

High selectivity flow sensor for gallic acid

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Abstract: A novel chemiluminescence (CL) sensor for gallic acid combined flow-injection analysis was presented. The analytical reagents involved in the CL reaction, including luminol and ferricyanide, were both immobilized on an anion-exchange resin mini-column. While a volume of sodium phosphate was passed through the column, two reagents were eluted from the resins and then mixed with a gallic acid stream. The fast oxidation reaction between gallic acid and ferricyanide inhibited the CL reaction of luminol and ferricyanide in alkaline solution. The CL emission was correlated with the gallic acid concentration in the range from 0.05~100 ng/mL and the limit of detection was 20 pg/mL (3σ). The whole process could be performed in 2 min with a relative standard deviation of less than 3.5%. The system was stable for over 300 analyses and has been applied for the determination of gallic acid in imitative samples successfully.

Key words: flow sensor; chemiluminescence; immobilized reagents; gallic acid

CLC number: O657.39 **Document code:** A **Article ID:** 1000-274 X (2001)03-0215-05

Gallic acid (3,4,5-trihydroxybenzoic acid) is a white crystalline powder. It is therefore particularly attractive since it occurs in many plant materials and acts as an antioxidant. Many methods have been developed for the determination of gallic acid, including liquid chromatography^[1~4], spectrophotometry^[5], polarography^[6]. Recently, some new methods have been proposed to predict the gallic acid in wine, fruits and vegetables, and HPLC-coulometric detection in a lipidic model system under strong oxidizing conditions^[7].

Chemiluminescence (CL) has attracted increasing attention owing to its high sensitivity, wide linear range and simple instrumentation. Evmiridis^[8,9] has reported the CL flow injection determination of pyrogallol with a pyrogallol-periodate system. Also reported was a method^[10] for the determination of trace amount of pyrogallol with

$\text{IO}_4^-/\text{CO}_3^{2-}/\text{OH}^-$ CL system and the linear range is 5~100 nmol/L. It is well known that luminol can be oxidized by ferricyanide in alkaline medium, producing a strong CL with a maximal intensity wavelength at 425 nm. A FI method using luminol-ferricyanide CL system to determine riboflavin was proposed previously^[11]. In this paper, a novel CL flow sensor for gallic acid determination is presented. It is based on the inhibition of gallic acid in the CL reaction between luminol and ferricyanide and the CL emission decrement is related to the amount of gallic acid. Considering the simplicity of FI system, the two CL reagents, luminol and ferricyanide were both immobilized on anion-exchange resins. Through the injection of 200 μL sodium phosphate, the reagents on the anion-exchange column are eluted from the resin and the CL reaction is inhibited in presence of gallic acid, by which

Received date: 2000-11-11

Foundation item: Supported under science Foundation of Ministry of Education, Shaanxi (98JK112)

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gallic acid can be sensed, and the decrease in CL intensity is linear with logarithm of the concentration of gallic acid range from 0.05 to 100ng/mL with a relative standard deviation of less than 3.5%.

1 Experimental

1.1 Reagents

All the reagents were of analytical grade; Doubly distilled water was used for the preparation of all solutions and dilutions. Luminol (Fluka, Switzerland) and gallic acid were obtained from Xi'an medical supply station. $K_3Fe(CN)_6$, Na_3PO_4 and KOH were the products of Xi'an chemical reagent plant. Finally, Amberlyst A-27 anion exchange resin (0.3 to 0.8 mm) used for reagent immobilization was purchased from Rohm and Haas Co.

1.2 Preparation of immobilized reagents

Amberlyst A-27 (1.0 g) was shaken with 50 mL of 0.06 mol/L luminol or 0.02 mol/L potassium ferricyanide in a flask for 72 h, then the resin was filtered, washed with doubly-distilled water and dry-stored. The most convenient method to determine the amounts of luminol and ferricyanide immobilized was to measure the loss of these reagents from the immobilization solutions UV-Vis. The concentration was monitored at 360 nm for luminol and at 420 nm for ferricyanide. The amounts of luminol and ferricyanide immobilized were 1.99 mmol/g and 0.92 mmol/g resin, respectively.

1.3 Apparatus and procedures

The flow injection system used in this work is shown in Fig. 1. A peristaltic pump was used to deliver all flow streams at a flow rate of 2.0 mL/min on each flow line. PTFE tubing (0.8 mm i. d.) was used between all components in the flow system. 200 μ L sodium phosphate solution was injected by a six-way valve through the anion-exchange column. The eluted luminol and ferricyanide merged with the sample stream in a mixing tubing and the resulting stream then joined with stream before reaching the CL cell. The CL signal

was transduced to an R456 photomultiplier tube (PMT) and recorded by an XWT-206 recorder (Shanghai Dahua Instrument and Meter Plant). The CL signal in the absence of gallic acid and in the presence of gallic acid were recorded as I_0 and I_1 , respectively. The concentration of gallic acid was quantified by the decreased CL intensity, $\Delta I = I_0 - I_1$.

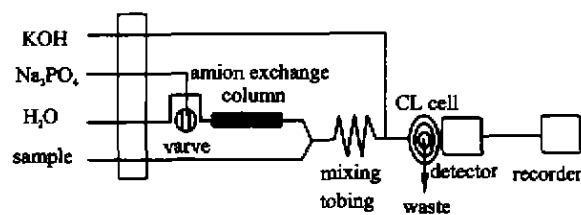


Fig. 1 Schematic diagram of the CL flow sensor for gallic acid determination

2 Results and discussion

2.1 Kinetic curves of CL reaction

The kinetic curves of the CL reaction was tested in a static system, using 0.05 μ mol/L luminol and 0.05 μ mol/L $K_3Fe(CN)_6$. It was found that the rate of the reaction in solution was fast; from the reagent mixing to the peak maximum 6s was needed and it took 60s for the signal to return to zero again as illustrated in Fig. 2 (1). It also showed that the CL intensity decreased sharply in the presence of gallic acid as Fig. 2 (2) shown.

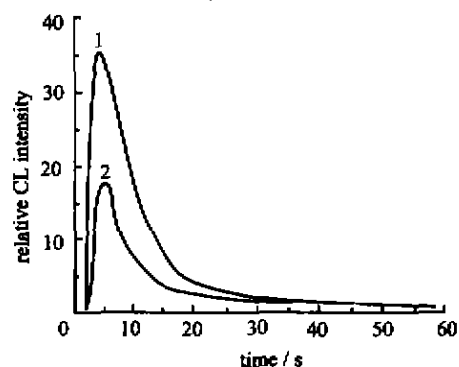


Fig. 2 CL kinetic curve of Lu- $K_3Fe(CN)_6$
1: in the absence of gallic acid
2: in the presence of 10ng/mL gallic acid

2.2 Selection of eluant

Different eluants were injected through the

resin column and released different amounts of luminol and ferricyanide, thus affecting CL intensity. The results were shown in Tab. 1. It was found that sodium phosphate was the better eluant with a maximal CL emission. Therefore, sodium phosphate was selected for subsequent work.

Tab. 1 Character of eluants for gallic acid determination

Type of CL intensity	Relative CL intensity ^a				
	NaCl	NaAc	NaNO ₃	Na ₂ SO ₄	Na ₃ PO ₄
I	75	72	89	92	99
II	44	46	44	42	30
III	31	26	45	50	69

^aThe concentration of each eluant was 0.1 mmol/L
(I): CL intensity in the absence of gallic acid
(II): CL intensity with 1.0 ng/mL gallic acid
(III): Decreased CL intensity

2.3 Effect of molar ratio of immobilized luminol and ferricyanide

To examine the influence of the mixing ratio, resins (0.18 g) with different mixing ratios were packed into a glass column of about 0.5 mL volume (i. d. 5 mm) and the two ends were furnished with glass wool. By injection of sodium phosphate (1.0 mmol/L) at a fixed volume of 200 μ L, different amounts of luminol and ferricyanide were eluted from the resins and generated different CL intensity in alkaline medium, which were shown in Tab. 2. The molar ratio of 1 : 1 (luminol to ferricyanide) was chosen in subsequent work.

Tab. 2 Effect of molar ratio of immobilized luminol and ferricyanide

Molar ratio luminol; K ₃ Fe(CN) ₆	Relative CL intensity		Decreased CL intensity
	Blank ^a	Signal ^b	
3 : 1	141	131	10
2 : 1	135	120	15
1 : 1	129	100	29
1 : 2	100	75	25
1 : 3	89	69	20

^a CL intensity in the absence of gallic acid

^b CL intensity with 1.0 ng/mL gallic acid

2.4 Effect of eluant concentration

The release of luminol and ferricyanide was controlled by the concentration of sodium phos-

phate. Various concentrations of sodium phosphate were injected through the anion exchange resin column with immobilized luminol and ferricyanide. The results were shown in Fig. 3. It is obvious that CL intensity increased with the increase of eluant concentration. To obtain long sensor lifetime and high CL intensity, 1.0 mmol/L sodium phosphate was used for the present system.

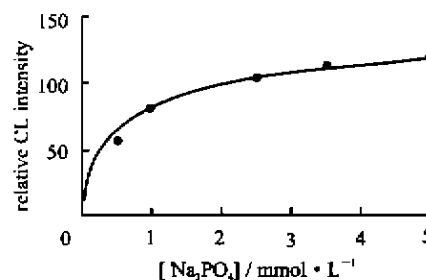


Fig. 3 Effect of concentration of Na₃PO₄ in the presence of 1.0 ng/mL gallic acid

2.5 Effect of acidity in sample solution

It was found that changes in sample acidity had a significant effect on CL intensity and this effect was examined by adjusting the pH of sample solution. The results showed that the decreased CL intensity reached its maximum with a sample solution of pH 6. Then this value was chosen as optimum.

2.6 Effect of potassium hydroxide concentration

Luminol reacts with ferricyanide in alkaline medium. Therefore, potassium hydroxide was added in the flow line to improve the sensitivity of the system. Since the concentration of potassium hydroxide versus CL intensity plot reached a maximum with potassium hydroxide around 0.2 mol/L (Fig. 4), this concentration was used in subsequent experiments.

2.7 Effect of mixing tubing length and flow rate

To ensure the efficient reaction between ferricyanide and gallic acid, mixing tubing was used in this system and its length was tested from 3.0 cm to 8.0 cm. It was found that a suitable length with a high CL intensity emission was 3.0 cm. Pump rate was a critical factor in the determination of gallic acid, the experiment demonstrated that the CL intensity increased with the increasing of pump

rate, to diminish the consumption of reagent and increase the stability of instrument, 2.0 mL/min of each flow line was selected in the following procedures.

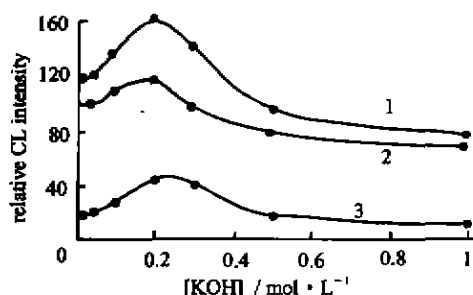


Fig. 4 Effect of KOH concentration with 1.0 ng/mL gallic acid

- 1. luminol- $K_3Fe(CN)_6$ system (I_0)
- 2. luminol- $K_3Fe(CN)_6$ -gallic acid system (I_s)
- 3. decrease in CL intensity ($\Delta I = I_0 - I_s$)

2.8 Performance of the system for gallic acid measurements

Under the optimal conditions, decreased CL intensity was linear over gallic acid concentration

range from 0.05 to 100 ng/mL, $\Delta I = 33.71 \log C_{\text{gallic acid}} + 42.81$, $R^2 = 0.9961$. The limit of detection was 0.02 ng/mL (3σ) with a relative standard deviation of less than 3.5% for 10 ng/mL gallic acid ($n=9$). At a flow rate of 2.0 mL/min, the determination of analyte could be performed in 2 min, including sampling and washing, giving a throughput of about 30 times per hour.

2.9 Interference study

The interference study for the proposed method for gallic acid was focused on some common metal ions and organic species. The tolerable limit of a foreign species was taken if it caused a relative error of less than 5% in the determination of gallic acid (5.0 ng/mL). The tolerable ratio of some interfering inorganic ions and organic compounds was listed in Tab. 3. The main interference was caused by the metal ions, and they could be masked by adding excess amount of EDTA.

Tab. 3 Tolerable concentration ratios with respect to gallic acid for some interfering species (<5% error)

Substance	Tolerable Conc.	Substance	Tolerable Conc.	Substance	Tolerable Conc.
Pb^{2+} , Cr^{6+}	52	Br^- , Cl^-	80	citric acid	9.6
Zn^{2+}	6.5	S^{2-}	3.2	malic acid	100
Mg^{2+}	12	I^- , NO_3^- , Ac^-	60	urea	300
Co^{2+} , Ni^{2+}	30	CO_3^{2-} , HCO_3^-	95	potassium oxalate	1 700
Ca^{2+} , SO_3^{2-}	4.0	SO_4^{2-}	95	sulfosalicylic acid	380
Fe^{3+} , Fe^{2+}	0.56	methanol	800	sodium benzenesulfonate	270
Mn^{2+}	0.56	ethanol	920	ethylhydroxybenzoate	1 000
Cu^{2+}	6.4	glucose	18		

2.10 Sample analysis

Following the procedure described in EXPERIMENTAL section, the proposed method was applied for the determination of gallic acid in two imitative samples. The results were shown in Tab. 4

Tab. 4 Results in imitative samples

Sample	Results by proposed method			
	Found/ ng · mL ⁻¹	Added/ ng · mL ⁻¹	Total/ ng · mL ⁻¹	Recover/ %
No. 1	3.24	1.00	4.32	108
No. 2	59.66	10.0	69.35	96.9

3 Conclusion

Compared with other CL methods, the proposed method offers advantages of simplicity, rapidity, low reagent consumption, high sensitivity as well as wide linear range for the determination of gallic acid.

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(编辑 杨丙雨)

高选择性流通式五倍子酸传感器的研究

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摘要: 采用流动注射和固定化试剂技术, 以五倍子酸对 luminol-K₃Fe(CN)₆ 化学发光体系的抑制作用为基础, 研制出简单、快速、灵敏度高、选择性好的流通式五倍子酸传感器。线性范围: 0.05~100 ng/mL; 检出限: 0.02 ng/mL; 相对标准偏差(RSD): 3.5%。该传感器可连续使用 300 次以上, 采样频率 30 次/h。

关键词: 流通式传感器; 化学发光; 固定化试剂; 五倍子酸