Flow-injection Chemiluminescence Sensor for the Determination of Gallic Acid by Immobilizing Luminol and Periodate on Anion-exchange Resin

ZHANG, Si-Chun(张四纯) ZHOU, Guo-Jun(周国俊) JU, Huang-Xian*(鞠熀先)

Department of Chemistry, Institute of Analytical Science, State Key Laboratory of Coordination Chemistry, Nanjing University, Nanjing 210093, China

A novel chemiluminescence (CL) sensor for the determination of gallic acid combined with flow injection analysis was developed by electrostatically immobilizing luminol and periodate on anion-exchange resins respectively. Gallic acid was sensed by its enhancing effect on the weak CL reaction between luminol and periodate, which were eluted from the ion exchange column. The possible reaction mechanism of the CL system was suggested and discussed. The response of the sensor to gallic acid concentration was linear over the range of 8.0×10^{-9}—1.0×10^{-6} mol/L with a detection limit of 6.5×10^{-9} mol/L (3σ). The relative standard deviation (RSD) for 7 repetitive determinations of gallic acid (1.0×10^{-7} mol/L) was 1.8%. The sensor could be used for over 400 times determination with a good reproducibility.

Keywords chemiluminescence sensor, gallic acid, luminol, periodate, flow-injection analysis

Introduction

Gallic acid exists in the leaves and fruits of many types of plants and is widely used in medicine for anti-oxidation and antibacterial activity, antiflammatory action and anti-cancer activity.[1-5] In spite of the health importance of gallic acid, its metabolism and kinetics in the human body have not been fully investigated due to lack of sensitive and simple method.[6] Several methods including spectrophotometry,[7,8] thermometric enthalpy titration,[9] electrochemiluminescence,[10] liquid chromatography with mass spectrometry,[11] high performance liquid chromatography and electrochemistry[12-14] have been used for the determination of gallic acid. But those methods are usually suffered from low sensitivity, time-consuming or complex instrumentation.

The gallic acid itself is a common luminescent reagent and exhibits chemiluminescence (CL) during the reaction with variety of oxidants such as hydrogen peroxide and permanganate in the presence of metal ions[15,16] or ozone in the presence of xanthene dyes.[17] But the oxidation of gallic acid has not been used for its determination since the emission intensity is too weak when it is at a relatively low concentration. An electrochemiluminescence[10] method for the determination of gallic acid has been proposed recently based on its potential dependent inhibition and enhancement effects on electrogenerated chemiluminescence of luminol. However, the fouling of electrode by organic compounds often affects the reproducibility of this method.

Our work here observed an enhancing effect of gallic acid on the CL reaction between luminol and KIO_4 in basic medium and developed a highly sensitive flow injection CL sensor for gallic acid by electrostatically immobilizing both luminol and IO_4^- on anion exchange resin. The sensor was placed in front of the detection window of a photomultiplier (PMT) tube, thus avoiding the unnecessary dilution of the eluted reagents and samples. The need to merge two streams prior to detection was not necessary therefore the

* E-mail: hxju@nju.edu.cn; Tel.: 025-3593593; Fax: 025-3317761

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configuration could further be miniaturized. The proposed sensor offered the advantages of simplicity, rapidity and high sensitivity and could be used for the determination of gallic acid in human urine.

Experimental

Reagents

All reagents were of analytical grade and all solutions were prepared with doubly distilled water. Gallic acid was obtained from Zunyi Chemical Plant (Zunyi, China). Luminol was purchased from Department of Chemistry, Shaanxi Normal University (Xi'an, China) and luminol solution (0.025 mol/L) was prepared by dissolving luminol (0.443 g) in NaOH solution (100 mL, 0.1 mol/L). The KIO_4 solution (0.01 mol/L) was stored in a brown bottle to avoid photochemical decomposition. D201 × 7 resin and other anion exchange resins, purchased from Nankai University (Tianjin, China), were used for the immobilization of luminol and KIO_4.

Preparation of the sensor

D201 × 7 ion-exchange resin (1.0 g) was dipped in HCl (1.0 mol/L) for an hour and then rinsed with water for 6 times. The resin was divided into two equal shares and stirred in 50 mL of luminol (0.025 mol/L) and KIO_4 (0.01 mol/L) solution for 24 h, respectively. The amounts of immobilized luminol and IO_4^- on the resins were determined by the changes of UV-vis absorbance of the immobilization solutions at 360 nm for luminol and 222 nm for IO_4^-, respectively.[20,22] The amounts of immobilized reagents were 2.05 mmol per gram resin for luminol and 0.94 mmol per gram resin for IO_4^-. Then the resins were filtered and washed with water for 6 times. The resins (about 0.1 g) immobilized with luminol and IO_4^- (1 :2, $w:w$) were packed into a mini glass column (50 mm × 3 mm I.D.) with glass wool at both ends.

Apparatus

The flow injection analysis system is shown in Fig. 1. One of the pumps of luminescence analyzer (IFFM-D, Remex Electronic Instrument Limited Co., Xi'an, China) was used to deliver flow streams at a flow rate of 2.0 mL/min. PTFE tube (0.8 mm i.d.) was used to connect all components in the flow system. The sample was injected into the carrier stream (water) via the six-way injection valve. The sensor was placed on the detection window of the PMT as a flow cell. The CL emission was converted by PMT to current signal and the output was fed to a computer via an A/D convert card and recorded by a special software. The measurement of gallic acid was carried out by continually pumping carrier water and injecting samples (30 μL) into the sensor at a frequency 60 h^{-1}.

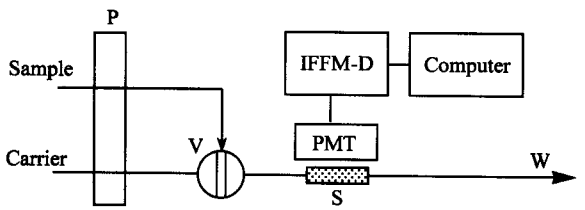


Fig. 1 Schematic diagram of flow injection system for the determination of gallic acid with the sensor. P, peristaltic pump; V, six-way valve; IFFM-D, IFFM-D luminescence analyzer; S, sensor; W, waste.

The UV-vis absorbance determination was carried out with a S2000 miniature fiber optic spectrometer (Ocean-Optics Inc., Florida, USA). The CL spectrum was recorded on a RF-1501 spectroflourimeter (Shimadzu, Japan).

Results and discussion

Preliminary study

The oxidation of gallic acid by KIO_4 in basic solution exhibited a weak CL emission. But it is too weak to be used for the sensitive determination of gallic acid. A very weak CL emission was also observed when gallic acid (1.0×10^{-7} mol/L) was injected into the basic solution of luminol (1.0×10^{-4} mol/L). At the same time, the mixed solution of luminol (1.0×10^{-4} mol/L) and KIO_4 showed a very low background emission. However, a strong CL emission was recorded when gallic acid was injected into the mixing solution of luminol and KIO_4.

Conditions optimization for sensor preparation

Since both luminol and IO_4^- were anion ions, anion exchange resin was selected as the substrate for their immobilization. Six styrene-PVB anion exchange resins were

compared and the signals of CL sensors prepared from those resins are shown in Table 1. It indicated that the strongest CL signal was obtained when the sensor was prepared with D201 × 7 anion resin, a strong basic resin.

Table 1 Comparison of different ion-exchange resins as substrate of the CL sensor[a]

Resin type	CL intensity[b]
D201 × 7 Styrene-DVB (strong basic)	896
D290 Styrene-DVB (strong basic)	624
D296 Styrene-DVB (strong basic)	637
D301R Styrene-DVB (weakly basic)	385
D396 Styrene-DVB (weakly basic)	344
D370 Styrene-DVB (weakly basic)	320

[a] Conditions: sample, gallic acid (1.0×10^{-7} mol/L) in NaOH (1.0×10^{-3} mol/L); carrier, water; flow rate, 2.0 mL/min. [b] Mean of three determinations.

The response and lifetime of the sensors depended on the KIO_4 concentration used in the immobilization step. No CL emission was detected with the sensor prepared solely by the resin immobilized with luminol. The intensity showed no significant difference when the D201 × 7 resin was treated in KIO_4 solution with the concentration from 1.0×10^{-3} to 1.0×10^{-2} mol/L but the lifetime of the sensor increased with increasing KIO_4 concentration. In consideration of the solubility of KIO_4 in water, a 0.01 mol/L KIO_4 solution was used for IO_4^- immobilization.

The effect of the ratio of resins immobilized with luminol to IO_4^- on CL emission intensity is shown in Table 2. The CL signal intensity was not sensitive to the ratio of the resins. But the sensor prepared with a mixing ratio of 1:2 (luminol : IO_4^- $w:w$) gave the longest lifetime. Therefore, it was selected for preparation of the sensor.

Table 2 Effect of mixing ratio of resins immobilized luminol to IO_4^- on the CL intensity and lifetime of the sensor[a]

Ratio ($w:w$)	CL intensity[b]	Lifetime of sensor (times)[c]
4:1	854	146
2:1	837	277
1:1	862	380
1:2	875	427
1:4	866	270

[a] Conditions: sample, gallic acid (1.0×10^{-7} mol/L) in NaOH (1.0×10^{-3} mol/L); pump rate, 2.0 mL/min; sampling frequency, 60 h^{-1}; sample volume, 30 μL. [b] Signal subtract blank. [c] The signal deviation was less than 5%.

Selection of determination conditions

The effects of eluants such as NaCl, $NaNO_3$, Na_2SO_4, CH_3COONa, Na_3PO_4, Na_2CO_3 and NaOH solutions were investigated for eluant selection. Although NaCl, Na_2SO_4 and $NaNO_3$ solutions could be used as an eluant with a freshly prepared sensor, the gradually decreased signal and baseline were observed due to the reduced OH^- concentration in the sensor. The results of CH_3COONa, Na_3PO_4, Na_2CO_3 and NaOH solutions used as the eluants are shown in Table 3. These solutions gave different emission intensities at the same concentration. It could be seen that NaOH solution was the best eluant with the highest CL intensity.

Table 3 Effect of eluant on the emission intensity[a]

Base	Concentration (mol/L)	CL intensity[b]
NaOH	1.0×10^{-3}	860
CH_3COONa	1.0×10^{-3}	217
Na_2CO_3	1.0×10^{-3}	140
Na_3PO_4	1.0×10^{-3}	265

[a] Conditions: sample, gallic acid (1.0×10^{-7} mol/L); pump rate, 2.0 mL/min; sampling frequency, 60 h^{-1}; sample volume, 30 μL. [b] Mean of three determinations.

The release of luminol and periodate from the resins, which had a significant effect on CL intensity, depended on the NaOH concentration injected into the sensor. The effect of NaOH concentration on CL intensity of the sensor is shown as Fig. 2. It was indicated that the CL intensity increased with the increase of NaOH concentration and trended to a constant value when its concentration was larger than 1.0×10^{-3} mol/L. The concentrations of NaOH higher than 1.0×10^{-3} mol/L caused a decrease in

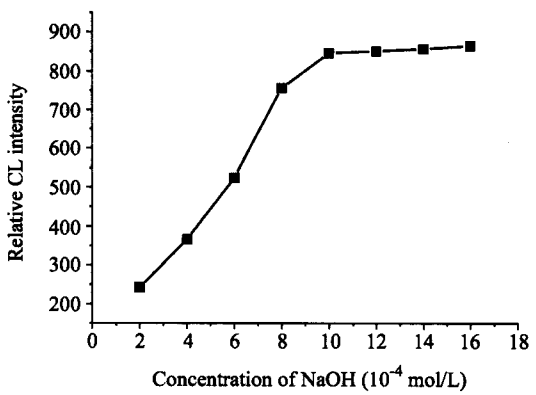


Fig. 2 Effect of NaOH concentration on CL intensity. Conditions: gallic acid, 1.0×10^{-7} mol/L; carrier, water; flow rate, 2.0 mL/min.

baseline during the determination.

The CL signal intensity was greatly affected by flow rate of carrier. The emission intensity increased with increasing flow rate, which impacted the rate of contact between the eluant molecule and resin and the mix of reactants. The lower flow rate caused lower CL signal and slowed down the sampling rate. But flow rates higher than 2.0 mL/min were not recommended because of the appearance of eccentric signals.

Interference

In order to assess the analytical applicability of the sensor, the interference of some organic compounds and metal ions were examined by analyzing synthetic samples containing gallic acid (1.0×10^{-7} mol/L) and various additives. The recovery results are given in Table 4. Common additives in drug formulation, such as glucose, sugar and starch showed no effect on gallic acid determination at 100 times that of gallic acid (1.0×10^{-7} mol/L). Metal ions such as Cu^{2+}, Fe^{2+}, Mn^{2+} Co^{2+} severely interfered to the determination, but they could be sheltered by the addition of EDTA (0.1 mmol/L). The existence of parogallol interfered to the determination of gallic acid even at the low concentration. Considering parogallol was a body metabolism product of gallic acid, separation step had to be performed for real samples.

Gallic acid determination

Under the selected conditions, the response of the sensor to gallic acid was linear over the concentration range of 8.0×10^{-9}—1.0×10^{-6} mol/L. The regression equation of CL intensity vs. gallic acid concentration was $I = 413 + 52.8c$ (where c is the concentration of gallic acid in 10^{-8} mol/L, $r = 0.9986$, $n = 7$). The relative standard deviation (RSD) for 7 determinations of gallic acid (1.0×10^{-7} mol/L) was 1.8%. The detection limit was 6.5×10^{-9} mol/L (3σ), which was lower than other existed method.[10]

Synthetic samples were prepared by adding 5.0×10^{-7} and 1.0×10^{-5} mol/L gallic acid to human urine, respectively. The interference of transition metal ion from the urine was diminished by adding EDTA (0.1 mmol/L). Other substances in urine, such as urea, uric acid and protein, did not interfere the determination of gallic acid. But the high concentration of NaCl in urine caused decrease of signals. Therefore the samples were diluted 10 times before determination. The determination results obtained by standard curve method are summarized in Table 5. In the synthetic samples 5.08×10^{-7} and 0.990×10^{-5} mol/L gallic acid were found respectively.

Table 4 Recovery of gallic acid from solutions containing various additive[a]

Additive	Concentration ratio (Additive/gallic acid)	Recovery (%) ($n = 3$)
Glucose, Sugar, Starch	100	102.0, 102.4, 99.5
Nicotinic acid, Lactose		99.2, 99.7
Sodium citrate,		101.2
Nicotinamide		102.0
Ascorbic acid, Riboflavin		95.2, 104.4
Thiamine hydrochloride	10	97.3
Pyrogallol	1	227.0
EDTA, $CaSO_4$, $MgSO_4$	1000	98.4, 99.5, 98.2
Cu^{2+}, Fe^{2+}	1.0	102.8, 103.5
Mn^{2+}, Co^{2+}	0.1	185.0, 246.0

[a] Conditions: sample, gallic acid (1.0×10^{-7} mol/L) in NaOH solution (1.0×10^{-3} mol/L).

Table 5 Determination of gallic acid in human urine with the CL sensor (10^{-7} mol/L)

Sample content	Detected sample	RSD (%)	Added sample	Found	Recovery (%)
5.00	5.08	1.6	3.00	2.98	99.3
100.0	99.0	1.3	50.0	49.4	98.8

Stability of the sensor

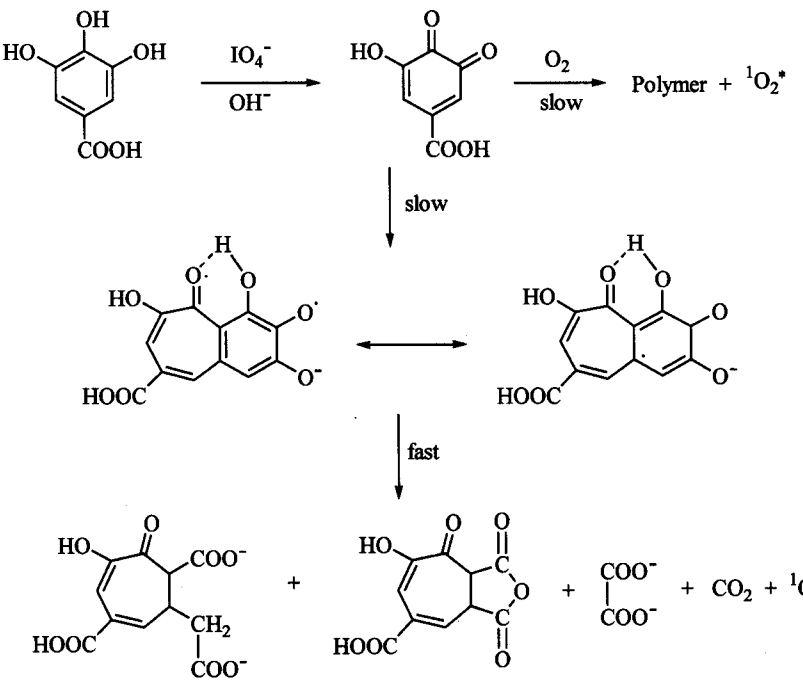
The peak height of 30 times repetitive injections of gallic acid (30 μL, 1.0×10^{-7} mol/L) in NaOH solution (1.0×10^{-3} mol/L) gave a relative standard deviation of 2.2%. The results of 400 repetitive injections showed good durability of the sensor (signal deviation was less than 5%). The CL intensity was decreased gradually after 400 times determination and a new sensor was needed when the signal deviation was larger than 5%. The sensor was stored in the diagram of flow injection system after flushing with water during experimental.

Discussion of the CL *reaction mechanism*

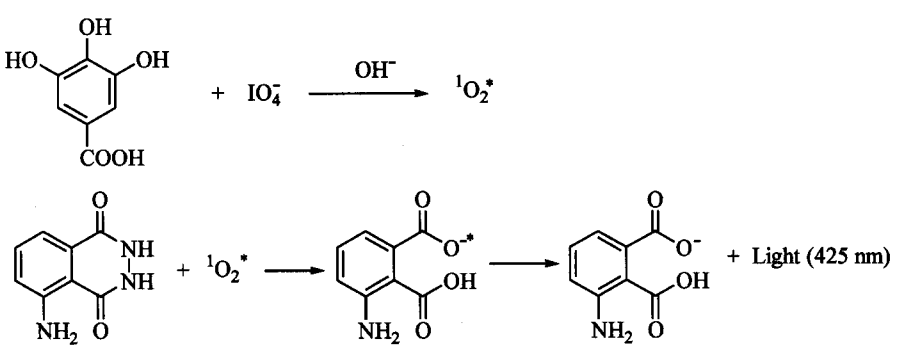
The oxidation of polyhydric phenol has been studied extensively.[21-25] The oxidation mechanism of gallic acid by H_2O_2 has been thoroughly investigated by spectrometric and chemical methods.[21-23] The mechanism involved singlet molecular oxygen. The excited singlet state molecular oxygen was also produced when pyrogallol was oxidized by KIO_4, which resulted in a CL emission.[24,25] Since gallic acid has the same function structure as pyrogallol, its oxidation process by KIO_4 should be similar to that of pyrogallol. Based on the mechanism of the oxidation of gallic acid by H_2O_2 and pyrogallol by KIO_4, the reaction steps can be proposed for the formation of singlet molecular oxygen (Scheme 1).

The maximum wavelength of the CL emission spectrum of luminol-KIO_4-gallic acid system was found at 425 nm, which suggested that the possible emission species was

Scheme 1



Scheme 2



the exited 3-aminophthalate.[25] It was reported that luminol could be oxidized by the singlet state oxygen to produce a strong CL emission.[26-29] Therefore the mechanism of CL reaction of luminol-KIO_4-gallic acid system could be suggested as Scheme 2.

Conclusions

Gallic acid can enhance significantly the weak CL emission from luminol and KIO_4 system. A novel sensor for the gallic acid determination combined with flow injection technique has been developed by electrostatically immobilizing the CL reagent luminol and periodate on anion-exchange resin. The possible reaction mechanism was suggested. The sensor avoids preparing large quantities of analytical reagents and continuously delivering them into the reaction zone as that in a common flow injection CL method. Therefore, it offers the advantages of simple configuration and detection device, convenience operation and high sensitivity.

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