Analytical Chemistry: An Indian Journal

Research | Vol16 Iss 13

Chemiluminescence Determination of L-Tryptophan in Compound Amino Acid Injection Based on Capillary Electrophoresis Analysis

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Abstract

A rapid and sensitive capillary electrophoresis chemiluminescence (CE-CL) method for the determination of L-tryptophan (L-Trp) in compound amino acid injection was developed. The method is based on the inhibitory effect of L-Trp on the CL signal of diperiodatoargentate (III)-luminol CL system in alkaline solution. Under optimized conditions, the relative CL intensity was linear with the L-Trp concentration in the range of 2.0×10–8 to1.0×10–6 M with a limit of detection of 9.0×10–9 M. The relative standard deviation at 7.0×10–8 M L-Trp (n =5) was 3.1%. A possible mechanism was investigated.

Keywords: *Capillary electrophoresis; Chemiluminescence; Diperiodatoargentate (III); L-tryptophan*

Introduction

Capillary electrophoresis (CE) is a micro-analytical technique that provides advantages of simplicity, high separation efficiency, low cost and short analysis time. Chemiluminescence (CL) in the liquid phase has provided a well-established and widely applied spectrometric branch of analytical chemistry [1]. Due to its simplicity, low cost, high sensitivity and selectivity, CL-based detection has become a quite useful detective tool in CE for resolving sensitivity from a short optical pathway in the small inner diameter of the electrophoretic capillary [2]. The combination of sensitive CL detection with effective CE separation can simultaneously achieve efficient separation and sensitive detection of trace components in complex sample, and the interference issue of coexisting component in CL detection can be solved, which can realized a good combination of high efficient separation in microscale and high sensitive detection in a simple way [3,4].

L-tryptophan (L-Trp, Figure 1), one of the indispensable amino acids for human, is a derivative of alanine with an indole substituent on the β carbon. It is necessary to use it as a food fortifier in human diet and pharmaceutical formulation for a normal growth and maintain the positive nitrogen balance since our body cannot synthesize it from other compounds through biochemical reactions. It is also a precursor for the neurotransmitter serotonin, melatonin, niacin and auxin [5] and played an important role in brain function and related regulatory mechanisms [6,7].

Citation: Yunfang Wu (2016) Chemiluminescence Determination of L-Tryptophan in Compound Amino Acid Injection Based on Capillary Electrophoresis Analysis, Analytical Chemistry: An Indian Journal, Vol: 16

Therefore, it is necessary to establish an easy, sensitive and selective method for the estimation of L-Trp. Up to now, several analytical methods have been established for the determination of L-Trp, such as spectrofluorimetry (FL) [8,9], electrochemistry (EC) [10-13], chemiluminescence (CL) [14,15], and high performance liquid chromatography (HPLC) [16-19].

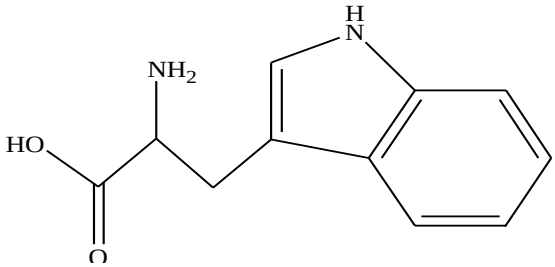

Figure 1: The structure of L-Tryptophan

Some transition metals in highest oxidation state can be stabilized by chelating with suitable polydentate ligands for their unstable character in an aqueous solution. Silver chelates such as diperiodatoargentate (III) (DPA) is good oxidant with a redox potential of 1.74 V in alkaline medium [20,21] In this work, DPA was found to react with luminol to generate a CL emission in a basic medium and L-Trp could greatly inhibit this CL reaction to produce the inhibitory CL signal. This CL system was utilized to develop a rapid and sensitive CE-CL method for the determination of trace amounts of L-Trp. Under the selected optimal experimental conditions, this method was successfully applied for detection of L-Trp in the compound amino acid injection.

Experimental

Chemicals and reagents

All the chemicals used in this work were analytical grade. Ultra pure water (Germany) was used to prepare all solutions. Luminol was obtained from Shannxi Normal University (Xi'an, China). L-Trp was purchased from Shanghai Institute of Pharmaceutical Industry (Shanghai, China). K2S2O8, KIO4, AgNO3 and sodium borate were obtained from Shanghai Chemical Reagent (Shanghai, China).

A 1.0×10−3 M luminol stock solution by solubilization of dilute NaOH solution was prepared in with water. The 1.0×10−3 M L-Trp stock solution was prepared in water. Working solutions were freshly prepared by diluting L-Trp stock solution with water. The 0.1 M borate buffer stock solution was prepared in water. All solutions were stored in a refrigerator. All solutions were filtered through 0.45 μm membrane filters before use.

CE-CL apparatus

All experiments were performed with a laboratory-assembled CE system with a CL detector, as shown in Figure 2. In principle, the CE–CL detection system was designed based on an on-column coaxial flow mode. A 0–30 kV high voltage power supply (Institute of Atom Nucleus, Shanghai, China) provided the high voltage. A 50 cm × 50 μm i.d. uncoated fused-silica capillary (Hebei Optical Fiber, China) was used for separation. The polyimide on 5 cm end section of the separation capillary was burned and removed. After etching with hydrofluoric acid for 50 min, this end of separation capillary was inserted into the 23 cm × 530 μm i.d. reaction capillary. A four-way connector held a separation capillary, a reaction

capillary, the reagent capillary (38 cm × 320 μm i.d.) and the grounding electrode. The detection window was made by burning the polyimide of the reaction capillary and was placed in front of the photomultiplier tube (PMT). The CL signal was collected with a BPCL ultra-weak luminescence analyzer (Institute of Biophysics, Chinese Academy of Science, Beijing, China) and then recorded using a computer with BPCL software. The whole CL detection system was held in a large light-tight box.

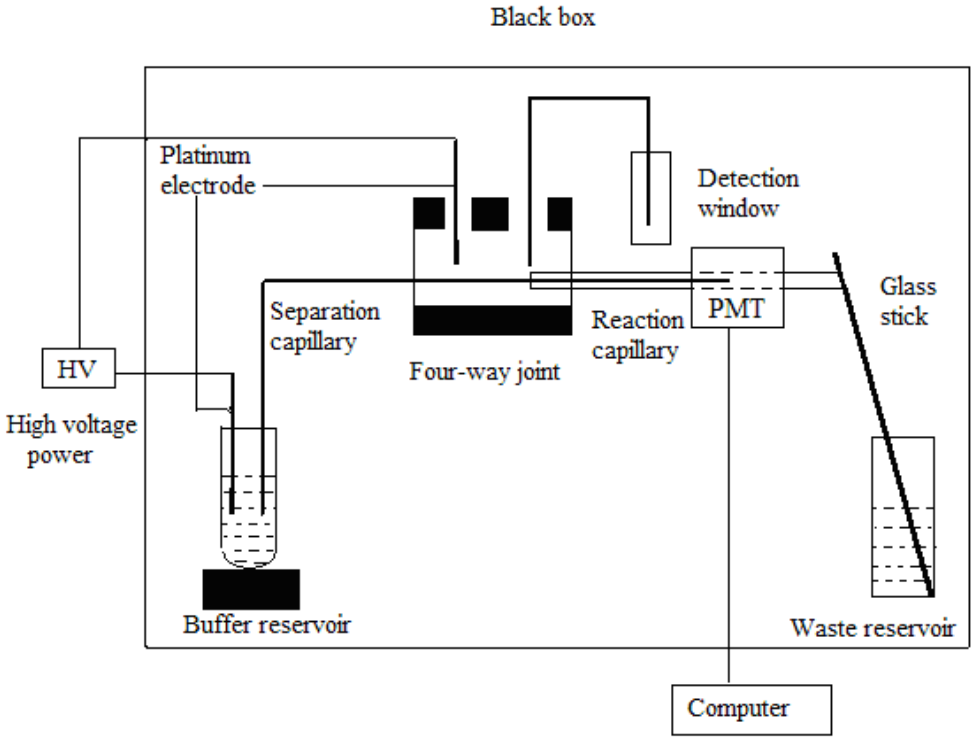

Figure 2: Schematic diagram of the CE-CL apparatus.

DPA complexes preparation

DPA complex was synthesized by slightly modifying Balikungeri method [22]. In brief, AgNO3 (1.36 g), KIO4 (3.68 g), K2S2O8 (3.0 g), KOH (8.0 g) and 100 mL water were taken in a 250 mL round bottomed flask. The solution was heated until boiling and kept in boil about 30 min with constant stirring. The mixture was left to cool to room temperature and then was cooled in an ice bath for filtrating. The obtained orangish-red clear filtrate was stored in the refrigerator. DPA was characterized by the UV-vis spectra (Model Cary 5000 Spectrophotometer, Varian, USA). DPA exhibited two absorption peaks near 254 and 362 nm, which was same as the reported [23]. The DPA concentration was 1.0×10−2 M which was determined by spectrophotometry at 362 nm [24].

CE procedure

At the beginning of each day, the capillary was sequentially rinsed using 0.1 M NaOH, 0.1 M HCl, and water, respectively, and each rinse lasted 10 min. The separation capillary was filled with running buffer while the four-way joint and reaction capillary were filled with CL solution. After each run, the separation capillary was treated with running buffer for 5 min.

During the electrophoresis, the reservoir containing the DPA solution was placed 25 cm above the reaction capillary outlet to continuously deliver the DPA solution by gravity. Luminol was added to the running buffer and introduced into the separation capillary by electroosmotic flow. The sample was introduced by hydrodynamically for 10 s at a height difference of 15 cm. The applied voltage was 20 kV. The determination of L-Trp was based on the linear relationship between the L-Trp concentration and the peak of the relative CL emission intensity, which was obtained by the CL signal decrease $\Delta I = I0 - Is$, (where Is is the CL signal of L-Trp and I0 is the blank).

Results and Discussion

Optimization of the reaction conditions

To maximize the sensitivity of indirect CL detection, several parameters including the concentrations of luminol, DPA, borate and NaOH, the applied voltage were optimized. In these experiments, a 2.0×10^{-7} M of L-Trp solution was injected into the CE-CL system and the CL intensity was recorded.

Because luminol reacts with DPA to produces CL in alkaline condition, the NaOH solution was selected as the reaction medium. The NaOH solution was added into the system by post-column as the DPA medium. The influence of the NaOH concentration on the CL intensity was investigated at different concentrations from 0.06 to 0.14 M. The maximum CL intensity was obtained at 0.1 M NaOH, and the CL intensity decreased dramatically at concentrations above or below this level. Therefore, 0.1 M NaOH was chosen for subsequent experiments. The effect of the concentration of luminol in the running buffer on the CL intensity was investigated over the range of 2.0×10^{-4}–6.0×10^{-4} M. The results indicated that the ΔI increased gradually with increasing luminol concentration up 4.0×10^{-4} M, where maximum ΔI was reached, and further increasing the luminol concentration resulted in a decrease in ΔI intensity. Thus, the luminol concentration of 4.0×10^{-4} M was chosen for sequent research work. The effect of the DPA concentration in the range of 0.6×10–5 to1.4×10–5 M on the ΔI was also investigated. The results demonstrated that the ΔI first increased and then decreased with increasing DPA concentration. The maximum ΔI was recorded when the DPA concentration was 1.0×10–5 M, therefore, this concentration was selected for subsequent experiments.

In the CE–CL detection system, L-Trp migrated in the separation capillary, where it mixed with luminol included in the running buffer. The running buffer concentration affects directly the migration rate of L-Trp and luminol in capillary, which may cause the change of CL intensity. The effect of the borate concentration in the range of 1.0×10^{-3}–1.0×10^{-2} M was investigated. Results showed that the increase of borate concentration shortened the migration time of L-Trp and increased the amount of luminol reaching the detection window of the reaction capillary, which led to the enhancement of the CL signal. However, the peak height was low and the peak shape became poor above 8.0×10^{-3} M borate solution because of baseline noise and Joule heat increased. Therefore, 8.0×10^{-3} M borate solution was employed.

Dependence of CL intensity on applied voltage was examined over the voltage ranges of 12–22 kV, and the experiment results showed that the applied voltage of 20 kV was the optimal one to obtain higher ΔI.

After a careful study on the effects of above several parameters, the CL conditions for the determination of L-Trp were selected as following: 20 kV applied voltage; running buffer consisted of 8.0×10^{-3} M borate and 4.0×10^{-4} M luminol; the oxidizer solution consisted of 1.0×10^{-5} M DPA in 0.1 M NaOH solution. Under optimum conditions, the electrophoregram of L-Trp was shown in Figure 3.

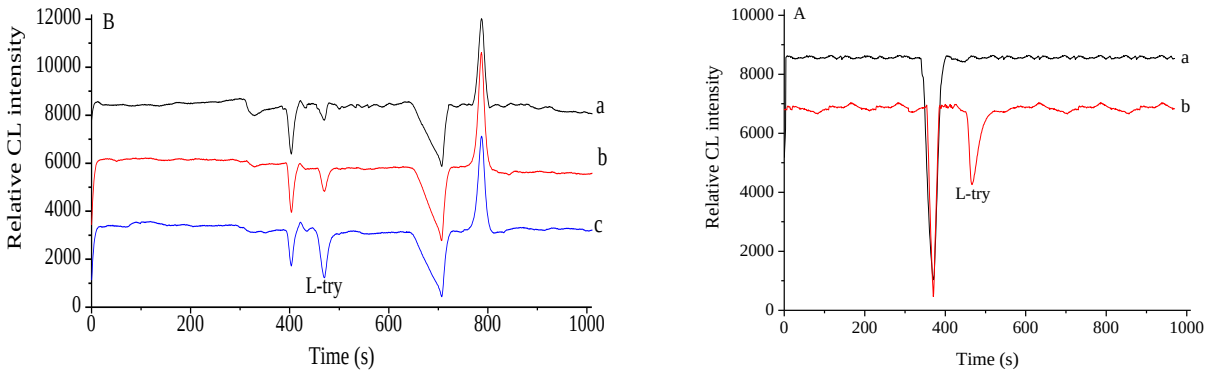

Figure 3: Electropherogram of blank (a) 1.0×10–6 M L-Trp standard L-Trp solution (b). (B) Electropherograms of compound amino acid injections of the compound amino acid injection (a); added with 2.2×10–7 M (b) and 4.4×10–7 M L-Trp (c).

Detection limit, linearity, and reproducibility

Under the optimum conditions, the CE-CL method was evaluated in terms of the response linearity, limit of detection and reproducibility. The calibration curve was linear over the concentrations range from 2.0×10–8 to 1.0×10–6 M, and the limit of detection (3σ) for L-Trp was 9.0×10–9 M. The regression equation of calibration curve for L-Trp was $\Delta I=246.19\ c + 22.47$ (c=1.0×10–7 M, R=0.999). The migration time were 6.0 min The relative standard deviation (RSD) was 3.1% for 7.0×10–8 M L-Trp (n=5).

Analytical application

The compound amino acid injections (18 amino acids) were purchased from a local market. The amount declared was 0.9 g/L. Without any pretreatment, 0.5 mL injection sample was diluted to 10 mL with water and further diluted to the working range of the determination of L-Trp, then analyzed according to the procedure described above. The analytical results for commercial L-Trp drug were compared with those declared on the injection label, i.e. the amounts found were between 0.89 g/L and 0.91 g/L. The RSD of reproducibility was ranged between 2.0% and 3.0%. The results of recovery were listed in Table 1.

Samples	Founded (10^{-7} M)	Added (10^{-7} M)	Total founded (10^{-7} M)	Recovery (%)	RSD (%)
Injection1	2.21	2.2	4.37	98.2	1.8
		4.4	6.59	99.5	2.2
Injection 2	2.19	2.2	4.41	100.9	1.9
		4.4	6.61	100.5	2.3

Table 1: The recoveries of the determination of L-Trp in compound amino acid injections.

Possible inhibition mechanism of CL reaction

The determination of L-Trp proposed in this work was based on its inhibiting effect on the light emission generated by the oxidation of luminol by DPA in a basic medium. In order to investigate the characteristics of the reaction, CL reaction kinetics were studied. As shown in Figure 4, after adding the DPA to the solution of luminol or to the mixing of luminol and L-Trp, the CL reaction took place immediately and reached a maximum within 2 s. It took about 10 s for the signal to return to baseline. There was no CL when the solutions of DPA and L-Trp or the solutions of luminol and L-Trp were mixed up, respectively, indicating that L-Trp only decreased the CL intensity from the reaction of luminol with DPA.

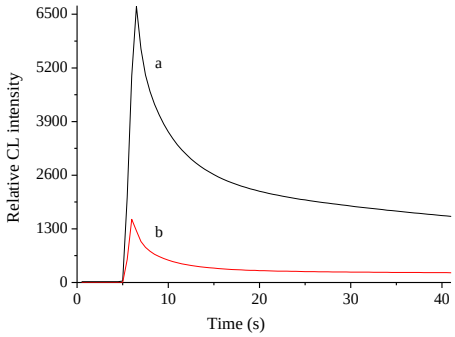

Figure 4: CL kinetic curves of absence (a) and presence of L-Trp (b).

The UV-vis absorption spectra of the luminol (curve a), DPA (curve b), L-Trp (curve f), DPA-L-Trp (curve e), luminol-DPA (curve d) and luminol-DPA-L-Trp (curve c) were recorded (Figure 5). Luminol has two absorption bands at 300 and 350 nm. DPA possesses a characteristic absorption band at 362 nm, and L-Trp has an absorption band at 280 nm. The addition of luminol or L-Trp to the DPA solution caused the color of the DPA solution to change from yellow to colorless, which resulted in a decrease of the absorbance at 362 nm. This result indicated that luminol and L-Trp can be oxidized by DPA.

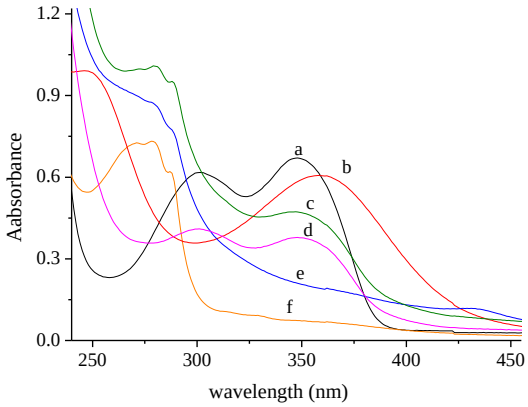

Figure 5: UV-vis spectra of luminol (a), DPA (b), DPA-luminol-L-Trp (c), DPA-luminol (d), DPA-L-Trp (e) and L-Trp (f).

The CL spectrum was obtained using a fluorospectrophotometer (Cary Eclipse Fluorescent spectrophotometer, Varian, USA), whose light entrance slot was shut. The maximum emission spectra of luminol-DPA CL reaction in the absence and presence of L-Trp appeared at 425 nm, and a lower CL peak was observed in the L-Trp presence (Figure 6). It is well known that 3-aminophthalate is the luminophor, and the maximum emission of CL reaction is at 425 nm. This indicated that the CL spectra were independent of L-Trp. Therefore the CL emitter in the both CL reactions between luminol and DPA with and without L-Trp is 3-aminophthalate, which is the oxidation product of luminol.

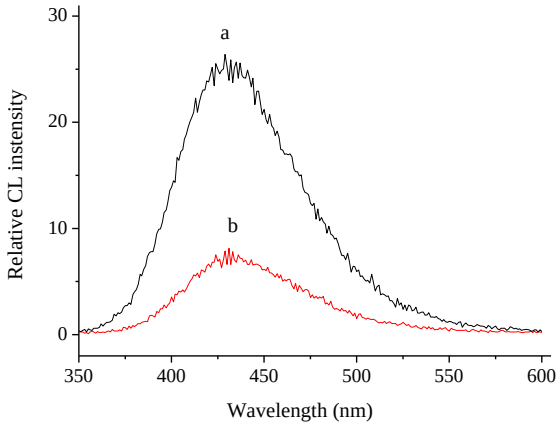

Figure 6: CL spectra of the absence (a) and presence (b) of L-Trp.

CL reaction kinetics showed that in the presence of L-Trp, CL emission of DPA-luminol system in alkaline medium was inhibited. UV-vis absorption spectra showed that the absorption peak of DPA at 362 nm in the presence of L-Trp or luminol resulted in a decrease. This suggested that a competitive oxidation reaction occurred between L-Trp and luminol with DPA. There action might consume DPA, leading to a decrease in CL intensity.

Conclusions

A comparison of previously reported methods, including FL, EC, CL, HPLC and the presented FI–CL method for L-Trp determination is summarized in Table 2. Based on the inhibition effect of L-Trp on the CL intensity of DPA-luminol system, a simple, rapid and sensitive CE-CL method for the determination of trace level L-Trp in compound amino acid injection is developed. It can be seen that this CE-CL analysis exhibits a higher selectivity and sensitivity, confirming this method is of practical value for the assay of L-Trp.

Method	Linear range (M)	Detection limit (M)	samples	Ref.
FL	1.0×10^{-5}–5.0×10^{-4}	6.8×10^{-6}	serum	8
	5.0×10^{-8}–8.0×10^{-6}	9.2×10^{-9}	human fluid	9
EC	2.0×10^{-3}–0.03	1.7×10^{-6}	pharmaceutical formulations	10
	5.0×10^{-6}–9.0×10^{-4}	4.0×10^{-6}	amino acid injection	11
	5.0×10^{-8}–1.0×10^{-5}	1.0×10^{-8}	amino acid injection	12
	5.0×10^{-7}–5.0×10^{-5}	5.0×10^{-8}	blood serum	13
CL	2.1×10^{-7}–7.1×10^{-4}	2.1×10^{-8}	drug	14
	6.0×10^{-7}–3.0×10^{-5}	1.8×10^{-7}	pharmaceutical preparations; human serum	15
HPLC	8.0×10^{-7}–1.6×10^{-4}	4.0×10^{-7}	human plasma	16
	4.9×10^{-7}–1.2×10^{-5}	–	male plasma	17
	3.1×10^{-9}–6.1×10^{-7}	–	water	18
	4.9×10^{-8}–4.9×10^{-5}	5.0×10^{-9}	serum of MDD patients	19
CE-CL	2.0×10^{-8}–1.0×10^{-6}	9.0×10^{-9}	amino acid injection	Our work

Table 2: Comparison of different methods for L-Trp determination.

Acknowledgememts

This work was financially supported by the Natural Science Foundation of Shanxi (Grant No. 2013011013-3); Undergraduate Training Programs for Innovation and Entrepreneurship of Shanxi Colleges and Universities (WL2015CXCY-SJ-02) and Shanxi Normal University of Modern Arts and Sciences (WL2015CXCY-YJ-25).

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