrow[H_2SO_4]{Ce(IV)} [{}^{\bullet}Rho\ B^{-}]^{*}_{OX} + H^{+} + A$$

Scheme 2. The mechanism of the cerium(IV)–rhodamine B–H_2A CL reaction.

an aromatic ring bearing one or more hydroxy substituents. These compounds mainly include phenols, polyphenols, phenolic acids, hydroxycinnamic acids and flavonoids. They have received considered attention as the potentially protective factors against cancer and heart disease in part because of their potent antioxidative properties and their ubiquity in a wide range of commonly consumed foods of plant origin [17,18]. Up to now, knowledge of the levels of these compounds in plants is of considerable interest but is limited by the problems of analysis [19]. Lately, more interest has been shown in PCs due to their possible additional health benefits. Moreover, PCs are environmentally important compounds. Therefore, it is very important to develop highly sensitive analytical methods for the analysis of PCs.

CL techniques have received much attention because high sensitivity and wide linear range can be reached with simple instrumentation. However, only limited analytes can be detected using CL techniques due to fewer CL reactions available. Therefore, it is a great challenge for chemists to develop new CL reactions with wide application and expand the applicable scope of the existent CL reactions. In our previous work, it was found that the oxidation reaction between cerium(IV) and rhodamine 6G in sulfuric acid medium generated weak CL, which could be strongly enhanced by parabens [20]. In this paper, the effects of 53 organic compounds of interest on the cerium(IV)-rhodamine 6G CL were examined by a flow injection (FI) procedure and 32 PCs were found to enhance CL. The correlation between CL and molecular structure was systematically studied. The kinetic process and the spectra of CL, fluorescence, and UV–vis absorption were analyzed, and a CL mechanism was proposed.

$$DNA + Ce(IV) \xrightarrow{H_2SO_4} [Ce(IV)\text{-}DNA]$$
$$[Ce(IV)\text{-}DNA] + Rho\ B \longrightarrow [Rho\ B\text{-}DNA\text{-}Ce(IV)]$$
$$\xrightarrow{electron\text{-}transfer} [DNA\text{-}Rho\ B^{-}]^{*}_{OX} + Ce(III)$$
$$[DNA\text{-}Rho\ B^{-}]^{*}_{OX} \longrightarrow [DNA\text{-}Rho\ B^{-}]_{OX} + h? \quad (\lambda = 300\ nm)$$

Scheme 3. The mechanism of the cerium(IV)–rhodamine B–DNA CL reaction.

2. Experimental

2.1. Reagent

All chemicals were of analytical grade, and redistilled water was used throughout. Cerium(IV) sulphate tetrahydrate was obtained from Shanghai Yaolong Metal Company (Shanghai, China) and prepared in sulfuric acid solution daily. Rhodamine 6G and 4-hydroxy-3,5-dimethoxybenzoic acid were obtained from Merck (Darmstadt, Germany). Chlorogenic acid was purchased from Roth Chem. (Karlsruhe, Germany). 3,5-Dihydroxybenzoic acid, *m*-hydroxybenzoic acid and 4-hydroxy-3-methoxybenzyl alcohol were purchased from Fluka Chemie (Bucks, Switzerland). 2,3-Dihydroxybenzoic acid, 2,5-dihydroxybenzoic acid and 4-hydroxy-3-methoxybenzoic acid were purchased from Acros Organics (Fairlawn, NJ, USA). All tested flavonoids were obtained from Chinese National Institute for the Control of Pharmaceutical and Biological Products (Beijing, China). The other chemicals were purchased from Shanghai Reagents (Shanghai, China). The stock solution of 1.0×10^{-3} mol/l rhodamine 6G was prepared with redistilled water. The stock solutions of 1.0×10^{-4} g/ml phenolic compounds were prepared with redistilled water or methanol and standard solutions by diluting with redistilled water. The solutions of phenolic compounds were all stored in the dark at 0–4 °C, which was stable during 1 month.

2.2. Apparatus

CL measurements were made using a flow injection CL analyzer consisting of a model FIA-2400 flow injection system (Xintong, Beijing, China), a model 1P-21 photomultiplier tube (Binsong, Beijing, China) biased at −850 V and a model GD-1 luminometer (Ruike, Xi′an, China). The flow cell was a flat coil placed in front of photomultiplier tube. An IBM compatible personal computer was used for data acquisition. The CL and the fluorescent spectra were measured by using a model RF-5301 fluorimeter (Shimadzu, Kyoto, Japan). The UV–vis spectra were conducted on a model UV-2401PC spectrophotometer (Shimadzu, Kyoto, Japan). pH measurement was carried out on a model pHS-3B meter (REX, Shanghai, China).

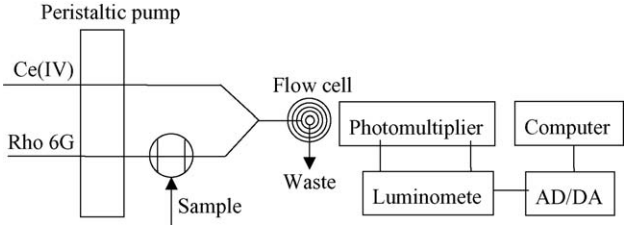


Fig. 1. Schematic diagram of FI–CL system.

2.3. Procedure

A schematic diagram of the FI–CL system is shown in Fig. 1. The solutions of 5.0×10^{-3} mol/l cerium(IV) containing 0.5 mol/l H_2SO_4 and 3.0×10^{-5} mol/l rhodamine 6G were pumped continuously at a rate of 3.0 ml/min into flow cell. The sample solution was introduced using a 100-μL loop valve injector. The CL emission was monitored by photomultiplier tube. Quantitative determination was based on the net CL intensity $\Delta I = I_S - I_0$, where I_S and I_0 are the CL signals in the presence and absence of phenolic compound, respectively. The assumed detection limit of the system is $\Delta I < 0.1$.

3. Results and discussion

3.1. CL of cerium(IV)–rhodamine 6G–PC system

The CL of cerium(IV)–rhodamine 6G reaction in the presence of 53 organic compounds of interest in sulfuric acid medium was explored by a FI–CL procedure. The structural formulae of some tested phenolic compounds are shown in Fig. 2. From the data of Table 1, the following conclusions can be drawn. There was no CL signal for the compounds without phenolic hydroxyl, including benzoic acid, phthalic acid, *p*-benzoquinone, *o*-phenylenediamine, *m*-phenylenediamine, *p*-phenylenediamine and sulphosalicylic acid. It implied that phenolic hydroxyls played a major role in the CL reaction. Altogether 32 PCs were found to enhance CL. The CL intensities of PCs might depend on their molecular structure such as electron delocalization and steric hindrance.

3.1.1. Phenols and polyphenols

Phenol was found to enhance the cerium(IV)–rhodamine 6G CL. However, CL signal disappeared, when substituent was an electron-withdrawing group $–NO_2$, such as *o*-nitrophenol, *m*-nitrophenol, *p*-nitrophenol and 2,4-dinitrophenol. For some compounds with the electron-donating groups including –OH, $–OCH_3$ and $–NH_2$, if these substituents were in the *ortho*- or *para*-position, no CL signal was found or CL intensity was very weak, e.g. eugenol, 4-hydroxy-3-methoxybenzyl alcohol, catechol, hydroquinone, *p-t*-butylpyrocatechol and adrenalin; If they were in the *meta*-position, CL emission was strong, e.g. *m*-aminophenol, resorcinol, pyrogallol and phloroglucin. Moreover, the CL intensity increased with increasing number of phenolic hydroxyls and followed the order: phloroglucin > pyrogallol > resorcinol > phenol. Phloroglucin generated stronger CL emission than pyrogallol because three phenolic hydroxyls of phloroglucin were in the *meta*-position. It was found that the effect of alkyl substituents on the CL emission of some compounds, such as 1-naphthol, thymol, tyrosine, was ambiguous.

3.1.2. Phenolic acids

When –COOH and –OH were in the *ortho*-position or two –OHs were in the *meta*-position, CL emission was strong. Thus 2,4-dihydroxybenzoic acid generated the strongest CL emission and 3,4-dihydroxybenzoic acid no CL emission. The CL intensities of some isomeric phenolic acids decreased in the following order: *o*-hydroxybenzoic acid > *p*-hydroxybenzoic acid > *m*-

Table 1
CL signal of some organic compounds[a]

Compound	ΔI
Benzoic acid	ND
Phthalic acid	ND
p-Benzoquinone	ND
o-Phenylenediamine	ND
m-Phenylenediamine	ND
p-Phenylenediamine	ND
Sulphosalicylic acid	ND
o-Nitrophenol	ND
m-Nitrophenol	ND
p-Nitrophenol	ND
2,4-Dinitrophenol	ND
Phenol	61.0 ± 0.9
1-Naphthol	143.6 ± 1.4
o-Aminophenol	ND
m-Aminophenol	34.4 ± 0.6
p-Aminophenol	ND
Thymol	186.8 ± 2.6
Tyrosine	45.6 ± 0.7
Eugenol	6.7 ± 0.1
trans-Ferulic acid	ND
4-Hydroxy-3-methoxybenzyl alcohol	ND
Catechol	ND
Resorcinol	148.2 ± 3.1
Hydroquinone	ND
p-t-Butylpyrocatechol	3.7 ± 0.1
Chlorogenic acid	17.1 ± 0.5
Adrenalin	13.4 ± 0.3
trans-Resveratrol	137.9 ± 2.4
Pyrogallol	163.6 ± 2.1
Phloroglucin	181.9 ± 2.2
o-Hydroxybenzoic acid	589.0 ± 8.5
m-Hydroxybenzoic acid	58.6 ± 1.2
p-Hydroxybenzoic acid	112.3 ± 1.7
4-Hydroxy-3,5-dimethoxybenzoic acid	3.7 ± 0.1
4-Hydroxy-3-methoxybenzoic acid	ND
2,4-Dihydroxybenzoic acid	1369.6 ± 24.7
3,4-Dihydroxybenzoic acid	ND
3,5-Dihydroxybenzoic acid	24.4 ± 0.4
2,3-Dihydroxybenzoic acid	236.8 ± 4.9
2,5-Dihydroxybenzoic acid	22.6 ± 0.3
Gallic acid	14.7 ± 0.2
Tannic acid	7.3 ± 0.1
Hesperidin	ND
Alpinetin	10.4 ± 0.1
Cardamonin	22.0 ± 0.3
Baicalin	24.4 ± 0.3
Kaempferol	145.3 ± 2.6
Quercetin	44.0 ± 0.5
Rutin	4.9 ± 0.1
Isorhamnetin	63.9 ± 0.8
Kaempferol-3-*o*-glucuronoside	35.0 ± 0.6
Calycosin	ND
Puerarin	495.6 ± 3.0

ND: not detected. ΔI: net CL intensity, mean ± S.D., $n = 5$.

[a] The sample concentration is 1.0×10^{-6} g/ml. CL conditions: Ce(IV), 5.0×10^{-3} mol/l (0.5 mol/l H_2SO_4); Rho 6G, 3.0×10^{-5} mol/l; Flow rate, 3.0 ml/min.

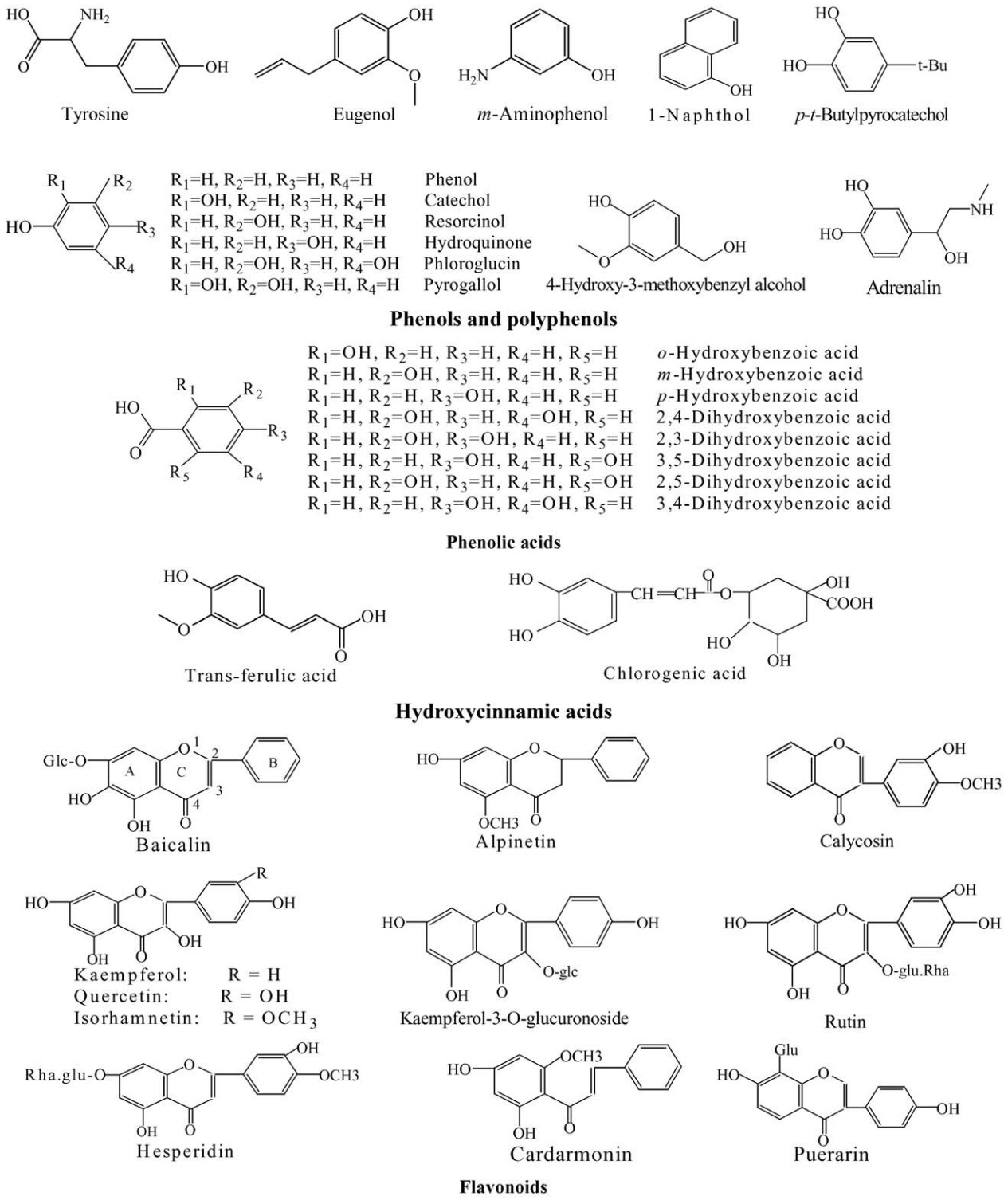


Fig. 2. Chemical structure of some tested phenolic compounds.

hydroxybenzoic acid; 2,4-dihydroxybenzoic acid > 2,3-dihydroxybenzoic acid > 3,5-dihydroxybenzoic acid > 2,5-dihydroxybenzoic acid > 3,4-dihydroxybenzoic acid.

3.1.3. Hydroxycinnamic acids

No CL signal was found for *trans*-ferulic acid, which is one of the most common hydroxycinnamic acid derivatives. But chlorogenic acid with glucose as a simple ester generated CL emission.

3.1.4. Flavonoids

First, no CL signal was found for calycosin, but baicalin was found to generate CL, which demonstrated that the hydroxyl groups in ring A were more important than the

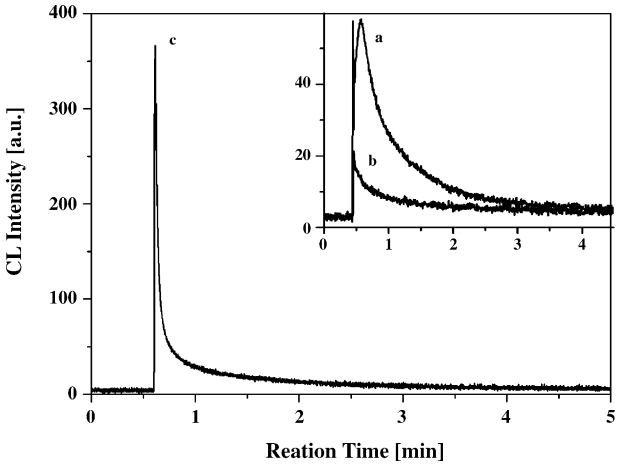


Fig. 3. Kinetic profile of the cerium(IV)–rhodamine 6G–phenol CL reaction. (a) Ce(IV) + Rho 6G; (b) Ce(IV) + phenol; (c) Ce(IV) + Rho 6G + phenol. Conditions: Ce(IV), 3.4×10^{-3} mol/l; Rho 6G, 1.4×10^{-5} mol/l; phenol, 2.9×10^{-5} g/ml.

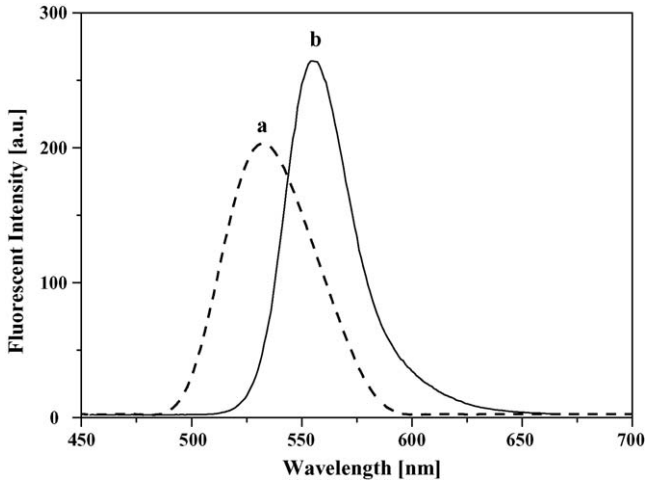


Fig. 4. Fluorescent spectra of rhodamine 6G with (a) and without (b) cerium(IV) solution. Conditions: (1) Ce(IV): 2.0×10^{-4} mol/l, Rho 6G: 1.0×10^{-5} mol/l; (2) Rho 6G: 8.0×10^{-7} mol/l.

ones in ring B. Second, for the flavonoids with hydroxyl group or glucoside on three positions of ring C, glycosides generated a weaker CL emission than aglycone, which may be due to steric hindrance. Thus, CL intensities were in the following order: rutin < quercetin; kaempferol-3-*o*-glucuronoside < kaempferol. Third, the structural types of flavonoids influence the CL intensity. Puerarin belongs to isoflavone and generates the strongest CL of all tested flavonoids.

The kinetic profile of cerium(IV)–rhodamine 6G–PC CL reaction was investigated with a static system. Herein phenol was discussed as a model compound. For the oxidation reaction between cerium(IV) and rhodamine 6G, weak CL was observed. The maximal peak was obtained at 6 s after mixing two reagents, and then the signal decreased slowly (Fig. 3a). When cerium(IV) and phenol were mixed, a weak light emission was also obtained (Fig. 3b). However, a strong enhancement of the CL emission of cerium(IV)–rhodamine 6G reaction was observed in the presence of phenol (Fig. 3c). Therefore, the addition of PCs could facilitate the cerium(IV)–rhodamine 6G CL reaction and greatly enhance the light emission.

3.2. CL mechanism of cerium(IV)–rhodamine 6G–PC system

The CL spectra of the cerium(IV)–rhodamine 6G reaction in the absence and presence of 32 PCs were recoded. All CL spectra are almost identical with the maximal wavelength at about 555 nm, which is in agreement with that of the fluorescent spectrum of rhodamine 6G. Thus, the luminophor could be ascribed to rhodamine 6G. Here the simplest PC phenol was chosen to explain the CL mechanism.

The fluorescent spectra of rhodamine 6G with and without cerium(IV) have been recorded and the maximal fluorescent wavelength was 530 and 555 nm, respectively (Fig. 4). Ma et al. reported that rhodamine B was oxidized by cerium(IV) to form a green fluorescent product ($\lambda_{ex}/\lambda_{em}$ = 467/535 nm) [21]. Rhodamine 6G and B have the same skeleton structure

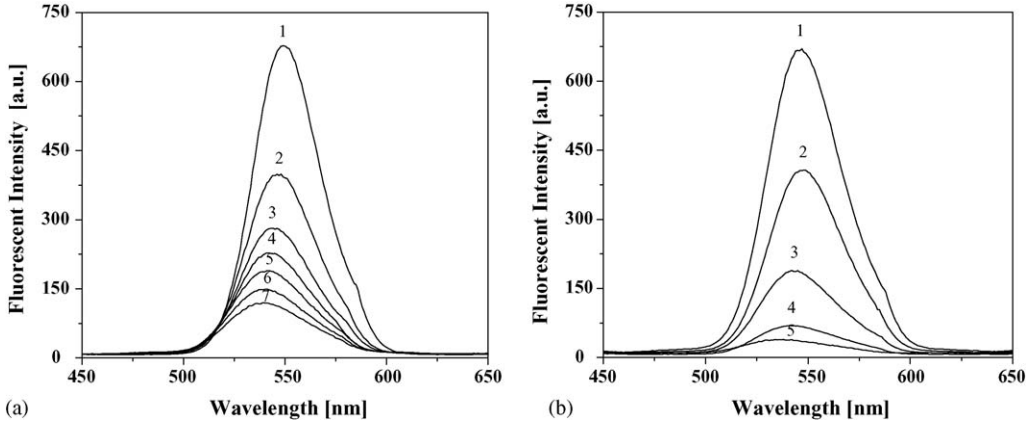


Fig. 5. Fluorescent spectra of the cerium(IV)–rhodamine 6G reaction. (a) Fluorescence spectrum vs. time profile. From spectra (1–7) the mixing time was 1, 2, 3, 4, 5, 6 and 7 min, respectively. Conditions: Ce(IV), 4.0×10^{-5} mol/l; Rho 6G, 5.0×10^{-7} mol/l; (b) Fluorescent spectra of cerium(IV) (8.0×10^{-5} mol/l) mixed with different concentrations of rhodamine 6G: (1) 1.3×10^{-6} mol/l; (2) 1.0×10^{-6} mol/l; (3) 7.5×10^{-7} mol/l; (4) 5.0×10^{-7} mol/l; (5) 2.5×10^{-7} mol/l. The mixing time was 1 min.

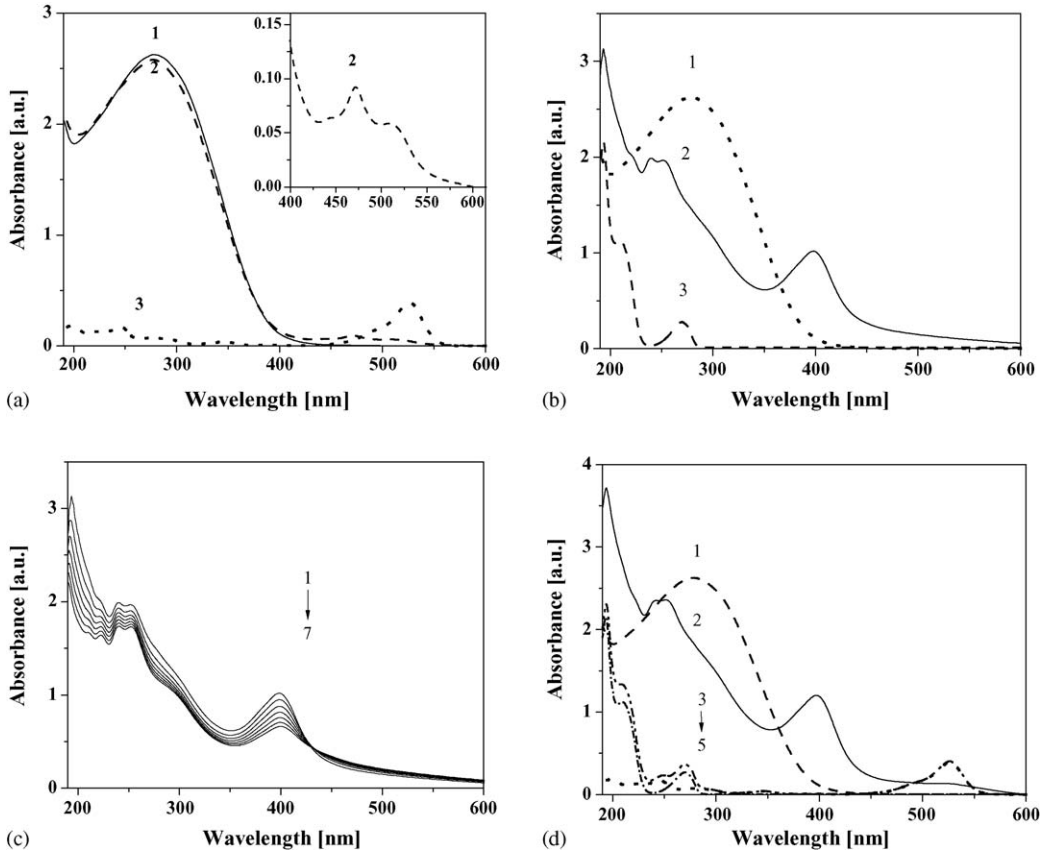
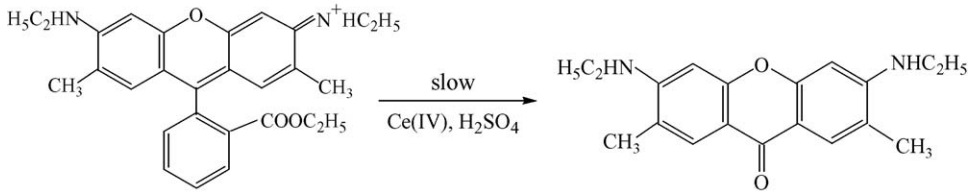


Fig. 6. Absorption spectra of the cerium(IV)–rhodamine 6G–phenol reaction. Reference solution, water. (a): cerium(IV)–rhodamine 6G reaction. (1) Ce(IV); (2) Ce(IV) + Rho 6G; (3) Rho 6G; (b): cerium(IV)–phenol reaction. (1) Ce(IV); (2) Ce(IV) + phenol; (3) phenol; (c): UV–vis spectrum vs. time profile of cerium(IV)–phenol reaction. From spectra (1–7) the mixing time was 1, 2, 3, 4, 5, 6 and 7 min, respectively; (d): cerium(IV)–rhodamine 6G–phenol reaction. (1) Ce(IV); (2) Ce(IV) + Rho 6G + phenol; (3) Rho 6G + phenol; (4) phenol; (5) Rho 6G. Conditions: Ce(IV), 8.0×10^{-4} mol/l; Rho 6G, 5.0×10^{-6} mol/l; phenol, 2.0×10^{-5} g/ml.

except a difference in substituents. Thus, the experimental data suggested the oxidized product of rhodamine 6G by cerium(IV) was almost the same as that of rhodamine B. Also, the cerium(IV)–rhodamine 6G reaction was monitored by the fluorescent detection. As shown in Fig. 5a, the fluorescent intensity at 555 nm decreases with an increase in mixing time, and the maximal wavelength shifts gradually from 550 to 538 nm. This indicated that the reaction between cerium(IV) and rhodamine 6G was a slow process. In addition, the fluorescent spectra of the cerium(IV)–rhodamine 6G reaction with different concentrations of rhodamine 6G were also recorded (Fig. 5b). The fluorescent intensity increased with an increase in rhodamine 6G concentration, and the maximal wavelength shifted from 535 to 547 nm. With an increase in rhodamine 6G concentration, the portion of the oxidized product of rhodamine 6G decreased, and the portion of rhodamine 6G increased. The above results confirmed that rhodamine 6G was oxidized by cerium(IV) to a fluorescent product at 530 nm. The UV–vis absorption of rhodamine 6G at 525 nm decreases after the addition of cerium(IV), and a new peak at 470 nm emerges (Fig. 6a), which is consistent with the one obtained by Navaratnam and Parsons [22]. Navaratnam found that the oxidant $Br_2^{\bullet -}$ reacted with rhodamine 6G to produce the species with distinctly maximum absorption at 470 nm by one-electron oxidation of rhodamine 6G. From these observations, it was concluded that the cerium(IV)–rhodamine 6G reaction would take place as Scheme 4.

It has been reported that the reaction of cerium(IV) with pyrogallol caused weak light emission [23]. In the present system, it was also observed that the cerium(IV)–PC reaction could



Scheme 4. The oxidation of rhodamine 6G.

produce weak CL, and the reactive product of PC was found to be nonfluorescent. The UV–vis absorption spectra of the reaction between cerium(IV) and phenol were analyzed. Fig. 6b indicates that the 270 nm peak of phenol disappears in the presence of cerium(IV), and a new compound is formed with an absorption band of 395 nm, which is almost identical with that of *o*-benzoquinone. It meant that phenol was converted into *o*-benzoquinone. Fig. 6c shows the absorption spectra of the cerium(IV)–phenol reaction at different time. It was found that the absorption at 395 nm decreased with an increase in mixing time, which demonstrated *o*-benzoquinone tends to decompose. Thus, the reaction is considered to be a rapid process. For cerium(IV) mediated phenolic oxidations, the mechanism has been discussed [24–26]. 2,6-Disubstituted phenol derivatives were oxidized by cerium(IV) perchlorate to the corresponding 1,4-benzoquinones, 4,4′-diphenoquinones, and oligomeric poly (1,4-phenylene) oxides, and nature and constitution of the oxidation products as a consequence of reaction conditions and physico-chemical properties of radicals and radical ions were reported [27].

Fig. 6d indicates the absorption spectra of the cerium(IV)–rhodamine 6G–phenol CL reaction. The absorption spectrum of the mixing solution of rhodamine 6G and phenol (curve 3) is exactly the addition of the spectrum of rhodamine 6G (curve 4) and phenol (curve 5), thus there is no reaction between rhodamine 6G and phenol. The UV–vis absorption spectrum of the mixture of cerium(IV), rhodamine 6G and phenol shows that the absorption of rhodamine 6G at 525 nm decreases and a new absorption band at 395 nm forms (curve 2). Therefore, it was evident that rhodamine 6G and phenol were oxidized by cerium(IV) to the oxidized product of rhodamine 6G and *o*-benzoquinone, respectively. On the other hand, the fluorescent spectrum taken from the mixture of cerium(IV), rhodamine 6G and phenol exhibits the characteristic emission of cerium(III) at 350 nm, indicating that cerium(IV) was reduced to cerium(III).

The CL emission wavelength of the cerium(IV)–rhodamine 6G reaction was at 555 nm, which demonstrated that the luminophor was not the oxidized product of rhodamine 6G but rhodamine 6G. Based on the above discussion, rhodamine 6G and PC are oxidized by cerium(IV) in sulfuric acid medium to form the excited-state cerium (III). The reaction rate between cerium(IV) and PC is faster than that of cerium(IV) with rhodamine 6G. Thus, the presence of PC 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. The mechanism of the cerium(IV)–rhodamine 6G–PC CL reaction can be summarized as Scheme 5. In Scheme 5 Rho 6G, PC and PC_{OX} are rhodamine 6G, phenolic compound and the oxidized form of PC.

According to the proposed mechanism, the CL emission of hydroquinone and catechol for the present CL reaction should be stronger than that of resorcinol because they more readily reacts with cerium(IV) to form cerium(III). However, our experimental data show the opposite results. Thus, it is supposed that this phenomenon is related to the oxidized products of hydroquinone,

$$\mathrm{Ce(IV) + Rho\ 6G \xrightarrow[H_2SO_4]{Slow} Ce(III)^* + Rho\ 6G_{ox}}$$
$$\mathrm{Ce(III)^* + Rho\ 6G \longrightarrow Ce(III) + Rho\ 6G^*}$$
$$\mathrm{Ce(IV) + PC \xrightarrow[H_2SO_4]{Fast} Ce(III)^* + PC_{ox}}$$
$$\mathrm{Ce(III)^* + Rho\ 6G \longrightarrow Ce(III) + Rho\ 6G^*}$$
$$\mathrm{Rho\ 6G^* \longrightarrow Rho\ 6G + hv}$$

Scheme 5. The mechanism of the cerium(IV)–rhodamine 6G–PC CL reaction.

catechol and resorcinol. The UV–vis data showed that two new peaks at 245 and 395 nm emerged when hydroquinone and catechol were oxidized by cerium(IV). The resulted products could be assigned to *p*-benzoquinone and *o*-benzoquinone, respectively. In this case, it is very difficult to identify characteristic peak of the oxidation products of resorcinol and phloroglucinol due to the spectral complexity, though it was reported that resorcinol and phloroglucinol could be oxidized to keto-enol tautomer or its dimmers [28,29]. The quenching of $Ru(bpy)_3^{2+}$ (bpy = 2,2′-bipyridine) electrogenerated chemiluminescence by benzoquinone was reported, and the mechanism was believed to involve energy transfer from the excited-state luminophore to benzoquinone [30]. We found that more than half of the fluorescent signal of rhodamine 6G was lost when 1000-fold *p*-benzoquinone was added to rhodamine 6G, which demonstrated that *p*-benzoquinone could quench the fluorescence of rhodamine 6G. The quenching mechanism was supposed to involve energy transfer from the excited-state rhodamine 6G to *p*-benzoquinone. Unfortunately, we could not obtain the oxidation products of catechol, resorcinol and phloroglucinol at this moment, thus it is impossible to carry out the studies on the fluorescent quenching of rhodamine 6G luminescence by these oxidation products. Based on the above discussion, we propose that there might be two competitive pathways in the present CL system. One is that cerium(IV) reacts with PCs to form the excited-state cerium (III), leading to strong light emission; Another one is that the oxidation products of PCs such as benzoquinone or ketone quench the emissive rhodamine 6G via energy transfer, as shown in Scheme 6. For hydroquinone and catechol, the quenching effect might be predominant, thus no CL signal was observed. For resorcinol and phloroglucinol, the quenching effect was very weak, thus they showed strong light emission. In Scheme 6 Rho 6G, PC and PC_{OX} are rhodamine 6G, phenolic compound and the oxidized form of PC.

$$\mathrm{Ce(IV) + Rho\ 6G \xrightarrow[H_2SO_4]{Slow} Ce(III)^* + Rho\ 6G_{ox}}$$
$$\mathrm{Ce(III)^* + Rho\ 6G \longrightarrow Ce(III) + Rho\ 6G^*}$$
$$\mathrm{Ce(IV) + PC \xrightarrow[H_2SO_4]{Fast} Ce(III)^* + PC_{ox}}$$
$$\mathrm{Ce(III)^* + Rho\ 6G \longrightarrow Ce(III) + Rho\ 6G^*}$$
$$\mathrm{Rho\ 6G^* + PC_{ox} \longrightarrow Quenching}$$

Scheme 6. The mechanism of the cerium(IV)–rhodamine 6G–PC reaction for compounds without CL.

4. Conclusions

The cerium(IV)–rhodamine 6G–phenolic compound reaction in sulfuric acid medium underwent strong CL. The magnitude of CL was related to the type and position of the substituents in the benzene ring. The maximal emission wavelength of CL spectra was at about 555 nm, and the luminophor was ascribed to rhodamine 6G. The CL mechanism was suggested that rhodamine 6G and phenolic compound are oxidized by cerium(IV) in sulfuric acid medium to form 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. If the oxidation products of phenolic compounds could significantly quench the emissive rhodamine 6G via energy transfer, no light emission would occur.

The primary survey shows that the CL system is capable of responding 32 phenolic compounds, thus this cerium(IV)–rhodamine 6G–PC CL reaction is ideal for the design of a CL detector in high-performance liquid chromatography (HPLC) and high-performance capillary electrophoresis (HPCE) to simultaneously detect PCs. The extension of this work is under way.

Acknowledgment

The support of this research by the Overseas Outstanding Young Scientist Program of China Academy of Sciences is gratefully acknowledged.

References

[1] H.N. Li, Y.X. Ci, L. Huang, Anal. Sci. 13 (1997) 821.
[2] X.R. Zhang, W.R.G. Baeyens, G. Van der Weken, A.C. Calokerinos, K. Nakashima, Anal. Chim. Acta 303 (1995) 121.
[3] Z.D. Zhang, W.R.G. Baeyens, X.R. Zhang, G. Van der Weken, J. Pharm. Biomed. Anal. 14 (1996) 939.
[4] Y.M. Huang, Z.H. Chen, Talanta 57 (2002) 953.
[5] Y. Rao, X.R. Zhang, G.A. Luo, W.R.G. Baeyens, Anal. Chim. Acta 396 (1999) 273.
[6] F.A. Aly, N.A. Alarfaj, A.A. Alwarthan, Anal. Chim. Acta 358 (1998) 255.
[7] A.A. Alwarthan, Anal. Sci. 10 (1994) 919.
[8] Y.M. Huang, C. Zhang, X.R. Zhang, Z.J. Zhang, Anal. Lett. 32 (1999) 933.
[9] T. Pérez-Ruiz, C. Martínez-Lozano, W.R.G. Baeyens, A. Sanz, M.T. San-Miguel, J. Pharm. Biomed. Anal. 17 (1998) 823.
[10] J. Ouyang, W.R.G. Baeyens, J. Delanghe, G. Van der Weken, A.C. Calokerinos, Talanta 46 (1998) 961.
[11] J.D. Yang, Y.M. Huang, S.P. Liu, Chin. J. Pharm. Anal. 22 (2002) 352.
[12] W.B. Liu, Y.M. Huang, Anal. Chim. Acta 506 (2004) 183.
[13] Y.J. Ma, M. Zhou, X.Y. Jin, B.Z. Zhang, H. Chen, N.Y. Guo, Anal. Chim. Acta 464 (2002) 289.
[14] H. Chen, M. Zhou, X.Y. Jin, Y.M. Song, Z.Y. Zhang, Y.J. Ma, Anal. Chim. Acta 478 (2003) 31.
[15] Y.J. Ma, M. Zhou, X.Y. Jin, Z.Y. Zhang, X.L. Teng, H. Chen, Anal. Chim. Acta 501 (2004) 25.
[16] J.J. Macheix, A. Fleuriet, J. Billot, Fruit Phenolics, CRC Press, Boca Raton, FL, 1990.
[17] F. Shahidi, M. Naczk, Food Phenolics, Technomic Publishing Co Inc., Lancaster, PA/Basel, Switzerland, 1995.
[18] V. Breinholt, Nature antioxidants and anticarcinogens, in: J. Kumpulainen, J. Salonen (Eds.), Nutrition, Health and Disease, Royal Society of Chemistry, Cambridge, UK, 1999.
[19] M. Antolovich, P. Prenzler, K. Robards, D. Ryan, Analyst 125 (2000) 989.
[20] A. Myint, Q.L. Zhang, L.J. Liu, H. Cui, Anal. Chim. Acta 517 (2004) 119.
[21] Y.J. Ma, X.Y. Jin, M. Zhou, Z.Y. Zhang, X.L. Teng, H. Chen, Anal. Chim. Acta 489 (2003) 173.
[22] S. Navaratnam, B.J. Parsons, J. Photochem. Photobiol. A 153 (2002) 153.
[23] K. Fujimori, N. Takenaka, H. Bandow, Y. Maeda, Anal. Sci. 17 (2001) 161.
[24] Y. Dzegets, K. Praviski, Izv. Vuz. Khim. Kh. Tekh. 35 (1992) 28.
[25] M. Ignachak, Y. Dzegets, S. Komisarski, G. Andrievski, M. Vodzinska, Zh. Obshch. Khim. 64 (1994) 1000.
[26] K. Pravitskii, Y. Dzegets, Zh. Obshch. Khim. 66 (1996) 2003.
[27] S. Domagala, V. Steglińska, J. Dziegieć, Monatsh. Chem. 129 (1998) 761.
[28] D.G. Wang, I. György, K. Hildenbrand, C.V. Sonntag, J. Chem. Soc. Perkin. Trans. 2 (1994) 45.
[29] M. Lohrie, W. Knoche, J. Am. Chem. Soc. 115 (1993) 919.
[30] J. McCall, C. Alexander, M.M. Richter, Anal. Chem. 71 (1999) 2523.