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Enhancing and inhibiting effects of aromatic compounds on luminol–dimethylsulfoxide–OH^- chemiluminescence and determination of intermediates in oxidative hair dyes by HPLC with chemiluminescence detection☆

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Abstract

The effect of 36 aromatic compounds on the luminol–dimethylsulfoxide–OH^- chemiluminescence (CL) was systematically studied. It was found that dihydroxybenzenes, and *ortho*- and *para*-substituted aminophenols and phenylenediamines inhibited the CL and phenols with three or more than three hydroxyls except phloroglucin tended to enhance the CL. The CL inhibition and enhancement was proposed to be dependent on whether superoxide anion radical ($O_2^{\bullet -}$) was competitively consumed by compounds in the CL system. Trihydroxybenzenes were capable of generating superoxide anion radical, leading to the CL enhancement, whereas dihydroxybenzenes were superoxide anion radical scavenger, causing the CL inhibition. Based on the inhibited CL, a novel method for the simultaneous determination of *p*-phenylenediamine, *o*-phenylenediamine, *p*-aminophenol, *o*-aminophenol, resorcinol and hydroquinone by high-performance liquid chromatography coupled with chemiluminescence detection was developed. The method has been successfully applied to determine intermediates in oxidative hair dyes and wastewater of shampooing after hair dyed.

Keywords: Aromatic compounds; HPLC; Luminol; Chemiluminescence

1. Introduction

High-performance liquid chromatography (HPLC) has been used as the most powerful separation technique in many fields. HPLC combining chemiluminescence (CL) detection represents an interface between the selectivity of an elegant separation method and the sensitivity of an ultrasensitive detection method. The advance of CL detection has greatly catalyzed the growth and popularity of HPLC–CL application, and made trace analysis possible owing to its capability of measuring picogram or femtogram quantities of compounds in the column eluate. Some CL reactions such as luminol and analogues [1–4], peroxyoxalates [1,5–9], tris(2,2′-bipyridyl)ruthenium [1,10–12], lucigenin [13–15] and polyphenols [16] have been used as practical detection in HPLC. Nevertheless, the analytical application of HPLC–CL detection has been restricted due to relatively fewer CL reactions available. Moreover, although some compounds are detectable by some CL reactions in a flow injection system, the mobile phases of HPLC are incompatible with the CL reactions, leading to that some compounds are undetectable in HPLC–CL detection. Therefore, it is important for chemists to expand the analytical potential of present CL reactions and explore new CL reactions so that the CL detection can become a popular detection technique suitable for numerous analytes.

In 1981, Hyland and Auclair [17] reported that $O_2^{\bullet -}$ could be generated by alkaline dimethylsulfoxide (DMSO) system. Later on, Zhao et al. [18] observed chemiluminescence of luminol–(DMSO)–NaOH system in 1987 and

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employed the new system for the determination of the activity of superoxide dismutase (SOD). However, to the best of our knowledge, there was no other research focusing on the analytical application of the luminol–DMSO–OH^-–H_2O and the analytical potential of the CL system has not been fully explored.

In this paper, the effects of 36 aromatic compounds on the CL reaction of luminol–DMSO–OH^-–H_2O in different media were examined. We found that the CL intensity of luminol–DMSO–OH^-–H_2O could be enhanced or inhibited by aromatic compounds. The correlation between such CL inhibition and enhancement and the pH of the medium and the structure of the compounds were studied. A possible mechanism of CL inhibition and enhancement was proposed according to CL spectra, UV-Vis absorption spectra, and variation of CL reaction conditions. Finally, analytical potential of the CL reaction for HPLC detector was explored.

Oxidation hair dyes are widely used in cosmetic products. The formulations for them consist essentially of aromatic diamines, aminophenols, polyhydroxybenzenes (such as resorcinol) and naphthol [19]. Owing to their sensitization and potential mutagenicity [20,21], the content of intermediates of oxidative hair dyes in hair coloring formulations is restricted according to Annex III of the EU Cosmetic Directive [22]. Despite the fact that thin layer chromatography (TLC) [23], gas chromatography (GC) [24,25], mass spectrometry [25], and micellar electrokinetic chromatography (MEKC) [26] have been used to detect oxidation hair dyes, most reports in the literature concerned HPLC methods [27–30]. However, UV-Vis detection was used in most of HPLC methods and the sensitivity was not good enough. It was found that the CL reaction was well suitable for the detection of intermediates of oxidative hair dyes separated by HPLC. Therefore, a sensitive HPLC–CL method was developed for the determination of intermediates such as *p*-phenylenediamine (PPDA), *o*-phenylenediamine (OPDA), *p*-aminophenol (PAP), *o*-aminophenol (OAP), resorcinol (RE) and hydroquinone (HQ) in oxidative hair dyes and wastewater of shampooing after hair dyed.

2. Experimental

2.1. Chemicals

Methanol was of HPLC grade. All other chemicals were analytical-reagent grade and the solutions were prepared with redistilled water. Luminol, 4-hydroxy-3,5-dimethoxybenzoic acid was purchased from Merck (Darmstadt, Germany). Phloroglucin, 3,5-dihydroxybenzoic 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 (New Jersey, USA). *p*-*tert*-Butyl catechol and *p*-aminophenol were purchased from Xingzhong Chemical Company (Shanghai, China). Chlorogenic acid was purchased from Roth Chem. (Karlsruhe, Germany). Catechol, hydroquinone, resorcinol, rutin, tannic acid, gallic acid, *m*-aminophenol, phenylenediamines, 1-naphthol and pyrogallol were purchased from China Medicine Group Shanghai Chemical Regent Corporation (Shanghai, China). Other organic compounds were purchased from Shanghai Reagents (Shanghai, China).

A 1×10^{-2} mol l^{-1} stock solution of luminol was prepared in 0.1 mol l^{-1} NaOH without further purification. The solution of NaOH and DMSO were prepared freshly. The HPLC mobile phases were fresh daily prepared, filtered through a 0.22 μm membrane filter (Xinya Company, Shanghai), and then degassed prior to use.

Stock solutions of 0.1 mg ml^{-1} aromatic compounds were prepared freshly by dissolving appropriate amount of aromatic compounds in redistilled water and were stored in dark at 4 °C. For HPLC–CL detection, the stock solutions of PPDA, OPDA, PAP, OAP, RE and HQ were prepared in mobile phase containing sodium sulfite (1%) and were stored in dark bottles at 4 °C and used within 2 days.

2.2. Apparatus

HPLC–CL detection system consisted of HPLC system and CL detection system (see supplementary material). The CL detection was conducted on a flow injection CL system (Remax, China) consisted of a model IFFM-D peristaltic pump, a mixing tee and a model IFFS-A CL detector equipped with a glass coil (used as reaction coil and detection cell) and a photomultiplier. This system was used for the CL detection in both FIA and HPLC systems. The HPLC system was Agilent 1100 series (Agilent Technologies, USA), including a binary pump, a thermostat column compartment, a diode array and multiple wavelength detector (DAD), a manual sample injection valve with a 100 μl loop, and an analytical column (Zorbax Eclipse XDB-C_8, 150 mm × 4.6 mm i.d, 5 μm; Agilent Technologies).

Absorption spectra were acquired at a Shimadzu UV-2401 spectrophotometer (Tokyo, Japan). CL spectra were measured on a Shimadzu RF-5301 spectrofluorometer (Tokyo, Japan). pH measurement was carried out on a REX pHS-3B meter (Shanghai REX, China).

2.3. Procedure

The schematic diagram shown in Fig. 1 illustrated FIA–CL detection system. The solutions of luminol, DMSO, NaOH and carrier water for samples were continuously pumped by the peristaltic pump at 2.0, 2.2, 2.1 and 1.8 ml min^{-1}, respectively. The sample solutions were injected with a sample loop of 100 μl. The light emission was monitored by the photomultipler tube.

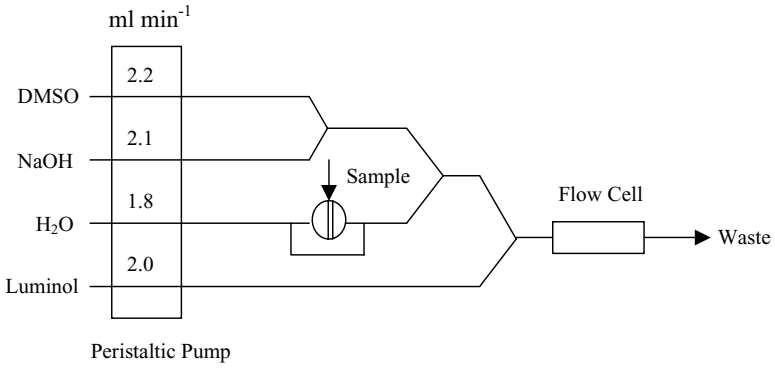


Fig. 1. Schematic diagram of the FIA–CL detection.

For HPLC–CL detection, the tested six aromatic compounds were separated by XDB-C_8 column at 25 °C with mobile phase at a flow rate of 1.0 ml min^{-1}. The manifold of post-column CL detection was same as that in FIA system shown in Fig. 1. The column effluent from DAD was first mixed with the combined stream of DMSO and NaOH solution at a mixing tee via a PEEK tube (600 mm × 0.25 mm i.d., Agilent technologies), then combined with luminol solution. The solutions of DMSO, NaOH and luminol were transported by a peristaltic pump with the same diameter of pump tubing and thus the same flow rate was obtained for the three reagent solutions. Detection by diode array was performed simultaneously at three different wavelengths: 254, 275, and 290 nm. The CL reagents were pumped into the flow cell at 2.8 ml min^{-1}, respectively. The data from the CL detector was acquired by Agilent Interface 35900E and processed by Agilent Chemstation (version A.08.03). For FIA–CL and HPLC–CL, each sample was injected in triplicate, the peak height was used for quantitative determination for DAD detection and the net CL intensity $\Delta I = I_S - I_0$ was used for quantitative determination for CL detection, where I_S was the CL intensity in the presence of aromatic compounds and I_0 was the intensity in the absence of aromatic compounds. The relative CL intensity ($\Delta I/I_0$) was used to study the effect of various factors on CL behavior.

2.4. Samples solution preparation

The main ingredients of the oxidative hair dyes were aromatic diamines, aminophenols and polyhydroxybenzenes. A 0.05 g aliquot of commercial hair dyes was dispersed in a mixture solution (20 ml methanol and 30 ml mobile phase B), containing 0.5 g sodium sulfite in a 50 ml dark volumetric flask. After 10 min ultrasound treatment, the mixture was cooled to the room temperature (25 °C). The sample suspensions were all filtered through a 0.22 μm membrane filter. A 0.5 ml aliquot of the filtrate was diluted to 5.0 ml with mobile phase containing 1% sodium sulfite and injected into HPLC within 24 h.

The wastewaters of shampooing after hair dyed were filtered by a 0.22 μm membrane filter and were analyzed within 24 h.

3. Results and discussion

3.1. Effect of aromatic compounds on the luminol–DMSO–OH^- –H_2O CL

The effect of 36 compounds on luminol–DMSO–OH^-–H_2O system was investigated in K_2HPO_4–KH_2PO_4, Na_2CO_3–$NaHCO_3$, NaOH and Na_2CO_3–NaOH media. The results are shown in Table 1. From these data the following conclusions can be drawn:

1. Dihydroxybenzenes except 2,4-dihydrobenzoic acid and *ortho*- and *para*-substituted aminophenols and phenylenediamines inhibited the CL independent of the tested pH. Whereas, phenols with three or more than three hydroxyls except phloroglucin such as pyrogallol, gallic acid and tannic acid enhanced the CL in all tested pH.
2. Aminophenols and phenylenediamines with substituents on *ortho*- and *para*-position showed strong inhibiting effects on luminol CL in all tested pH, however, there was no effect on CL with substituents on *meta*-position.
3. Two or three peaks were observed in CL kinetic curves for pyrogallol, tannic acid and hydroquinone, which implied that the CL reaction in the presence of phenolic compounds involved several pathways.
4. The results from Table 1 implies that it is ideal for the detection of trihydroxybenzenes by using the enhanced CL and for the detection of dihydroxybenzenes and their derivatives by using the inhibited CL. Additionally, for the determination of one of the aromatic compounds, other aromatic compounds are potential interference species. Therefore, luminol–DMSO–OH^-–H_2O CL reaction is well suitable for the detection of aromatic compounds separated by various separation techniques such as high-performance liquid chromatography and capillary electrophoresis (CE).

3.2. Analytical application

Oxidation hair dyes are widely used in cosmetic products. This work demonstrates that most intermediates

Table 1
Effect of the organic compounds on luminol–DMSO–NaOH CL

Medium	ΔI^a (K_2HPO_4–KH_2PO_4)			ΔI^a (Na_2CO_3–$NaHCO_3$)			ΔI^a (NaOH)	ΔI^a (NaOH–Na_2CO_3)
pH	7.13	8.29	9.00	9.26	10.10	11.21	12.13	13.05
Phenol	ND	ND	ND	ND	ND	ND	ND	ND
4-Hydroxy-3-methoxybenzyl alcohol	−330 ± 7	−533 ± 12	−323 ± 12	231 ± 1	287 ± 3	35 ± 1	25 ± 1	ND
4-Hydroxy-3-methoxybenzoic acid	ND	ND	ND	ND	ND	ND	ND	ND
Eugenol	−388 ± 2	−599 ± 9	−421 ± 3	−244 ± 1	−335 ± 1	−55 ± 3	−40 ± 1	ND
4-Hydroxy-3,5-dimethoxybenzoic acid	−366 ± 18	−578 ± 12	−402 ± 9	−250 ± 3	−329 ± 5	−54 ± 1	−38 ± 1	ND
trans-Ferulic acid	ND	ND	ND	ND	ND	ND	ND	ND
1-Naphthol	−418 ± 3	−599 ± 4	−419 ± 10	−322 ± 2	−359 ± 2	−42 ± 1	−21 ± 1	19 ± 1
2-Naphthol	ND	ND	ND	ND	ND	51 ± 1	27 ± 1	20 ± 1
1-Amino-2-naphthol-4-sulfonic acid	−292 ± 3	−473 ± 14	−210 ± 2	−232 ± 2	−210 ± 1	69 ± 1	39 ± 11	53 ± 1
Hydroquinone	−367 ± 2	−700 ± 4	−775 ± 8	−147 ± 1	−404 ± 4	−71 ± 8 66 ± 1	−58 ± 1 44 ± 1	54 ± 1
Catechol	−390 ± 2	−711 ± 7	−783 ± 4	−151 ± 1	−390 ± 5	−79 ± 1	−67 ± 1	−20 ± 1
Resorcinol	−356 ± 3	−661 ± 4	−550 ± 7	−124 ± 1	−813 ± 9	−67 ± 1	−57 ± 1	−18 ± 1
Pyrogallol	1717 ± 24	1663 ± 8	2017 ± 25	527 ± 16 610 ± 13 528 ± 4	1182 ± 10	1285 ± 23	859 ± 6	578 ± 5
Phloroglucin	−223 ± 7	−319 ± 5	−174 ± 3	−81 ± 2	−91 ± 3	−29 ± 1	ND	ND
4-*t*-Butylpyrocatechol	−406 ± 2	−603 ± 4	−527 ± 4	−250 ± 2	−803 ± 8	−88 ± 1	−57 ± 1	−1258 ± 25
Dopamine	−393 ± 2	−650 ± 13	−568 ± 2	−250 ± 2	−357 ± 2	−67 ± 1	−34 ± 1	ND
Adrenaline	−377 ± 2	−653 ± 4	−572 ± 4	−234 ± 2	−381 ± 2	−57 ± 1	−30 ±	ND
Noradrenaline	−383 ± 1	−657 ± 4	−497 ± 6	−250 ± 2	−345 ± 4	−70 ± 1	−23 ± 1	ND
Chlorogenic acid	−388 ± 4	−632 ± 14	−449 ± 7	−224 ± 6	−349 ± 7	−61 ± 1	−46 ± 1	−14 ± 1
Rutin	−300 ± 6	−588 ± 4	−320 ± 14	−148 ± 2	−230 ± 2	−55 ± 1	−44 ± 1	−15 ± 1
2,5-Dihydroxybenzoic acid	−402 ± 2	−678 ± 7	−599 ± 4	−226 ± 2	−373 ± 2	−83 ± 1	−42 ± 1	−16 ± 1
2,3-Dihydroxybenzoic acid	−397 ± 4	−659 ± 3	−564 ± 3	−215 ± 1	−366 ± 4	−80 ± 1	−40 ± 1	−14 ± 1
Protocatechuic acid	−402 ± 2	−678 ± 14	−599 ± 4	−226 ± 2	−373 ± 2	−83 ± 1	−42 ± 1	−16 ± 1
2,4-Dihydrobenzoic acid	ND	ND	ND	ND	ND	ND	ND	ND
3,5-Dihydrobenzoic acid	−157 ± 4	−275 ± 6	−128 ± 5	−120 ± 3	−104 ± 1	−25 ± 1	ND	ND
Gallic acid	973 ± 11	876 ± 31	740 ± 13	1860 ± 20	1084 ± 6	1061 ± 10	511 ± 15	4197 ± 99
Tannic acid	151 ± 8 −200 ± 3 307 ± 5	−451 ± 4	−291 ± 3 100 ± 1	582 ± 13 567 ± 15	309 ± 4 −82 ± 4 358 ± 9	285 ± 4 288 ± 4	107 ± 3 107 ± 1	1269 ± 51
Resveratol	−189 ± 4	−467 ± 10	−237 ± 6	−74 ± 1	−128 ± 1	−22 ± 1	ND	ND
p-Aminophenol	−306 ± 2	−706 ± 7	−820 ± 20	−152 ± 2	−356 ± 2	−72 ± 1	−43 ± 1	−17 ± 1
o-Aminophenol	−311 ± 6	−709 ± 17	−822 ± 24	−152 ± 1	−366 ± 3	−79 ± 1	−57 ± 1	−24 ± 1
m-Aminophenol	ND	ND	ND	ND	ND	ND	ND	ND
p-Phenylenediamine	−371 ± 7	−709 ± 4	−798 ± 10	−152 ± 1	−379 ± 2	−72 ± 1	−70 ± 1	−18 ± 1
o-Phenylenediamine	−261 ± 5	−584 ± 7	−398 ± 14	−125 ± 1	−225 ± 7	−36 ± 1	−31 ± 1	−12 ± 1
m-Phenylenediamine	ND	ND	ND	ND	ND	ND	ND	ND
Chloromycetin	ND	ND	ND	ND	ND	ND	ND	ND
Ascorbic acid	407 ± 26	255 ± 8	ND	108 ± 1	93 ± 7	51 ± 1	17 ± 1	15 ± 1

Working conditions: luminol, 1.5×10^{-4} mol l^{-1}; DMSO, 1.4 mol l^{-1}; NaOH, 0.1 mol l^{-1}; compound, 1 μg ml^{-1}. ND: not detected.

[a] Mean value ± S.D. ($n = 3$).

of oxidative hair dyes can inhibit the CL reaction of luminol–DMSO–OH^-–H_2O at lower pH values. Accordingly, we developed an HPLC–CL method for the simultaneous determination of PPDA, OPDA, PAP, OAP, RE and HQ in hair dyes and wastewater of shampooing after hair dyed.

3.2.1. Optimization of chromatographic separation

Several chromatographic methods have been used to analyze intermediates of oxidative hair dyes [27–30], however, most methods were not suitable for the separation of the target intermediates in the HPLC–CL system. For example, complete separation of these compounds could not be obtained with the methods reported by Shao et al. [27] and baseline drift of CL detection was caused by gradient program. The ingredients of elution led to unexpected effects on the CL behaviors. For example, the CL intensity was intensely inhibited by solvents such as methanol and acetonitrile used for separation in chromatographic system and pH value of the CL system was changed by the amino modifier and ion-pair reagents. Therefore, much attention was paid to the compatibility of chromatographic system and CL detection. Finally, a modified method based on Andrisano et al. [30] was adopted to separate these compounds. According to the basic properties of the hair dyes examined, triethylamine (TEA) was used as an amine modifier to suppress the adverse silanol effect and improved the peak shape. The mobile phase, a binary mixture of methanol (A)–ammonium acetate buffer solution (B) containing tetrabutyl ammonium bromide (TBAB) and TEA was found to be suitable for the separation of these compounds. Under the condition of complete separation, the concentrations of methanol, TBAB, TEA and the pH of buffer solution were investigated to obtain maximum CL intensity. The chromatographic peaks of PPDA and PAP overlapped when the concentration of methanol was 5%, whereas, peak overlap of HQ and OPDA occurred with 15% methanol. Therefore, 10% was chosen for the methanol concentration for this method.

The concentration of TBAB from 0 to 10^{-2} mol l^{-1} was studied. Baseline separation could not be obtained with concentration lower than 10^{-3} mol l^{-1}, whereas, maximal relative CL intensity was obtained when the concentration was chosen as 5×10^{-3} mol l^{-1}.

In the range of 0.05–1.5% (v/v), the effect of concentration of TEA on peaks shape and CL intensity was tested. With increasing the concentration of TEA, the peaks shape improved but the relative CL intensity decreased. Therefore, the concentration of 0.1% (v/v) was used.

The effect of ammonium acetate buffer solution on CL behaviors was studied. When the concentration of ammonium acetate was greater than 0.01 mol l^{-1}, the baseline separation could be obtained. Maximal relative CL intensity was obtained when the concentration was chosen as 0.025 mol l^{-1}. pH was tested at 5–8. When pH was 5, the tested compounds could not be separated from each other. When pH was higher than 5, good separation could be achieved and the relative CL intensity got maximum at pH 6.

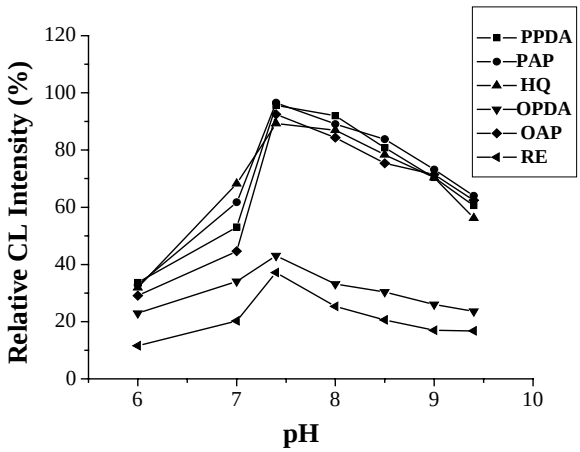


Fig. 2. Effect of pH of luminol solution on the relative CL intensity for the HPLC–CL detection. Separation conditions: mobile phase, A:B = 10:90 (A: methanol; B: 0.1% TEA + 0.025 mol l^{-1} NH_4Ac + 5×10^{-3} mol l^{-1} TBAB); pH 6.0; flow rate, 1.0 ml min^{-1}. CL reaction conditions: luminol, 1×10^{-4} mol l^{-1}; DMSO, 1.4×10^{-4} mol l^{-1}; NaOH, 0.1 mol l^{-1}; flow rate, 2.8 ml min^{-1}; compound, 100 ng ml^{-1}.

Therefore, the optimal mobile phase was 10% methanol solution containing 5×10^{-3} mol l^{-1} TBAB, 0.1% (v/v) TEA and 0.025 mol l^{-1} ammonium acetate (pH 6).

3.2.2. Optimization of the CL reaction conditions

The factors influencing the CL reaction such as the pH of solution and the concentration of luminol, NaOH and DMSO and the flow rate of solutions were studied in order to obtain maximal relative CL intensity.

It is well known that the luminol CL can only be produced in alkaline media. K_2HPO_4–KH_2PO_4, Na_2CO_3–$NaHCO_3$, NaOH, NaOH–Na_2CO_3 buffer solutions were used to study the effect of media. From Table 1, we can conclude that the CL intensity was the strongest in K_2HPO_4–KH_2PO_4 medium than other media. Thus, K_2HPO_4–KH_2PO_4 was chosen in this research. The effect of the pH on the CL intensity was studied ranging from 6.0 to 9.0. As shown in Fig. 2, the maximal relative CL intensity could be reached at pH 7.4. Thus, a K_2HPO_4–KH_2PO_4 buffer solution with pH 7.4 value was considered to be optimal.

The effect of the concentration of luminol solution was evaluated from 1×10^{-5} to 1×10^{-3} mol l^{-1} and the results are shown in Fig. 3. When luminol was 1×10^{-4} mol l^{-1}, the maximal relative CL intensity was obtained.

In the range of 0.1–3.0 mol l^{-1}, the effect of the concentration of DMSO on CL was tested. As shown in Fig. 4, the maximal relative CL intensity could be achieved at 1.4 mol l^{-1} DMSO. Therefore, 1.4 mol l^{-1} DMSO was chosen in the work.

Fig. 5 shows the effect of NaOH concentration on CL. All the tested compounds inhibited the CL in the test range. The maximal relative CL intensity was obtained with 0.1 mol l^{-1} NaOH.

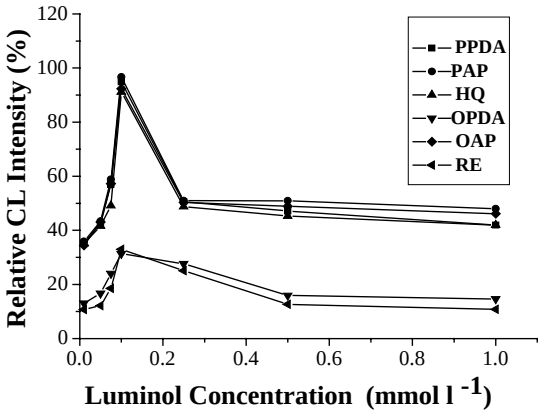


Fig. 3. Effect of luminol concentration on the relative CL intensity for the HPLC–CL detection. Separation conditions are the same as described in Fig. 2. CL reaction conditions: pH 7.4; DMSO, 1.4×10^{-4} mol l^{-1}; NaOH, 0.1 mol l^{-1}; flow rate, 2.8 ml min^{-1}; compound, 100 ng ml^{-1}.

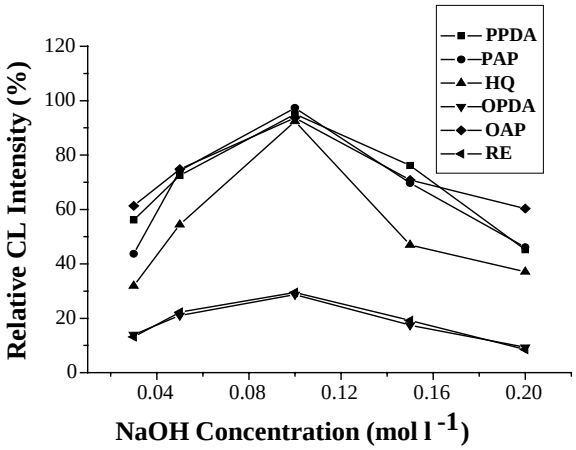


Fig. 5. Effect of NaOH concentration on the relative CL intensity for the HPLC–CL detection. Separation conditions are the same as described in Fig. 2. CL reaction conditions: luminol, 1×10^{-4} mol l^{-1}; DMSO, 1.4×10^{-4} mol l^{-1}; pH 7.4; flow rate, 2.8 ml min^{-1}; compound, 100 ng ml^{-1}.

The flow rate of DMSO, NaOH and luminol solutions was same in this case because they were controlled by the same pump and the same diameter of pump tubing. The effect of flow rate on CL was studied over the range 0.9–4.6 ml min^{-1} and the results are shown in Fig. 6. The relative CL intensity increased sharply in the range 0.9–2.8 ml min^{-1}, and then decreased dramatically beyond 2.8 ml min^{-1}. The optimal flow rate was 2.8 ml min^{-1}.

Under the optimal conditions described earlier, the chromatograms of standard solutions using DAD and CL detection are shown in Fig. 7A and B, respectively. The retention time for PPDA, PAP, HQ, OPDA, OAP, and RE was 1.67, 2.57, 4.91, 5.50, 7.82 and 12.22 min, respectively. The elution time of last peak was less than 14 min.

3.3. Method validation

Linearity, detection limit, precision, selectivity, and recovery were established to evaluate the method performance. The parameters of the regression equations and detection limits and precisions are shown in Tables 2 and 3, respectively. For all tested compounds, linear ranges of the CL detection were about two orders of magnitude. The detection limits (DLs) ($N + 3$S.D.) were in the range 0.21–32.35 ng ml^{-1} and the relative standard deviations ($n = 11$) were in the range 0.9–2.9%. From Table 3, one can conclude that the DLs of the CL detection for tested compounds are lower than that of DAD except that of OPDA.

In comparison with literature methods as shown in Table 4, the DLs of tested compounds using the proposed method were one to three orders of magnitude lower than

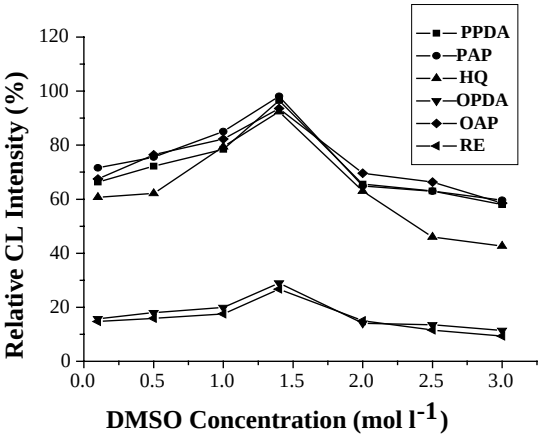


Fig. 4. Effect of DMSO concentration on the relative CL intensity for the HPLC–CL detection. Separation conditions are the same as described in Fig. 2. CL reaction conditions: luminol, 1×10^{-4} mol l^{-1}; pH 7.4; NaOH, 0.1 mol l^{-1}; flow rate, 2.8 ml min^{-1}; compound, 100 ng ml^{-1}.

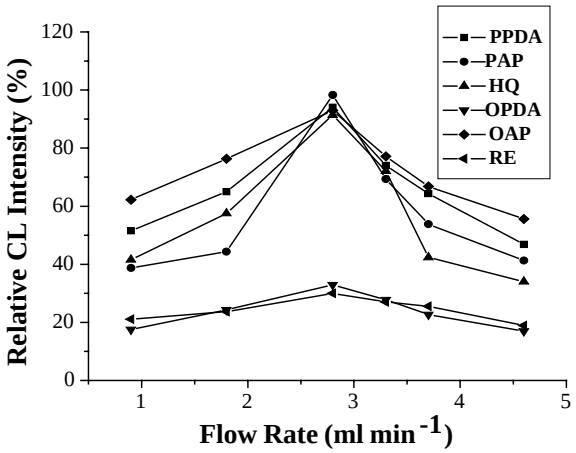


Fig. 6. Effect of flow rate on the relative CL intensity for the HPLC–CL detection. Separation conditions are the same as described in Fig. 2. CL reaction conditions: luminol, 1×10^{-4} mol l^{-1}; DMSO, 1.4×10^{-4} mol l^{-1}; pH 7.4; NaOH, 0.1 mol l^{-1}; compound, 100 ng ml^{-1}.

Table 2
Parameters of regression for tested compounds

Compound	Linear range (ng ml^{-1})	Regression equation ($\log \Delta I = a \log C + b$)	Correlation coefficient (r)
PPDA	1–250	$\log \Delta I = 0.355 \log C + 2.003$	0.993
PAP	1–100	$\log \Delta I = 0.342 \log C + 2.124$	0.994
HQ	10–1000	$\log \Delta I = 0.312 \log C + 1.734$	0.993
OPDA	100–10000	$\log \Delta I = 0.334 \log C + 1.398$	0.995
OAP	5–1000	$\log \Delta I = 0.359 \log C + 1.788$	0.994
RE	100–10000	$\log \Delta I = 0.365 \log C + 1.341$	0.996

C: concentration of compounds (ng ml^{-1}).

those methods in literatures and present HPLC–DAD. The results demonstrate that the HPLC–CL method is sensitive for the detection of all tested compounds.

3.4. Application

In order to validate the utility of the proposed method in real samples, the tested compounds in oxidative hair dyes and in wastewaters of shampooing after hair dyed were detected by the HPLC–CL method. As shown in Tables 5 and 6, good recoveries (90.6–105.2%) were obtained for the tested compounds. From data in Table 5, the results from the CL detection were comparable with those from DAD. Furthermore, lower concentrations of PPDA and PAP in wastewaters could be detected by CL but not by DAD detector. Therefore, the proposed method was applicable for the detection of tested compounds in hair dyes and wastewaters of shampooing after hair dyed. Chromatograms of typical hair dye (Xiandai) of DAD and CL detector are showed in Fig. 7C and D, respectively.

3.5. Mechanism of CL reaction

p-Aminophenol and pyrogallol were chosen as model compounds in the luminol CL system to elucidate the CL mechanism. It is well known that 3-aminophthalate

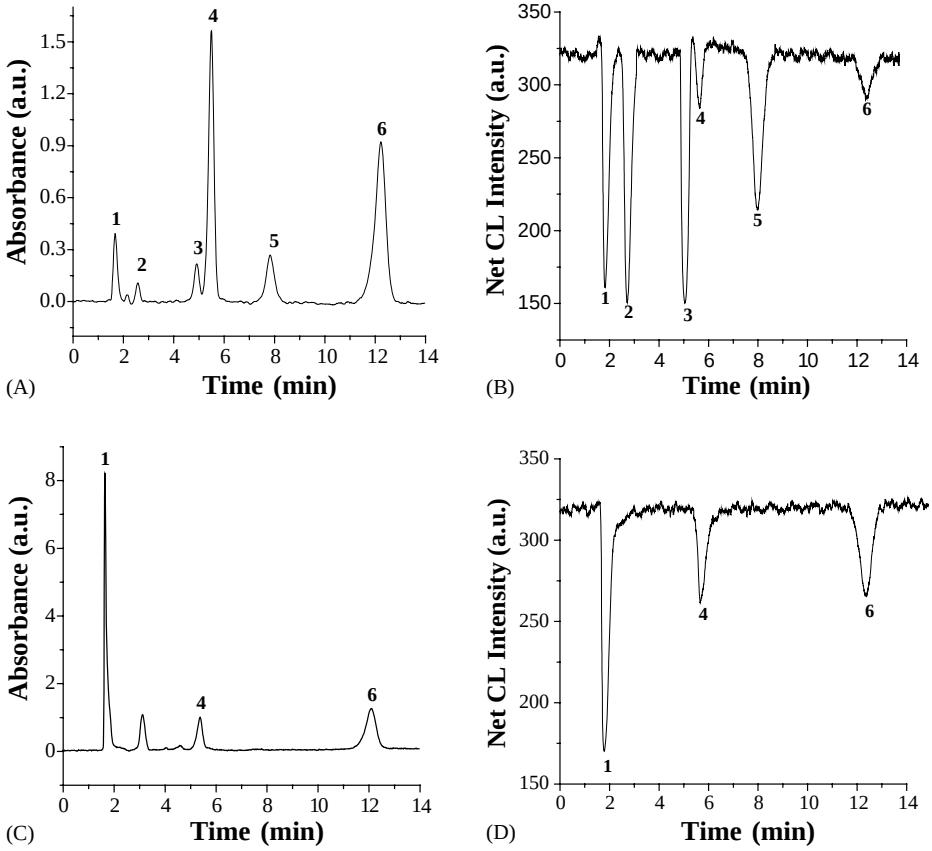


Fig. 7. Chromatograms of tested compounds with DAD at 275 nm and CL detection. CL reaction conditions: luminol, 1×10^{-4} mol l^{-1}; pH 7.4; DMSO, 1.4×10^{-4} mol l^{-1}; flow rate, 2.8 ml min^{-1}. Separation conditions are the same as described in Fig. 2. Peaks: 1 = PPDA; 2 = PAP; 3 = HQ; 4 = OPDA; 5 = OAP; 6 = RE. (A) Chromatogram of standard solution by DAD detection (compound, 100 ng ml^{-1}); (B) chromatogram of standard solution by CL detection (compound, 100 ng ml^{-1}); (C) chromatogram of Xiandai hair dyes by DAD detection; (D) chromatogram of Xiandai hair dyes (diluted 10 times) by CL detection.

Table 3
Detection limits and precision for tested compounds

Compound	Detection limit (ng ml^{-1})		Precision ($n = 11$)	
	CL	DAD	ng ml^{-1}	R.S.D. (%)
PPDA	0.48	30.01[a]	100.00	2.0
			5.00	1.7
PAP	0.21	54.11[a]	50.00	2.2
			5.00	2.7
HQ	3.21	44.62[b]	200.00	0.9
			25.00	2.2
OPDA	29.62	27.94[b]	500.00	2.6
			200.00	2.9
OAP	1.99	29.73[b]	100.00	2.9
			25.00	2.5
RE	32.35	48.54[c]	500.00	2.6
			200.00	1.7

[a] Wavelength: 254 nm.
[b] Wavelength: 290 nm.
[c] Wavelength: 275 nm.

(3-APA) is the luminophor for the two systems of luminol in alkaline aqueous solution and luminol in DMSO. The spectra of the CL reaction in the presence and absence of *p*-aminophenol and pyrogallol in Fig. 8 showed that the CL spectra were independent of *p*-aminophenol and pyrogallol, respectively, which revealed that the luminophor of the luminol–DMSO–OH^-–H_2O system in the presence of *p*-aminophenol and pyrogallol was still 3-APA.

UV-Vis absorption spectra in Figs. 9 and 10 showed that light absorption of the mixed system was different from the sum of the light absorption of the individuals system, which suggested that *p*-aminophenol and pyrogallol were involved in the luminol CL reaction.

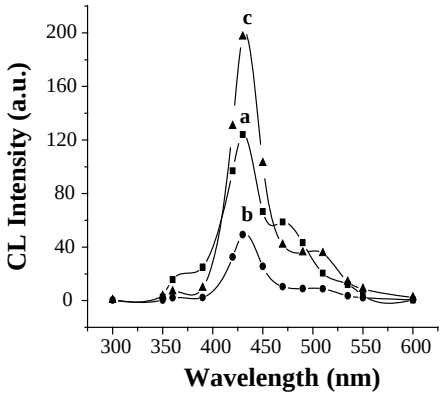


Fig. 8. Chemiluminescence spectra of luminol–DMSO–OH^- system. CL reaction conditions: luminol, 1.0×10^{-4} mol l^{-1}; pH 8.20; NaOH, 0.1 mol l^{-1}; DMSO, 1.4 mol l^{-1}. (a) Luminol–DMSO–OH^-; (b) luminol–DMSO–OH^-–PAP (5 ng ml^{-1}); (c) luminol–DMSO–OH^-–pyrogallol (10 ng ml^{-1}).

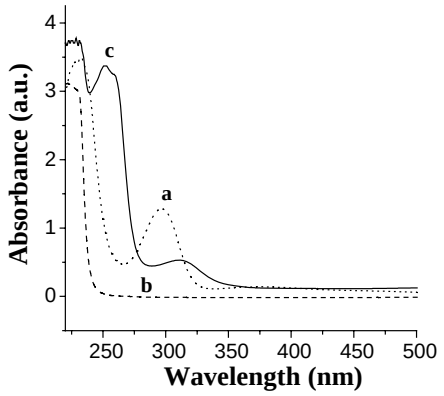


Fig. 9. UV-Vis absorption spectra. CL reaction conditions: PAP, 1.0×10^{-4} mol l^{-1}; DMSO, 1.4 mol l^{-1}; NaOH, 0.1 mol l^{-1}. (a) PAP; (b) DMSO + NaOH; (c) PAP + DMSO + NaOH.

Table 4
Detection of tested compounds with different methods

Compound	Method	Linear range (ng ml^{-1})	Detection limit (ng ml^{-1})	Reference
PPDA	This method	1–250	0.48	
	HPLC–ECD	10.8–1080	1.0	[31]
	Bioelectrochemistry		1.08	[32]
PAP	This CL method	1–100	0.21	
	HPLC–ECD	1–55	1.0	[33]
	Colorimetry	2000–100000	900	[34]
	Bioelectrochemistry		4.36	[32]
HQ	This method	10–1000	3.21	
	HPLC–CL	5–1300	2.0	[2]
	Biosensor	8250–176500	891	[35]
OPDA	This method	100–10000	29.62	
	Bioelectrochemistry		1.08	[32]
OAP	This method	5–1000	1.99	
	Amperometry	5450–43600	2180	[36]
	Bioelectrochemistry		1.09	[32]
RE	This method	100–10000	32.35	
	HPLC–CL	1000–50000	680	[2]
	FIA–CL		572	[37]

Table 5
Recovery of tested compounds in oxidative hair dyes

Brand	Compound	HPLC–CL ($\mu g\,ml^{-1}$)				HPLC–DAD ($\mu g\,ml^{-1}$)			
		Original[a]	Added	Found[a]	Recovery (%)	Original[a]	Added	Found[a]	Recovery (%)
Savol	PPDA	1.27 ± 0.03	1.00	2.18 ± 0.03	91.3	1.39 ± 0.02	1.00	2.46 ± 0.05	106.3
	OPDA	1.47 ± 0.02	1.00	2.39 ± 0.07	94.7	1.35 ± 0.01	1.00	2.26 ± 0.05	91.4
	RE	0.29 ± 0.01	0.50	0.76 ± 0.02	94.4	0.30 ± 0.01	0.50	0.80 ± 0.01	100.8
Zhanghua	PPDA	0.99 ± 0.02	1.00	1.91 ± 0.05	91.6	1.03 ± 0.03	1.00	2.11 ± 0.04	107.8
	OPDA	1.27 ± 0.03	1.00	2.20 ± 0.01	92.5	1.20 ± 0.02	1.00	2.23 ± 0.06	103.3
	RE	0.25 ± 0.01	0.50	0.70 ± 0.01	90.6	0.26 ± 0.01	0.50	0.75 ± 0.01	98.6
Xiandai	PPDA	1.92 ± 0.03	2.00	3.74 ± 0.05	91.2	2.06 ± 0.04	2.00	4.13 ± 0.03	106.3
	OPDA	1.57 ± 0.03	1.00	2.57 ± 0.05	100.0	1.59 ± 0.05	1.00	2.54 ± 0.05	94.7
	RE	0.45 ± 0.01	0.50	0.91 ± 0.02	92.6	0.46 ± 0.02	0.50	0.95 ± 0.02	98.2
Guangming	PPDA	12.66 ± 0.24	10.00	22.02 ± 0.42	93.6	14.48 ± 0.04	10.00	24.84 ± 0.55	103.6
	RE	8.46 ± 0.16	5.00	13.72 ± 0.39	105.2	8.21 ± 0.03	10.00	17.99 ± 0.18	97.9

ND: not detected.
[a] Mean value ± S.D. ($n = 3$).

The superoxide anion radical plays a key role in the formation of the electronically excited state in luminol CL reactions [38,39]. Hyland and Auclair [17] found that $O_2^{\bullet -}$ could be generated by a reaction among O_2, OH^- and dimethylsulfoxide. The possible pathways for luminol–DMSO–OH^-–H_2O are described as follows:

$$2(CH_3)2SO + 2OH^- + 3O_2 \rightarrow 2CH_3SOOH + CH_3OOCH_3 + 2O_2^{\bullet -} \quad (1)$$

$$LH_2 + OH^- \rightarrow LH^- + H_2O \quad (2)$$

$$LH^- + O_2^{\bullet -} \rightarrow L^{\bullet -} + HO_2^- \quad (3)$$

$$L^{\bullet -} + O_2^{\bullet -} \rightarrow LO_2^{2-} \quad (4)$$

$$LO_2^{2-} \rightarrow AP^{2-*} + N_2 \quad (5)$$

$$AP^{2-*} \rightarrow AP^{2-} + h\nu \quad (6)$$

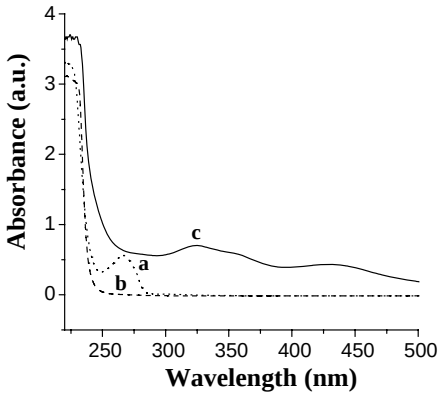


Fig. 10. UV-Vis absorption spectra. CL reaction conditions: pyrogallol, 1.0×10^{-4} mol l^{-1}; DMSO, 1.4 mol l^{-1}; NaOH, 0.1 mol l^{-1}. (a) pyrogallol; (b) DMSO + NaOH; (c) pyrogallol + DMSO + NaOH.

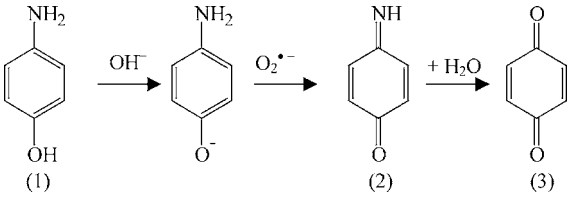
Table 6
Recovery of tested compounds in wastewaters of shampooing after hair dyed

Brand	Compound	HPLC–CL ($ng\,ml^{-1}$)				HPLC–DAD
		Original[a]	Added	Found[a]	Recovery (%)	
Shengshida	PAP	5.93 ± 0.01	5.00	11.23 ± 0.02	106.0	ND
Yuanhua	PPDA	106.12 ± 2.12	50.00	157.33 ± 1.58	102.4	ND
	PAP	3.67 ± 0.11	5.00	8.78 ± 0.15	102.2	ND
Lambia	PPDA	62.62 ± 1.25	50.00	115.03 ± 0.12	104.8	ND
	PAP	3.30 ± 0.08	50.00	50.93 ± 1.48	95.3	ND
Youngrace	PPDA	47.34 ± 0.95	50.00	95.26 ± 1.98	95.8	ND
	PAP	2.14 ± 0.04	50.00	53.14 ± 1.06	102.0	ND
Weicaili	PPDA	73.14 ± 1.05	50.00	123.10 ± 1.48	99.9	ND
	PAP	10.92 ± 0.10	50.00	61.13 ± 1.73	100.4	ND
Vincente	PPDA	36.23 ± 0.87	50.00	83.14 ± 2.18	93.8	ND
	PAP	2.70 ± 0.07	50.00	48.73 ± 0.57	92.1	ND

ND: not detected.
[a] Mean value ± S.D. ($n = 3$).

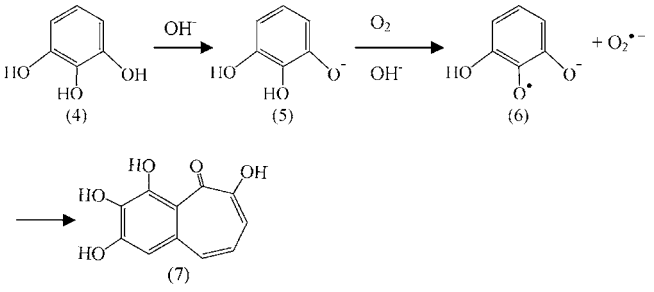
The symbols LH_2, LH^-, $L^{\bullet -}$, LO_2^{2-}, AP^{2-*} refer to luminol, luminol monoanion, luminol radical, luminol endoperoxide, and excited state 3-aminophthalate dianion.

Alanko et al. [40] found that trihydroxybenzenes were capable of generating superoxide anion radical, whereas dihydroxybenzenes were superoxide anion radical scavenger. We presumed that the inhibiting and enhancing effect might depend on whether or not $O_2^{\bullet -}$ was competitively consumed by compounds in the CL system. If $O_2^{\bullet -}$ was consumed by the compounds, the inhibition occurred. By contraries, if the compounds could accelerate the formation of $O_2^{\bullet -}$, enhancement would appear. For PAP (1), the absorption intensity at 295 nm decreased greatly after mixing with DMSO + NaOH solution and the absorption peak at 249 nm appeared. This demonstrated that the reaction between *p*-aminophenol and DMSO + NaOH took place. The CL inhibition is likely due to that $O_2^{\bullet -}$ generated by DMSO + NaOH was competitively consumed by *p*-aminophenol, similar to dihydroxybenzene, to produce *p*-benzoquinone (3) [41], resulting in a decrease in the CL intensity:



(7)

Pyrogallol (4) is a trihydroxybenzene. Abrash et al. [42] reported that pyrogallol reacted with oxygen in alkaline solution to produce purpurogallin (5) with absorption maximum at 320 nm. After mixing with DMSO + NaOH solution, the absorption intensity of pyrogallol at 270 nm decreased greatly and the absorption peak at 322 nm appeared, which implied that pyrogallol was probably oxidized by the dissolved oxygen to purpurogallin in this CL system. The reaction may be as follows:



(8)

During the oxidation of pyrogallol, $O_2^{\bullet -}$ was generated, which accelerated the luminol CL reaction, leading to the CL enhancement.

4. Conclusion

More than 30 aromatic compounds were found to inhibit or enhance the CL from luminol–DMSO–OH^-–H_2O system, depending on whether or not $O_2^{\bullet -}$ was competitively consumed by compounds in the CL system. Trihydroxybenzenes, capable of generating superoxide anion radical, exhibited the CL enhancement, whereas dihydroxybenzenes, as superoxide anion radical scavenger, demonstrated the CL inhibition. This study implies that it is good for the detection of dihydroxybenzenes and their derivatives by using the inhibited CL and for the detection of trihydroxybenzenes by using the enhanced CL. Based on the inhibited CL reaction, a sensitive HPLC–CL method was established for the simultaneous determination of *p*-phenylenediamine, *o*-phenylenediamine, *p*-aminophenol, *o*-aminophenol, resorcinol and hydroquinone in oxidative hair dyes.

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