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Short communication

Flow injection determination of adenine at trace level based on luminol–$K_2Cr_2O_7$ chemiluminescence in a micellar medium

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Abstract

A novel flow injection chemiluminescence (CL) analysis method for the determination of adenine in the presence of sodium dodecylbenzene sulfonate (SDBS) surfactant micelles is described. This method is based on the luminescent properties of the luminol–$K_2Cr_2O_7$–adenine in alkaline medium sensitized by SDBS. The optimized experimental conditions were evaluated and the possible mechanism was discussed by examining CL emission spectrum and the effect of various free radical scavengers on CL emission intensity. The CL increase is linearly related to the concentration of adenine in the range of 2.92×10^{-6} to 4.38×10^{-10} mol l^{-1} with a detection limit of 2.46×10^{-10} mol l^{-1} (S/N = 3). The relative standard deviation for 2.92×10^{-7} mol l^{-1} samples was 1.67% ($n = 12$). The proposed method has been applied to the determination of adenine in human serum.

Keywords: Flow injection; Chemiluminescence; Determination; Adenine; SDBS

1. Introduction

Adenine (Fig. 1), an integral part of deoxyribonucleic acid and ribonucleic acid, is one of the most important organic molecules for life and its quantification is critical for many biological studies [1]. For instance, adenine metabolism in physiological fluids, tissues and cells are related to the catabolism of nucleic acids, enzymatic degradation of tissues, dietary habits, and various salvage pathways. Therefore, the quantitative determination of adenine can provide important information on certain disease [2] and genetic diagnosis [3]. Most methods for the determination of adenine are based on electrochemical analysis, which include adsorption stripping voltammetry [4,5], electrophoresis with adsorption-voltammetric detection [6], second order derivative adsorption chronopotentiometry [7], differential pulse voltammetry method [8], overlapping of linear scan voltammetry and differential pulse polarography [9]. In addition, colorimetric assay [1] and some spectroscopic methods such as surface-enhanced Raman scattering (SERS) and fluorescence [3,10] are also suitable for the analysis of adenine. However, the electrochemical methods encounter the problem of low voltammetric peaks and the fouling of irreversible adsorption on the electrode surface. Though SERS methods offer a higher sensitivity, the complicated instrumentation is required.

Chemiluminescence (CL) has received much attention in various fields, especially with flow injection (FIA). The method of CL relying on the effects related to the chemical reaction only, i.e. without the need of external energy supply, has been found to be more advantageous than other luminescence methods. It is characterized by high sensitivity, a large dynamic range of concentrations of the substances determined, minimum background, no disturbances and light scattering, reproducibility and the possibility of simple and quick analysis. But to the best of our knowledge, no CL method coupled with FI analysis has been previously reported for the measurement of adenine in the open literature.

The purpose of this work is to develop a simple, rapid and sensitive FI–CL method for the determination of trace adenine for the first time. Due to various advantageous properties of surfactants, which have been found to improve CL measurement efficiently [11], so we investigated the effects of surfactants on emission intensity in luminol CL system and reported significant and useful improvements in relative intensity. The method possesses a good accuracy and precision and

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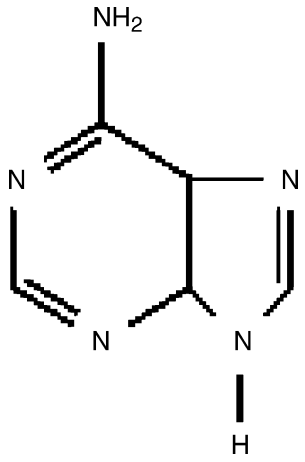

Fig. 1. The structure of adenine.

has been successfully used to determine adenine in human serum.

2. Experimental

2.1. Reagents

All reagents were of analytical grade and all solutions were prepared with doubly distilled water. The stock solutions of 2.92×10^{-4} mol l^{-1} adenine were prepared by directly dissolving it (Beijing Bio Life Science and Technology Co. Ltd.) in doubly deionized water and stored in the refrigerator (4 °C). Luminol was used as supplied to prepare a 0.03 mol l^{-1} stock solution by dissolving 1.3258 g luminol (synthesized by Department of Chemistry, Shaanxi Normal University, China) with 0.05 mol l^{-1} sodium hydroxide to 250 ml in an amber-colored measuring flask. Potassium dichromate, SDBS, sodium hydroxide solutions were prepared by water.

2.2. Apparatus

Fig. 2 shows the schematic diagram of flow injection system. The two pumps of the ultra weak luminescence analyzer (IFFM-D, Remex Electronic Instrument Limited Co., Xi'an, China) were used to deliver flow streams. Polytetrafluoroethylene (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 with sample loop of 78 μl. The mixture of luminol, sodium hydroxide and potassium dichromate were merged into the first single stream through the first Y-shaped mixing element, then mixed the sample through the second Y-shape element, which positioned just before the flow cell inlet. The flow cell was a coil of glass tubing (1.3 mm i.d.) spiraled to a diameter of 35 mm with five turns, located in front of the detection window of the photomultiplier tube (PMT). The CL emission was converted by PMT to current signals and the output was fed to luminescence analyzer, recorded with a computer via special software.

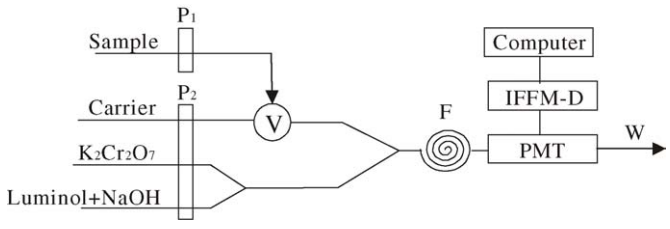

Fig. 2. Schematic diagram of flow injection CL system for the determination of adenine. S, sample; C, carrier (water); R, reagent; P, peristaltic pump; V, six-way valve; F, flow cell, W, waste. IFFM-D, ultra weak luminescence analyser; PMT, photo multiplier tube.

The absorption spectra were performed on a model WZF-2A UV–vis spectrophotometer (Tianjin Optical Instrument Corporation, Tianjin, China).

The CL spectrum was obtained with a series of interference filters (CC143-13, Beijing Hamamatsu Photon. Com, Beijing, China). The filters were inserted between the sample cuvette and the photomultiplier tube (PMT).

3. Results and discussion

3.1. Kinetic curve

The rate of CL reaction plays an important role in the design of a flow CL system. The kinetic curve of CL reaction in the presence of SDBS was investigated with a static method (Fig. 3A). This system showed a fast-type luminescence and the CL intensity reached a maximum value within 0.7 s after the addition of adenine. Compared with that without SDBS (Fig. 3B), the emission intensity increased significantly. This showed the characteristic of this system is very suitable for FI–CL determination of adenine.

3.2. Selection of oxidant

Imidazole group and oxidizable amide group in the molecule structure of adenine make the redox reaction between adenine and some typical oxidants used in CL reactions take place possibly. Thus, the characteristics of several oxidants including permanganate, periodate, ferricyanide, dichromate and hydrogen peroxide of the same concentration reacting with luminol

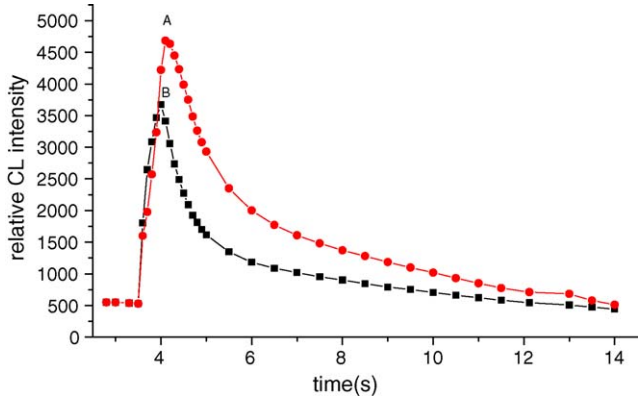

Fig. 3. Kinetic curve: (A) in the presence of adenine, (B) in the absence of adenine. Luminol, 3×10^{-4} mol l^{-1}; $K_2Cr_2O_7$, 1×10^{-4} mol l^{-1}; NaOH, 0.3 mol l^{-1}; SDBS, 2.5×10^{-4} mol l^{-1}; adenine, 2.92×10^{-7} mol l^{-1}.

in the presence of adenine were evaluated. It was found that the most significantly increased CL signal was recorded when potassium dichromate was used as oxidant in basic medium. Therefore, luminol–potassium dichromate system was selected for subsequent experiment.

3.3. Optimization

3.3.1. Effect of luminol concentration

The influence of luminol concentration on CL was initially examined from 7.2×10^{-5} to 1.2×10^{-3} mol l^{-1}. The result indicated that 3×10^{-4} mol l^{-1} luminol gave the highest relative CL intensity and the sensitivity decreased on either side of this value. Therefore, 3×10^{-4} mol l^{-1} luminol was chosen for the subsequent experiment.

3.3.2. Effect of NaOH concentration

The influence of sodium hydroxide concentration on the CL intensity was investigated at different concentrations from 0.09 to 0.75 mol l^{-1} and the maximum CL intensity was obtained at 0.3 mol l^{-1} sodium hydroxide. At concentrations above or below this, the CL intensity decreased dramatically. Therefore, 0.3 mol l^{-1} sodium hydroxide was selected for the present work.

3.3.3. Effect of $K_2Cr_2O_7$ concentration

The effect of $K_2Cr_2O_7$ concentration over the range of 2×10^{-5} to 5×10^{-4} mol l^{-1} on the CL emission was examined. The peak height increased steeply with raising $K_2Cr_2O_7$ concentration up to 1×10^{-4} mol l^{-1}, above which CL intensity decreases sharply probably because of self-absorption of the $K_2Cr_2O_7$. Therefore, 1×10^{-4} mol l^{-1} $K_2Cr_2O_7$ was used for subsequent work.

3.3.4. Effect of SDBS concentration

The utilization of surfactant in the CL reaction system offered many advantages in comparison with those systems in the absence of surfactant. In order to investigate whether surfactant media function effectively in the present CL reaction system, some organized systems, including a non-ionic surfactant (Tween 80), two cationic surfactant (CTMAB, CTAB) and two anionic surfactants (SDBS, SDS) were added to the present system. The results showed SDBS enhanced the signal dramatically. Other surfactants, such as SDS gave a somewhat enhanced signal whereas other surfactants were less effective.

The effect of SDBS concentration on the changes in relative CL intensity was studied at different concentrations from 5×10^{-5} to 3×10^{-4} mol l^{-1}. The CL intensity was found to increase with the increasing concentration of SDBS in the range of 5×10^{-5} to 2.5×10^{-4} mol l^{-1} and decreased when the concentration of SDBS was higher than 2.5×10^{-4} mol l^{-1}. Hence, 2.5×10^{-4} mol l^{-1} SDBS was selected for the following experiments.

3.3.5. Effect of flow rate

The flow rate is an important parameter in CL detection because the time taken to transfer the excited product into the flow cell is critical for maximum collection of the emitted light.

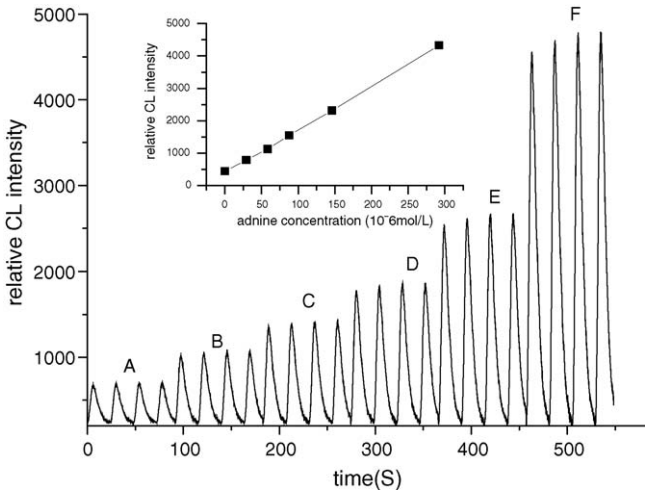

Fig. 4. Typical CL signal of adenine at different concentrations. Inset: calibration curve for adenine. (A) 4.38×10^{-10} mol l^{-1}, (B) 2.92×10^{-8} mol l^{-1}, (C) 5.84×10^{-8} mol l^{-1}, (D) 8.76×10^{-8} mol l^{-1}, (E) 1.46×10^{-6} mol l^{-1}, (F) 2.92×10^{-6}. Luminol, 3×10^{-4} mol l^{-1}; $K_2Cr_2O_7$, 1×10^{-4} mol l^{-1}; NaOH, 0.3 mol l^{-1}; SDBS, 2.5×10^{-4} mol l^{-1}; flow rate, 1.4 ml min^{-1}.

Too low or too high flow rates result in a decrease or even absence of CL signal in the flow cell. The rate of 1.4 ml min^{-1} was chosen as a suitable condition with superior sensitivity, precision and reducing reagent consumption.

3.4. Calibration

Under the selected experimental conditions, a linear calibration graph between 2.92×10^{-6} and 4.38×10^{-10} mol l^{-1} was obtained, typical CL signals was shown in Fig. 4. The calibration equation was $\Delta I = 13.416 \times 10^{-8} c + 387.08$, $r^2 = 0.9993$, with a detection limit of 2.46×10^{-10} mol l^{-1} (S/N = 3). The relative standard deviation (R.S.D.%) for 12 determinations of 2.92×10^{-7} mol l^{-1} was 1.67%. The sample measurement frequency was calculated about 90 samples h^{-1}.

3.5. Interference

The interference of foreign substances were tested by analyzing a standard solution of 2.92×10^{-7} mol l^{-1} adenine into which increasing amounts of interfering analyte was added. The tolerable concentration of a foreign species was taken as a relative error <5% and the results indicate that 1000-folded NaCl, $NaHCO_3$, carbamide, glucose, 100-folded $CaSO_4$, $MgCl_2$, lysine, 50-folded glutamic acid, 10-folded $MnCl_2$, $FeCl_3$, five-folded creatinine, creatine and two-folded leucine had no interference on the determination of the adenine.

3.6. Applications

The proposed CL method was applied to determine adenine in human serum at trace level. Three serums were kindly supplied by the local hospital and analyzed after dilution with water without any pretreatment process and checked by the standard

Table 1
The results of sample analysis ($n = 5$)

Sample no.	Found[a] ($\times 10^{-8}$ mol l^{-1})	Added ($\times 10^{-8}$ mol l^{-1})	Total ($\times 10^{-8}$ mol l^{-1})	Recovery (%)	R.S.D. (%)	Content[a] (10^{-6} mol l^{-1})
1	3.07, 6.14	2.33, 2.33	5.36, 8.61	98.34, 106.01	2.23, 2.51	6.14
2	3.56, 7.12	2.33, 2.33	5.81, 9.59	97.85, 106.01	2.07, 1.46	7.12
3	2.65, 5.30	2.33, 2.33	5.11, 7.60	105.64, 98.85	2.35, 2.63	5.30

Luminol, 3×10^{-4} mol l^{-1}; $K_2Cr_2O_7$, 1×10^{-4} mol l^{-1}; NaOH, 0.3 mol l^{-1}; SDBS, 2.5×10^{-4} mol l^{-1}; flow rate, 1.4 ml min^{-1}.
[a] Mean ± S.D. ($n = 3$).

addition method. The results of determination were listed in Table 1, with recovery from 97.85 to 106.01%.

4. CL mechanism

4.1. The nature of CL emitter

In order to elucidate the possible mechanism of the CL reaction, the following experiments were performed with adenine working solution.

Firstly, a series of optical filters were placed between flow cell and PMT to ascertain the nature of the emitting species. The results showed the maximum emission wavelength in the presence of adenine was the same as that in the absence of adenine and the maximum light emission was at about 425 nm. The emitter was 3-aminophthalate, the oxidization product of luminol.

Furthermore, the effects of various free radical scavengers such as ascorbate, formate, thiourea were investigated to illustrate if free radical participated in the CL reaction and the results were summarized in Table 2. The striking quenching effect was observed upon the addition of ascorbate, a common scavenger of free radicals. The complete quenching of the emission was achieved even at 10^{-3} mol l^{-1} level of ascorbate. The results indicated that free radicals were involved in the present CL process. As formate, a scavenger for $^{\bullet}OH$, was added to the system it was noticed that the intensity was greater than that adenine alone at the same concentration. A similar effect was also observed by Zhang et al. in the determination of hemoglobin. Formate ion was known to react with $^{\bullet}OH$ radical and subsequently with O_2 to form $O_2^{\bullet}$ radical. In this way, the efficiency of $O_2^{\bullet}$ generation could be doubled. But thiourea, another scavenger for $^{\bullet}OH$, did not completely inhibit the light emission even at high concentrations [12]. The effects of scavengers on CL emission intensity indicated the formation of $^{\bullet}OH$ radical and possibly $O_2^{\bullet}$ radical, during the CL reaction process.

Table 2
The influence of free radical scavengers on CL emission intensity of luminol–$K_2Cr_2O_7$–adenine

Scavengers	Concentration (mol l^{-1})	Relative CL intensity
None	0	792
Ascorbate	10^{-5}, 10^{-4}, 10^{-3}	389, 265, 38
Sodium formate	0.1, 0.5, 1.0	837, 1806, 6271
D–Mannitol	8×10^{-6}, 8×10^{-5}, 4×10^{-4}	515, 419, 106

Luminol, 3×10^{-4} mol l^{-1}; $K_2Cr_2O_7$, 1×10^{-4} mol l^{-1}; NaOH, 0.3 mol l^{-1}; SDBS, 2.5×10^{-4} mol l^{-1}; flow rate, 1.4 ml min^{-1}; adenine, 2.92×10^{-7} mol l^{-1}.

Though the detail describing the effect of adenine on the oxidation of luminol is still unknown, we can conclude that the possible mechanism of the CL reaction could be best explained by the following pathway: reaction of the adenine with potassium dichromate in basic solution could produce hydroxyl radicals and superoxide radicals; superoxide radicals could oxidize luminol to form excited intermediate product 3-aminophtalate ion, when 3-aminophtalate ion relaxes to its ground state, light was emitted. A schematic reaction process could be proposed as follows:

$$\text{adenine} + K_2Cr_2O_7 + OH^- \rightarrow \text{adenineoxide} + {}^{\bullet}O_2 + {}^{\bullet}OH$$

$$\text{luminol} + {}^{\bullet}O_2 + {}^{\bullet}OH \rightarrow [\text{3-aminophthalate}]^*$$

$$[\text{3-aminophthalate}]^* \rightarrow [\text{3-aminophthalate}] + h\nu$$

4.2. The role of SDBS in the CL process

The principles of micellar enhancement including solubilization and solute organization, altering the local microenvironment and changes to the light-emitting pathways that affect the quantum yield and reaction rate have already been discussed by Townshend et al. [11].

In present work, the enhancement of SDBS could arise in the following possible ways. The first is the effect of SDBS micelles, when the concentration of SDBS reached its critical micelle concentration (CMC) value (2.87×10^{-4} mol l^{-1}), the SDBS molecules tend to form micelles in aqueous solution, which promoted better contact, provided a protective environment for the excited singlet state and led to a significant increase in the CL quantum yield. The second is the facilitating energy transfer, the benzene sulfonate group of the SDBS has fluorescent characteristics, and it can act as acceptors of the energy in the CL reaction. This was also confirmed by experiments that showed that SDBS enhances the CL almost to the same extent when their concentrations are below the CMC of SDBS [13].

In the presence of SDBS, the UV–vis absorption spectra showed similar location and appearance of the band to that without SDBS, but relatively weak, likely due to the coordination effect (Fig. 5). It also indicated that SDBS might not involve in the ultimate emission step but be favorable to one or more intermediate steps.

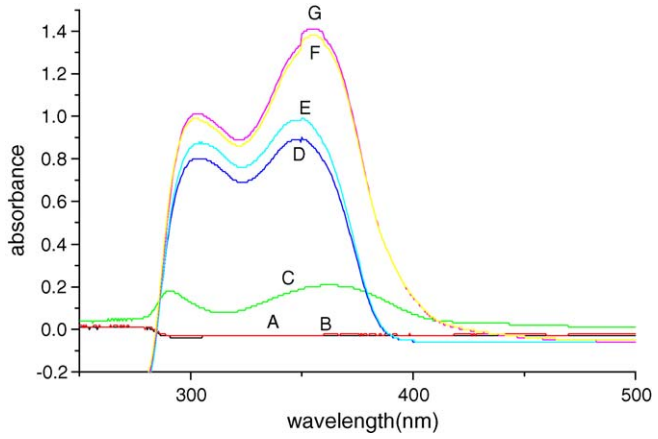

Fig. 5. UV–vis absorption spectra: (A) adenine, (B) adenine-SDBS, (C) $K_2Cr_2O_7$, (D) luminol–NaOH, (E) luminol–$K_2Cr_2O_7$–NaOH–adnine-SDBS, (F) luminol–$K_2Cr_2O_7$–NaOH, (G) luminol–$K_2Cr_2O_7$–NaOH–adenine. Luminol, 3×10^{-4} mol l^{-1}; $K_2Cr_2O_7$, 1×10^{-4} mol l^{-1}; NaOH, 0.3 mol l^{-1}; SDBS, 2.5×10^{-4} mol l^{-1}; adenine, 2.92×10^{-5} mol l^{-1}.

5. Conclusions

This paper firstly reports a FIA-CL method for the determination adenine in SDBS micelles. Compared with the other methods, the present work is sensitive and rapid and precludes the treatment of the sample. The desirable recovery ratio ensured the accurate detection of adenine in human serum and can be used in clinical diagnosis and pharmaceutical quality control.

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