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Lucigenin-pyrogallol chemiluminescence for the multiple detection of pyrogallol, cobalt ion, and tyrosinase

Islam M. Mostafa^{a,b,c}, Muhammad Rehan Hasan Shah Gilani^{a,d}, Yequan Chen^e, Baohua Lou^{a,b,*}, Jianping Li^{e,*}, Guobao Xu^{a,b,*}

^a State Key Laboratory of Electroanalytical Chemistry, Changchun Institute of Applied Chemistry, Chinese Academy of Sciences, Changchun, Jilin 130022, PR China

^b University of Science and Technology of China, Hefei, 230000, PR China

^c Department of Analytical Chemistry, Faculty of Pharmacy, Minia University, 61519, Minia, Egypt

^d Institute of Chemical Sciences, Bahauddin Zakariya University, Multan 60800, Pakistan

^e College of Chemistry and Bioengineering, Guilin University of Technology, Guilin, 541004, China

Abstract

Developing new, cheap, sensitive and selective chemiluminescence (CL) systems with multiple detection properties is still a big challenge for biological and environmental applications. Here, we report a new CL system having multiple detection applications (environmental and biological). The developed lucigenin-pyrogallol system gave an enhancement (190 times) over the conventional lucigenin-H₂O₂ CL system. Cobalt ion and tyrosinase can inhibit lucigenin-PG CL intensity. Based on these phenomena, we have developed new CL methods for the detection of pyrogallol (LOD = 0.94 μM), lucigenin (LOD = 0.42 μM), cobalt (LOD = 68 nM), and tyrosinase (LOD = 1.8 μg/mL). Furthermore, the developed system gave excellent recoveries in real samples within the range of 93.59–103.12% for the assay of pyrogallol, Co²⁺ and tyrosinase in coffee, lake water, and human plasma, respectively.

Keywords: Chemiluminescence, Cobalt, Lucigenin, Pyrogallol, Tyrosinase

1. Introduction

Challenges in the development of rapid, sensitive and selective chemiluminescence (CL) systems for numerous detecting applications have attracted tremendous research awareness for many years. Inspired by CL inherent features which include short analysis time, low background noise, wide linear dynamic range, lower detection limit, and easy availability of the instrumentation [1,2], many researchers have been studying CL. Recently, most of the researchers are looking for novel and stable cor-reactants for different types of luminophores like

luminol and lucigenin to develop selective CL systems with multiple detections ability.

Following the discovery of luminol in 1920, another luminophore called lucigenin was discovered in 1935 and it is considered one of the most effective luminophores with extensive scope of scientific applications. Lucigenin have been paired with H₂O₂ for the first attempt in 1935 as a novel CL system [3]; however, this CL system suffered from several serious disadvantages including weak stability and poor selectivity for its real applications and implementation especially in a matrix containing metal ions as example plasma and urine [4]. Recently, researchers incorporated different synthesized nanomaterial such as quantum dots and

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* Corresponding author: State Key Laboratory of Electroanalytical Chemistry, Changchun Institute of Applied Chemistry, Chinese Academy of Sciences, 5625 Renmin Street, Changchun, Jilin 130022, China. Tel/fax: +86 431 85262747.
E-mail addresses: loubh@ciac.ac.cn (B. Lou), likianping@263.net (J. Li), guobaouxu@ciac.ac.cn (G. Xu).

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nanoclusters into CL platforms to develop and extend the CL applications [5–7]. Despite that the numerous efforts have been made, the search for a practical and broadly useful CL system with multiple detection applications has remained very challenging.

Meanwhile the quality of food, beverages, and juices are very important issue in our life because it mainly affects our health. Phenolic compounds are commonly found in food, beverages, coffee, oils and juices. The quantification of polyphenols is important owing to their antioxidant health benefits. Also, phenolic compounds are responsible for the self-oxidation stability and organoleptic properties of foods [8–10]. What's more, in the course of recent decades, numerous specialists have concluded that eating routine food rich in polyphenolic compounds assumes a significant therapeutic role in lessening the danger of malignant growth, cardiovascular infection, inflammation, diabetes, and other degenerative diseases [11].

Pyrogallol (PG) is a natural polyphenolic compound that extensively found in different fruits, tea, crops, coffee, and many kinds of plants. It plays an important role in their quality. It is also found in many pharmaceutical dosage forms as active pharmaceutical ingredient [12]. Moreover, it is used in photography, hair dying, dying of suturing substances, and in gas analysis for adsorption of oxygen [13]. In addition, PG structural group is found in several important natural molecules such as gallic acid (GA) and tannic acid (TA). Different analytical techniques were reported for detection of PG such as spectrophotometry [14–16], chromatographic techniques [17–20], and electrochemical methods [21–24]. However, most of these methods especially chromatographic techniques involve tedious sample preparation and complicated analytical procedures that are time-consuming and use toxic organic solvents. Additionally, a little consideration has been paid to PG as a CL reagent and only a few papers have dealt with the CL oxidation of pyrogallol with different oxidants, such as periodate and H_2O_2 [13,25–27]. The reason for this is that CL intensity was very weak and should be enhanced by using enhancer such as surfactant, aldehyde and acid.

Cobalt ion (Co^{2+}) was first discovered in 1735 by George Brandt in Stockholm Sweden. It is used in many places today, such as glasses, paint pigments, magnets materials and even cancer therapy. It is one of the most important metal ions that present in our body fluid and in the surrounding environment. It plays a significant role in different biological processes and also act as a nutrient for the human body. It is the main constituent of vitamin B12 as well as

certain other metalloproteins [28]. Pernicious anemia is an example of dangerous disease related to Co^{2+} deficiency [29]. Other different dangerous diseases may affect human as rhinitis, lung cancer, asthma, allergic dermatitis, and pathogenic infections by the high exposure to Co^{2+} ions polluted environment.

Tyrosinase is a typical phenol oxidase that oxidize monophenols to o-diphenols and also oxidize o-diphenols to corresponding o-quinones in the existence of molecular oxygen [30,31]. Its high expressing level in melanoma cancer cells makes tyrosinase a valuable biomarker for the diagnosis of melanoma cancer [32,33]. Additionally, tyrosinase possesses more functions than phenol oxidases such as catalase, peroxygenase, and catecholase [34–36]. It has broad substrate specificity toward many kinds of catechols, phenols, and quinones [37–39]. Also, PG is acting as tyrosinase substrate [37,38,40].

As mentioned before, PG was used as a CL luminophore to generate a very weak CL intensity [25–27]. Also PG was detected by its enhancing effect on weak and slow lucigenin-hydroxylamine CL system in a very high concentration of sodium hydroxide (5 M) which caused corrosion of the CL sample cell tube [41]. However, hydroxylamine is toxic.

In this work, we exploited for the first time the utility of PG as a new promising CL coreactant for lucigenin CL without using hydroxylamine. The developed lucigenin-PG system displayed a strong CL emission in carbonate buffer, and its CL signals were strongly decreased in the presence of Co^{2+} and tyrosinase. Thus, it allows the multiple determination of PG, lucigenin, Co^{2+} and tyrosinase activity with low detection limit and excellent selectivity. Finally, the real implementation of the developed CL system is checked for the assay of PG, Co^{2+} and tyrosinase in coffee, lake water, and plasma samples, respectively.

2. Experimental

2.1. Chemicals and materials

PG and bovine serum albumin (BSA) were acquired from Sinopharm Chemical Reagent Co. Ltd. which located in Beijing, China. Lucigenin was acquired from TCI (Shanghai, China) and H_2O_2 was bought from Beijing Chemical Reagent Company. Tyrosinase, glucose oxidase (GOx), uric acid, dopamine, pyridoxal-5'-phosphate (PLP) and ascorbic acid were obtained from Sigma–Aldrich. Trypsin was obtained from Beijing Dingguo Changsheng Biotechnology Co., Ltd. The standard solution of lucigenin (1.0 mM)

was obtained by dissolving precisely 0.0510 g lucigenin powder in 100 mL of water. Various pH values of carbonate buffers (CB) were achieved by using sodium carbonate, bicarbonate, and small quantity of NaOH to control the final pH.

Regarding the instrument used for counting the CL intensity, we have recorded the CL intensity by using a flow injection CL system (FIA-CL). FIA-CL is consisting of three compartments including; a Biophysics Chemiluminescence ultra-weak luminescence analyzer (the Institute of Biophysics, Chinese Academy of Sciences), an intelligent flow injection sampler (IFIS–C mode) (ReMax Inc., Xi'an, China), and a hand-made flow cell. In a light-tight box of the luminescent analyzer, the flow cell was placed. Injection loop with 50 μ L capacity for sample injection.

2.2. Analytical procedure for PG determination

In our experiment, we used FIA-CL system (Scheme S1) for all sample measurements. Channels A and B were loaded with water and 20 μ M lucigenin in 0.10 M CB solution of pH adjusted at 12.0, respectively. After adjusting the pump flow rate at 1.25 mL/min, two solutions (water and lucigenin) were run through the channels. Different concentrations of daily freshly prepared PG in water were injected for its determination.

2.3. Analytical procedure for lucigenin determination

Two channels cylinders A and B respectively were charged with 0.10 M CB solution of pH 12.0 and 200 μ M PG in water. After that, two solutions were pumped with the same above mentioned flow rate. Various lucigenin solutions with various concentrations were injected for its determination.

2.4. Analytical procedure for Co^{2+} determination

Lucigenin (20 μ M) in 0.10 M CB solution (pH 12.0) was pumped in channel A, while double distilled water was pumped into channel B at the same flow rate. Different Co^{2+} solutions with different concentrations were mixed well with 200 μ M PG in water first. After that the mixtures were injected immediately for its analysis.

2.5. Procedure of sensing tyrosinase activity

Double distilled water and 20 μ M lucigenin in 0.10 M CB solution (pH 12.0) were used in our FIA cylinders A and B, respectively. Into 10 mL test tubes, different concentrations of tyrosinase was

mixed well with 350 μ L borate buffer (pH 8.0) and 200 μ L of PG (3 mM). Subsequently, the mixture was completed to 3 mL with distilled water and incubated at 37 $^{\circ}$ C for 2 h. After the incubation period the mixture was injected through the loop injector for its analysis.

2.6. Procedure of real sample preparation

Lucigenin-PG system was further used for the assay of PG, Co^{2+} and tyrosinase activity in coffee, lake water and human plasma samples, respectively.

For PG assay in coffee sample, coffee powder was obtained from local market and converted to fine powder with a mortar for its analysis. For Co^{2+} assay in lake water, water sample was obtained from the Changchun South Lake, Jilin, China. Regarding tyrosinase assay in real sample, human plasma sample was obtained from Jilin University First Hospital. The plasma sample was diluted 100 times to eliminate the interference of other components in the plasma sample. Standard addition method was applied for different real samples and the percentage recoveries were evaluated by utilizing lucigenin-PG CL system.

3. Results and discussion

3.1. CL of lucigenin-PG system

Fig. 1 represents the CL intensity of the developed lucigenin-PG system and the traditional lucigenin- H_2O_2 . Lucigenin-PG system shows a remarkable CL intensity (~190 times) compared with lucigenin- H_2O_2 system. It is one of the most enhanced lucigenin CL systems by comparing with other reported

Fig. 1. The relationship between CL intensities and time for the lucigenin- H_2O_2 system (blue line), and lucigenin-PG system (red line). Inset: shows the enlarged version of lucigenin- H_2O_2 curves. C(lucigenin), 20 μ M; C(PG), 1 mM; C(H_2O_2), 1 mM; pH, 12.0; PMT, 900 V.

lucigenin-coreactants systems [42–46]. Besides PG, we have also investigated the effect of two other polyphenolic compounds, GA and TA. Fig. S1 shows that GA and TA gave very weak CL intensity. A comparison of the structures of PG, GA and TA indicates that PG does not have carboxylic group and GA and TA have carboxylic group. The absence of electron-withdrawing carboxylic group in PG makes its autoxidation easy, resulting in intense lucigenin-PG CL. The wavelength-dependent emission spectrum was recorded by utilizing various filters ranged from 400 to 640 nm (Fig. S2). The highest CL intensity for the developed system was estimated at ~490 nm, which is compatible with the spectrum of lucigenin CL reported previously in the literature. It has been reported that PG autoxidized in alkaline medium to generate superoxide anion radical ($O_2^{\bullet-}$) [13,47–49]. $O_2^{\bullet-}$ can react with lucigenin to produce an excited state of N-methyl-acridone that decays to the ground state after generating strong CL emission. Therefore the CL mechanism is proposed as seen in Scheme 1.

3.2. Effect of dissolved oxygen on CL

Lucigenin CL reaction can be initiated by superoxide anion radical ($O_2^{\bullet-}$) which resulted from dissolved O_2 . So it is important to check the impact of dissolved oxygen on the developed CL system. Therefore, we have measured the CL intensity of the developed system in presence and absence of O_2 . Purging N_2 gas into lucigenin and PG solutions for 30 min was utilized for the removal of the dissolved O_2 . As appeared in Fig. S3, CL intensity decreases by about 22% after the removal of O_2 in the two solutions.

Scheme 1. Suggested reaction mechanism for lucigenin-PG CL system.

3.3. Effect of the ionic strength and pH of CB solution on CL

To check the effect of ionic strength of CB solution, different molar concentrations of CB were used (0.01M, 0.05M, 0.07M, 0.10M, 0.15 and 0.20M), while the pH of CB solution was kept constant. As shown in Fig. S4A, the highest CL intensity was achieved at 0.1M. After 0.1M no much increase in the CL intensity. In addition, the effect of pH of 0.1 M CB solution (10–12.5) was also examined to see its effect on lucigenin-PG CL system. It was clearly seen that pH has a great effect. CL intensity increased by increase pH value, as at higher pH values the protons of the phenolic groups were deprotonated to give phenolate anions. Phenolates anions are very rich with electron density, thus increase the autoxidation of PG and higher generation of superoxide radical that reaches its maximum at pH 11 (Fig. S4B). After pH 11 no much increase in the CL intensity. So, pH 12 of 0.1M CB solution was selected as the optimum values for further measurements.

3.4. Determination of lucigenin

Using the optimized conditions, lucigenin-PG proposed system was successfully used for the determination of lucigenin. After plotting the relation between logarithm of CL intensity ($\log I$) and concentrations of lucigenin ($\log C$), a linear relationship was achieved from 0.5 to 120 μM (Fig. 2B). The linear regression equation is $\log I = 2.06 + 0.916 \log C$, while correlation coefficient is = 0.9944. The LOD was estimated to be 0.42 μM for lucigenin ($S/N = 3$). The RSD for the repeated measurements of 50 μM lucigenin ($n = 9$) is 3.70% (Fig. S5).

3.5. Determination of PG

Dependent on the high obtained CL signal intensity from the CL reaction between PG and lucigenin, the new CL system permits its detection with wide linear range in the concentration range from 1 to 100 μM (Fig. 3B). The linear equation is $\log I = 1.36 + 1.23 \log C$ with a correlation coefficient of 0.9950 (where C is the PG concentration in μM). Applying signal-to-noise ratio of 3 ($S/N = 3$), LOD was estimated to be 0.94 μM for PG. The developed CL method shows good repeatability ($RSD = 2.91\%$) for the repeated measurements ($n = 9$) of 50 μM PG (Fig. S6).

3.6. Interference study for PG detection

To further assess the selectivity of proposed system for the assay of PG in coffee sample, different

Fig. 2. (A) CL emission–time curves recorded with different concentrations of lucigenin from 0.5 to 120 μM and (B) linear relationship between CL intensity and concentration of lucigenin. C(PG), 200 μM ; pH, 12.0; PMT, 900 V.

potential interference species in coffee were examined. To a solution containing PG, a different species were added such as TA, GA, catechin (CC), glucose (Glu), sucrose (Suc), fructose (Fru), glycine (Gly), cysteine (Cys), arginine (Arg), maleic acid (MA), citric acid (CA), Ca^{2+} , Cu^{2+} and Pb^{2+} . The variation of CL intensity in samples corresponding to all the species is nearly negligible (Fig. 3C). This indicates that the new system has excellent selectivity toward PG assay in coffee sample.

3.7. Determination of Co^{2+}

As shown in Fig. 4A, the CL intensities decreased linearly by increasing Co^{2+} concentrations from 0.3 to 80 μM . The quenching effect is due to the interaction between cobalt and PG that leads to a decrease in the autoxidation of PG to produce superoxide anion radical. Based on the quenching effect of Co^{2+} , the proposed system was used for

sensitive and selective determination of Co^{2+} . The decrease in CL intensity vs. Co^{2+} concentration follows Stern–Volmer equation:

$$I_0/I = 1 + K_{\text{SV}} C_{\text{Co}^{2+}}$$

where I_0 and I refer to the CL intensities of the control sample and after the addition of Co^{2+} , respectively, K_{SV} is the Stern–Volmer quenching constant, and $C_{\text{Co}^{2+}}$ is the concentration of cobalt ion in μM . The linear equation is $I_0/I = 0.9886 + 0.307 C$ ($r = 0.9951$). LOD was estimated to be 68 nM ($S/N = 3$) that is lower than the guideline reported by Department of Environmental Protection for drinking water (1.7 μM) [50]. The RSD for the repeated measurements of 5 μM Co^{2+} ($n = 9$) is 3.40% (Fig. S7). By comparison the present work with some different reported analytical methods for assay of Co^{2+} , the developed method is cost-effective and simple (Table S1).

Fig. 3. (A) Repeated CL emission–time curves recorded with different concentration of PG from 1.0 to 100 μM and (B) linear relationship of CL enhancement versus concentration of PG from 1.0 to 100 μM and (C) selectivity for the detection of PG. The concentration of PG is 200 μM and the interfering species (tannic acid, gallic acid, catechin (CC), glucose, sucrose, fructose, glycine, cysteine, arginine, maleic acid, citric acid, Ca^{2+} , Cu^{2+} and Pb^{2+} and were 100 μM for the selectivity study. C(lucigenin), 20 μM ; pH, 12.0; and PMT, 900 V.

Fig. 4. (A) Repeated CL emission–time curves recorded in different concentrations of Co^{2+} , (B) linear relationship of CL quenching versus concentration of Co^{2+} from 0.3 to 80 μM , and (C) selectivity for the detection of Co^{2+} . The concentration of Co^{2+} and all the interfering metal ions were 10 μM for the selectivity test. Numbers 1–19 refer to control, Hg^{2+} , Cu^{2+} , Cd^{2+} , Zn^{2+} , K^+ , Mg^{2+} , Ca^{2+} , Fe^{2+} , Sn^{2+} , Li^+ , Na^+ , Mn^{2+} , Pb^{2+} , Cr^{3+} , Ag^+ , Al^{3+} , Ni^{2+} and Co^{2+} . C(lucigenin), 20 μM ; C(PG), 200 μM ; and pH, 12.0; PMT, 900 V.

3.8. Selectivity study for Co^{2+} detection

Selectivity study of the new system was checked by studying the potential interfering of 17 typical metal ions including Hg^{2+} , Cu^{2+} , Cd^{2+} , Zn^{2+} , K^+ , Mg^{2+} , Ca^{2+} , Fe^{2+} , Sn^{2+} , Li^+ , Na^+ , Mn^{2+} , Pb^{2+} , Cr^{3+} , Ag^+ , Al^{3+} and Ni^{2+} . Fig. 4C exhibits that, the CL intensities of lucigenin-PG system quenched significantly only by the presence of Co^{2+} . Conversely, the controlled sample CL intensity stayed unchanged or lightly decreased by the presence of other metals. Consequently, the new system has achieved an obvious selectivity toward Co^{2+} determination over other metals. This remarkable selectivity is attributed to the strong and selective interaction between cobalt and PG [51,52].

3.9. Detection of tyrosinase activity

Tyrosinase detection involved in two steps. The first step is the catalytic oxidation of PG by tyrosinase. To measure tyrosinase activity, PG was dissolved in borate buffer (pH 8.0), instead of water. The interaction of PG with borate buffer can avoid the autoxidation of PG [53,54]. Tyrosinase can catalyze the oxidation of PG in borate buffer and decrease the PG concentration. In the second step, the pH is increased by mixing with carbonate buffer (pH 12.0) and PG autoxidation can occur at higher pH. Since tyrosinase decreases the PG concentration, the CL intensity decreases.

Fig. 5A and B shows CL intensities and standard calibration plot for tyrosinase detection, respectively. It was observed that upon increasing tyrosinase concentrations, the CL intensity decreased and the linear range was 2–50 $\mu\text{g/mL}$ with a limit of detection 1.8 $\mu\text{g/mL}$. The linear equation is I_0/I

$\text{I} = 0.0529 + 0.9044 \text{ C}$ and r^2 is 0.9889. The developed CL method shows good repeatability ($\text{RSD} = 2.88\%$) for the repeated measurements ($n = 9$) of 25 $\mu\text{g/mL}$ tyrosinase (Fig. S8).

3.10. Selectivity study for detection of tyrosinase activity

To further examine the selectivity of the proposed CL platform, some common interfering species, such as Glu, lactose (Lac), sucrose (Suc), Gly, glutathione (GSH), cysteine (Cys), histidine (Hist), ascorbic acid (AA), uric acid (UA), pyridoxal-5'-phosphate (PLP), dopamine, BSA, trypsin, glucose oxidase (GOx), Cu^{2+} , Zn^{2+} , Ca^{2+} and Mg^{2+} were tested. Fig. 5C confirms that all the mentioned interfering species and metal ions have no significant interference on tyrosinase detection. The obtained data reveals the appropriate selectivity for the sensing of tyrosinase in biological fluid.

3.11. Determination of PG, Co^{2+} and tyrosinase in real samples

The proposed system was also used for assay of PG, Co^{2+} and tyrosinase in coffee, lake water and in human plasma samples, respectively.

For assay of PG in coffee; coffee sample was obtained from local market and converted to powder. 0.1 g was accurately weighed and boiled in 50 mL of double distilled water for 10 min. Then the boiled solution was cooled, filtered, diluted to 100 mL with water and then injected in the injection loop to evaluate the percentage recovery of the real presence of PG in coffee sample. After that, different concentrations of PG (2, 5, 10 μM) were spiked in the coffee sample using standard addition technique.

Fig. 5. (A) Repeated CL emission–time curves recorded in different concentrations of tyrosinase, (B) linear relationship of CL quenching versus concentration of tyrosinase from 2 to 50 $\mu\text{g/mL}$, and (C) selectivity for the detection of tyrosinase. The concentration of tyrosinase is 50 $\mu\text{g/mL}$ and the interfering species (glucose, lactose, sucrose, glycine, histadine, glutathione, cysteine, ascorbic acid, uric acid, PLP, dopamine, BSA, Cu^{2+} , Zn^{2+} , Ca^{2+} and Mg^{2+} were 100 μM and 10 $\mu\text{g/mL}$ for GOx and trypsin for the selectivity test. Numbers from 1 to 20 refer to control, glucose, lactose, sucrose, glycine, histadine, glutathione, cysteine, ascorbic acid, uric acid, PLP, dopamine, BSA, trypsin, glucose oxidase, Cu^{2+} , Zn^{2+} , Ca^{2+} and Mg^{2+} and tyrosinase. C(lucigenin), 20 μM ; C(PG), 200 μM ; and pH, 12.0; PMT, 900 V.

Table S2 shows the obtained percentage recoveries for the estimated samples (97.0–103.1%).

For assay of Co^{2+} in lake water sample; lake water sample was obtained from the Changchun, Jilin, China. Three different concentration of standard Co^{2+} solutions (7, 10, 50 μM) were spiked into water samples. Table S3 represents that, the calculated recoveries of the sample were estimated to be in the range of 94.8–100.2%. The achieved results show that lucigenin-PG CL system is convenient for real lake water application.

For assay of tyrosinase in real human plasma; fresh human plasma was used for the real assay of tyrosinase activity. Three different concentrations in the calibration linear range of tyrosinase (10, 25, 50 $\mu\text{g/mL}$) were added to the plasma. After that, the percentage recoveries were calculated and listed in Table S4. The results reflect that lucigenin-PG developed system is suitable and promising for the detection of tyrosinase activity in biological samples.

4. Conclusions

In conclusion, lucigenin-PG CL has been found for the first time and was successfully developed for the multiple detections of lucigenin, PG, Co^{2+} and tyrosinase. Furthermore, the proposed CL system was also used for the assay of PG, Co^{2+} and tyrosinase in real samples with high obtained recoveries. Notably, this system achieves excellent selectivity for PG, Co^{2+} and tyrosinase detections in real samples without interference from the presence of several species in different matrixes.

Declaration of competing interest

The authors declare no conflicts of interest.

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Supplementary Material.

Scheme S1. Diagram of the flow injection lucigenin-PG CL system. A and B: flow channels; C: IFIS-C mode intelligent flow injection sampler; D: loop injector; E: CL detector; F: waste cup.

Fig. S1. CL emission–time curves recorded in the presence of 1.0 mM TA (black color), 1 mM GA (red color), and 100 μM PG (blue color). C(lucigenin), 20 μM ; pH, 12; PMT, 900 V.

Fig. S2. CL intensity versus wavelength spectrum plotted from 400 to 640 nm. C(lucigenin), 50 μ M; C(PG), 2 mM; pH, 12.0; and PMT, 900 V.

Fig. S5. CL emission-time curves at the lucigenin concentration of 50 μ M. C(PG), 200 μ M; pH, 12.0; PMT, 900 V.

Fig. S3. CL emission-time curves recorded in the presence (green) and absence of oxygen (blue). C(lucigenin), 50 μ M; C(PG), 3 mM; pH, 12.0; PMT, 900 V.

Fig. S6. CL emission-time curves at PG concentration of 50 μ M. C(lucigenin), 20 μ M; pH, 12.0; PMT, 900 V.

Fig. S4. CL intensity plotted as a function of (A) the ionic strength and (B) pH of CB solution. C(lucigenin), 20 μ M; C(PG), 200 μ M; PMT, 900 V.

Fig. S7. CL emission–time curves for Co^{2+} (5 μM). C(lucigenin), 20 μM ; pH, 12.0; PMT, 900 V.

Fig. S8. CL emission–time curves for tyrosinase (25 $\mu\text{g/mL}$). C(lucigenin), 20 μM ; pH, 12.0; PMT, 900 V.

Table S1. Comparison of different methods for the detection of Co^{2+}

Analytical Method	LOD	Ref.
Silicon nanoparticles/Fluorescence	0.14 μM	[55]
N-CDs/Fluorescence	0.68 μM	[56]
Ag NCs/Luminescence	100 nM	[57]
Peptide modified Au NPs/Absorbance	2000 nM	[58]
Carboxyl-functionalized CdS QDs/Absorbance	390 nM	[59]
Photoelectrochemical Detection	0.3 μM	[60]
Fluorescence detection	0.97 μM	[61]
Colorimetric detection	12.4 μM	[62]
Fluorescence detection	0.26 μM	[63]
NBS-reduced carbon QDs/Chemiluminescence	3.25 μM	[64]
Nitrogen and sulfur co-doped graphene quantum dots/Fluorescence	1.25 μM	[65]
Colorimetric detection	0.88 μM	[66]
Lucigenin-PG with FIA system/Chemiluminescence	68 nM	Present work

Table S2. Analytical results for the detection of PG in coffee.

Sample	Concentrations of analyte		Recovery	RSD (n = 3)
	Amount added ^a	Amount found ^b		
Coffee	0.0 μM	4.60 μM	—	3.6%
	2.0 μM	6.57 μM	99.5%	2.5%
	5.0 μM	9.90 μM	103.1%	3.0%
	10.0 μM	14.16 μM	97.0%	4.9%

^{a,b} average of three determinations.

Table S3. Analytical results for the detection of Co^{2+} in lake water.

Sample	Concentrations of analyte			Recovery	RSD (n = 3)
	Amount detected	Amount added	Amount found ^a		
Lake water	N.D	7.0 μM	6.63 μM	94.8%	2.4%
		10.0 μM	9.95 μM	99.5%	3.9%
		50.0 μM	50.11 μM	100.2%	2.9%

N.D: Not detected.

^a Average of three determinations.

Table S4. Analytical results for the detection of tyrosinase in plasma.

Sample	Concentrations of analyte			Recovery	RSD (n = 3)
	Amount detected	Amount added	Amount found ^a		
Plasma	N.D	10 μM	9.35 μM	93.6%	2.9%
		25 μM	24.78 μM	99.1%	3.3%
		50 μM	49.02 μM	98.0%	1.4%

N.D: Not detected.

^a Average of three determinations.

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