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# A Novel Chemiluiminescence System Based on Ag(III) Complex Oxidation Sensitized by Rhodamine 6G and the Application for the Determination of Cefazolin Sodium in the Injectable Powder and Human Urine

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A novel chemiluminescence (CL) system for the determination of cefazolin sodium is developed based on the direct chemiluminescence reaction of $[Ag(HIO_6)_2]^{5-}$-Rhodamine 6G (Rh6G)-cefazolin sodium system in sulphuric acid medium. The possible mechanism of chemiluminescence emission and enhancing effect was discussed. Rhodamine 6G can be oxidized by Ag(III) complex to be its excited form (Rh6G*), which could produce chemiluminescence emission at about 550 nm. Otherwise, Rhodamine 6G can be oxidized by Ag(III) complex to be Rh6G oxide. Cefazolin sodium in acid solution could be oxidized by Ag(III) complex to be its excited form, which could excite Rh6G oxide to be its excited form (Rh6G oxide*), which could produce chemiluminescence emission at about 420 nm. The regression equations of the calibration curves in the range of $4.0 \times 10^{-8}$-$4.0 \times 10^{-6}$ g/mL can be shown as follows: LgI = 0.0018 C + 1.9848, with a correlation coefficient ($R^2$) of 0.9902. The limit of detection (LOD) was $1.73 \times 10^{-8}$ g/mL. The proposed method was applied satisfactorily for the determination of cefazolin sodium in the injectable powder and patient urine.

**Key Words: Chemiluminescence, Ag(III) complex, Rhodamine 6G, Cefazolin sodium, Injectable powder, Human urine.**

## INTRODUCTION

Cefazolin sodium is β-lactam antibiotic, which has a potential antibacterial activity against a broad spectrum of microorganisms, gram-positive and gram-negative bacteria and has a curative effect for the control of pneumonia, faucitis and urinary tract infection[1]. A great deal of attention has been paid to its determination in various biological fluids.

A comprehensive review for the analysis of members of cephalosporin antibiotics covers most of the methods described for the analysis of these drugs in pure forms, in different pharmaceutical dosage forms and in biological fluids[2]. Several spectrophotometric methods were used for the determination of cefazolin sodium in pure samples and in pharmaceutical preparations[3-5]. A spectrofluorometric method was reported for the determination of cefazolin sodium[6]. Capillary zone electrophoresis had been used to determine cefazolin sodium in pig serum and human plasma[7]. A series of liquid chromatography (LC) methods with UV detection have been reported for the determination of cefazolin sodium in biological fluids, animal tissues, food, *etc*.[8-17]. However, the reported LC methods showed lower sensitivity and narrow linearity range, in some cases, measurement conditions, such as the selection of ion-exchanger and the amount of ion-pair reagent, should be carefully controlled to achieve good selectivity.

The chemiluminescence (CL) method shows the advantages of simplicity, rapidity and high sensitivity and has been applied extensively for the analysis of pharmaceutical compounds[18]. However, there are few reports for the determination of cefazolin sodium by chemiluminescence method. Two flow-injection chemiluminescence methods were used for the determination of cefazolin sodium, respectively, based on the reaction of the analyte with permanganate and the reaction of luminol with permanganate, with high detection limits (1.6 mg/L and 400 μg/L)[19,20]. A permanganate-glyoxal chemiluminescence system was used for flow-injection analysis of cefazolin sodium in pharmaceutical preparations with high sensitivity[21]. In order to enhance chemiluminescence, rhodamine 6G (Rh6G) was used for determination of phenolic compounds, furosemide and chlorpromazine hydrochloride using cerium(IV) as an oxidant[22-24] and for determination of terbutaline sulfate based on potassium ferricyanide oxidation[25].

In previous works a novel chemiluminescence reaction system with (Ag(III) complex, $[Ag(HIO_6)_2]^{5-}$), was developed for the determination of some fluoroquinolones[26-28]. The main purpose of this work is to develop a new chemiluminescence

system for the determination of cefazolin sodium based on (Ag(III) complex oxidation sensitized by rhodamine 6G. The possible chemiluminescence mechanism was described. The proposed method was applied for the determination of cefazolin sodium in the injectable powder and urine samples with satisfactory result.

## EXPERIMENTAL

The flow injection system (Fig. 1), is a IFFM-D FICL analysis system (Xi'an Remex Electronic Science Tech Co. Ltd., Xi'an, China) consisted of two peristaltic pumps working at a constant flow rate (60 rpm, 3.0 mL/min) and a six-way injection valve with a sample loop (120 μL), which is automatically operated by a computer equipped operation system of IFFM-D flow injection analysis. PTFE tubing (0.8 mm i.d.) was used to connect all components in the flow system. The flow cell was placed close to the window of the photomultiplier tube (PMT, operated at -800 V).

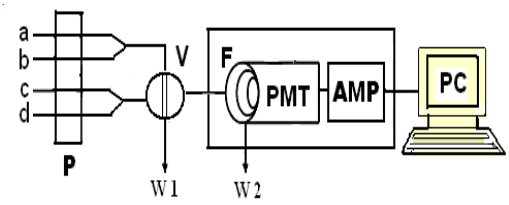


Fig. 1. Schematic diagram of flow injection chemiluminescence analysis system P-peristaltic pump; V- sampling inlet valve; F-flowing cell; PMT-photomultiplier tube; AMP-amplifier; PC-recorder; W-waste; a-Rh6G solution; b-sulfuric acid solution; c-sample; d-$[Ag(HIO_6)_2]^{5-}$ solution

Cefazolin sodium was purchased from the North China Pharmaceutical Co. Ltd. (Shijiazhuang, China). All reagents were of analytical grade. Purified water was prepared by an XGJ-30 highly pure water machine (Yongcheng purification Science & Technology Co. Ltd., Beijing, China).

Sodium periodate ($NaIO_4$, 99.5 %) was purchased from Tianjin Kermel Chemical Reagent Company (Tianjin, China). Potassium peroxydisulfate ($K_2S_2O_8$, 99.5 %) was purchased from Beijing Chemical Reagent Company (Beijing, China). Silver nitrate ($AgNO_3$, 99.8 %) and potassium hydroxide (KOH, 82 %) were purchased from Tianjin Damao Chemical Reagent Company (Tianjin, China). All chemicals were of analytical reagent grade and used without further purification and deionized water was used throughout.

The Ag(III) complex, *bis*(hydrogenperiodato) argentate(III) complex anion $[Ag(HIO_6)_2]^{5-}$, stock solution was prepared by oxidizing Ag(I) in the alkaline medium according the known method[29]. The concentration of Ag(III) complex solutions prepared was determined accord to literature[30].

A stock solution (2 mg/mL) of cefazolin sodium was prepared by dissolving an accurately weighed amount of 0.1 g in a 50 mL volumetric flask and adjusting to the volume with water and stored in refrigerator at -4 ºC. The stock solutions are steady within 6 months. The standard solution of 100 μg/mL was prepared by diluting the stock solution with water. All standard working solutions were prepared weekly and filtered through 0.45 μm cellulose acetate filters (Shanghai Xinya Purification Material Factory) prior to injection.

**Procedures:** The investigation of the chemiluminescence intensity-time profiles was performed with the static chemiluminescence analysis. In a 10 mL calibrated flask, standard solution and $[Ag(HIO_6)_2]^{5-}$ solution were mixed and added in a reaction tube, then rhodamine 6G and sulfuric acid solutions were injected into the reaction tube by a quantitative injector. The chemiluminescence intensity was measured without stirring.

In the flow injection system, both sample solution and $[Ag(HIO_6)_2]^{5-}$ solution were pumped through the flow lines (b) and (c), respectively, when the injection valve was switched to the position of injection, rhodamine 6G solution and sulfuric acid solutions through the flow lines (a) and (b) were injected, producing chemiluminescence emission. The concentration of cefazolin sodium was quantified by the decrement of peak height of the chemiluminescence signals.

**Sample treatment:** Urine sample was provided by Hospital of Hebei University. 1 mL of urine sample was taken and diluted to 10 mL with deionized water. 1 g $PbO_2$ powder was added and followed by stirring for 10 min to eliminate urine acid, thiourea and ascorbic acid, *etc*. After centrifugation for 10 min at 10000 rpm, the supernatant was filtrated, then the filtrate was applied to a cation exchange column (4 cm × 1.2 cm) for clean up. The clear liquid was diluted by 100 fold with deionized water. A 0.4 mL of the diluted sample solution was taken in a 25 mL calibrated flask and then diluted to the volume, making the concentrations of cefazolin sodium in the linear range for chemiluminescence analysis.

## RESULTS AND DISCUSSION

**Characteristic and mechanism of chemiluminescence:** Before carrying out the flow-injection method, the kinetic characteristics of the proposed chemiluminescence reaction were studied by static injection method. The response curve (intensity *versus* times) of $[Ag(HIO_6)_2]^{5-}$-Rh6G -cefazolin sodium system in sulphuric acid medium was recorded in term of different order of addition of the three reagents to study the kinetic characteristic of the chemiluminescence reaction. The result showed that the chemiluminescence emission observed in the addition sequence of $[Ag(HIO_6)_2]^{5-} \rightarrow$ Rh6G $\rightarrow$ cefazolin sodium $\rightarrow$ $H_2SO_4$ was higher and sharper than that in other two addition sequences and the reaction rate in solution was very fast. The kinetic curves (Fig. 2) indicated that from reagent mixing to peak maximum only 0.3 s was needed and it took 5 s for the signal to return to zero again. The result obtained by static injection and flow injection methods indicated the chemiluminescence method is rapid and sensitive enough and suitable to perform determination of cefazolin sodium.

The preliminary experiment showed that cefazolin sodium could react with $[Ag(HIO_6)_2]^{5-}$ to produce a weak chemiluminescence emission and this chemiluminescence reaction could be sensitized by rhodamine 6G. In order to get an idea about the reaction product generating chemiluminescence, the chemiluminescence spectra of $[Ag(HIO_6)_2]^{5-}$-Rh6G-cefazolin sodium system in sulphuric acid medium were recorded by Hitachi F-7000 fluorescence spectrophotometer (taken off lamp-house), as shown in Fig. 3. It is observed that this reaction system shows two chemiluminescence peaks at *ca.* 550 and *ca.* 420 nm.

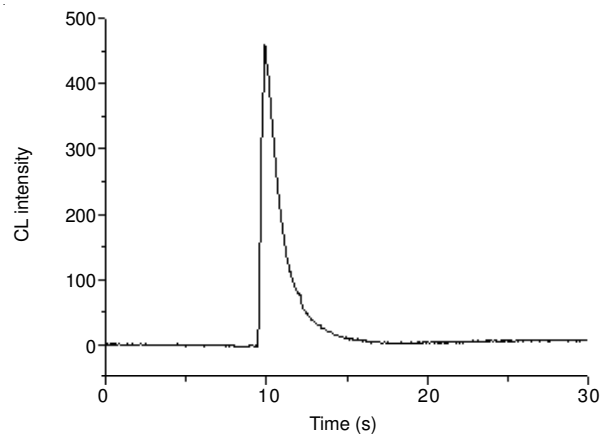


Fig. 2. Kinetic characteristic of the chemiluminescence (CL) reaction of $[Ag(HIO_6)_2]^{5-}$ - Rh6G -cefazolin sodium-$H_2SO_4$ system

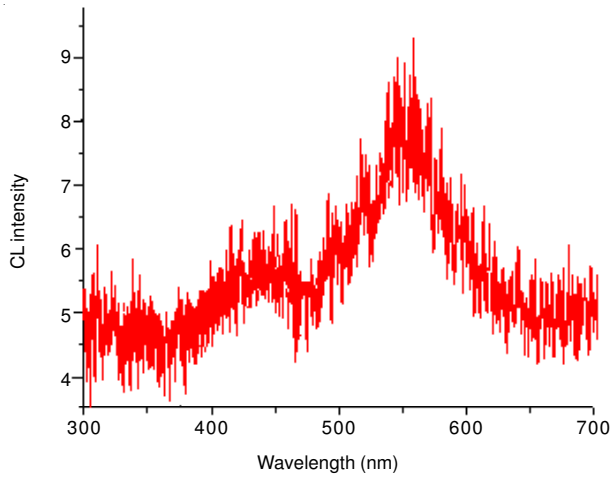


Fig. 3. Chemiluminescence spectra of $[Ag(HIO_6)_2]^{5-}$ -Rh6G -cefazolin sodium-$H_2SO_4$ system

The previous work showed that Ag(III) complex ion has two forms, $[Ag(HIO_6)_2]^{5-}$ (Ag(III)) and $[Ag(HIO_6)(OH)(H_2O)]^{2-}$ (Ag(III)*) and the last could be active center and could take place oxidation reaction[31]. Rhodamine 6G in sulphuric acid medium can be oxidized by the oxidant Ag(III)* as following reaction equation.

Rhodamine 6G can be oxidized by Ag(III)* to be excited form (Rh6G*), then de-excited to its ground state, producing the chemiluminescence emission (*ca.* 550 nm)[22]. Rh6G oxide could be oxidized by Ag(III)* further to be excited form (Rh6G oxide*). Otherwise, cefazolin in acid solution could be oxidized by Ag(III)* to be excited form (cefazolin*), which also could excite Rh6G oxide to the excited form (Rh6G oxide*). Then Rh6G oxide* de-excited to its ground state, producing the chemiluminescence emission (420 nm). This chemiluminescence emission of Rh6G oxide is same as rhodamine B oxide because both have same molecular structure[22].

Based on the discussion above, the possible chemiluminescence mechanism for the reaction system can be described as follows:

$$[Ag(HIO_6)_2]^{5-} + H_2O \rightarrow [Ag(HIO_6)(OH)(H_2O)]^{2-} + H_2IO_6^{3-}$$

$$[Ag(HIO_6)(OH)(H_2O)]^{2-} + \text{Rh6G} \rightarrow \text{Ag(I)} + \text{Rh6G}^* + \text{Rh6G oxide}$$

$$\text{Rh6G}^* \rightarrow \text{Rh6G} + h\nu \text{ (}ca.\text{ 550 nm)}$$

$$\text{Rh6G oxide} + [Ag(HIO_6)(OH)(H_2O)]^{2-} \rightarrow \text{Ag(I)} + \text{Rh6G oxide}^*$$

$$\text{Cefazolin} + [Ag(HIO_6)(OH)(H_2O)]^{2-} \rightarrow \text{Cefazolin}^* + \text{Ag(I)}$$

$$\text{Cefazolin}^* + \text{Rh6G oxide} \rightarrow \text{Rh6G oxide}^* + \text{Cefazolin}$$

$$\text{Rh6G oxide}^* \rightarrow \text{Rh6G oxide} + h\nu \text{ (}ca.\text{ 420 nm)}$$

**Choice of acid medium and concentration:** An attempt was made to research and develop a new and sensitive chemiluminescence system that could be applied for the chemiluminescence determination. The kind of acid used in the reaction has a significant influence on the chemiluminescence emission intensity. Therefore, several acids, such as HCl, $H_2SO_4$, $HNO_3$, $H_3PO_4$ and $H_6P_4O_{13}$, were added in the $[Ag(HIO_6)_2]^{5-}$ solution to test the effect of acidic medium on the chemiluminescence signal, respectively. The results indicated that chemiluminescence signal could be produced by the direct chemiluminescence reaction of $H_2SO_4$ and $[Ag(HIO_6)_2]^{5-}$, with the highest and stable emission. The reason why $H_2SO_4$ could have best result compared to other acids tested needs to be researched further.

The concentration of $H_2SO_4$ used in the reaction has a very significant influence on the chemiluminescence emission intensity. The chemiluminescence intensity increased remarkably with the increase of concentration of $H_2SO_4$ in the range from 0.005-0.20 mol/L, then decreased with further increase of $H_2SO_4$ concentration. So 0.20 mol/L $H_2SO_4$ was selected as optimal concentration.

**Effect of $[Ag(HIO_6)_2]^{5-}$ concentration:** In this chemiluminescence system, $[Ag(HIO_6)_2]^{5-}$ was used as the oxidant, its concentration influenced the sensitivity. Therefore, the dependence of the $[Ag(HIO_6)_2]^{5-}$ concentration on the chemiluminescence intensity was investigated for $1.0 \times 10^{-6}$ g/mL analyte. The chemiluminescence intensity increased remarkably with the increase of $[Ag(HIO_6)_2]^{5-}$ concentration in the range from $1.0 \times 10^{-5}$-$2.5 \times 10^{-5}$ mol/L, then decreased obviously with further increase of $[Ag(HIO_6)_2]^{5-}$ concentration. So the optimized concentration of $2.5 \times 10^{-5}$ mol/L was selected.

**Effect of rhodamine 6G concentration:** The preliminary experiment showed that cefazolin sodium could react with $[Ag(HIO_6)_2]^{5-}$ to produce a weak chemiluminescence emission and this chemiluminescence reaction could be sensitized by rhodamne 6G. The effect of rhodamne 6G was further examined. Fig. 4 shows that the maximum chemiluminescence intensity was obtained when the concentration of rhodamne 6G was $4.0 \times 10^{-6}$ mol/L. So rhodamne 6G of $4.0 \times 10^{-6}$ mol/L was chosen for the subsequent study.

**Effect of sample volume and flow rate:** The role of sample volume and flow rate is critical. For instance, if the sample volume is too small or too large, the maximum chemiluminescence could not be obtained. The highest emission was obtained when the injected sample volume was 120 µL. The chemiluminescence intensity increased with increasing flow rate. A flow rate of 3.0 mL/min for all solutions was recommended because of greater precision and economy in the use of reagents.

**Analytical performance of chemiluminescence systems:** The influence of some common excipients on the chemiluminescence intensity was investigated for determining cefazolin sodium by comparing with the chemiluminescence emissions.

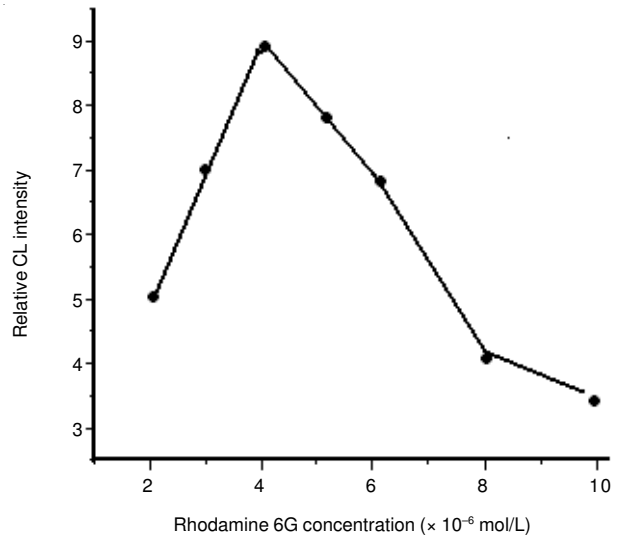


Fig. 4. Effect of rhodamine 6G concentration on relative chemiluminescence (CL) intensity. $[Ag(HIO_6)_2]^{5-}$; $2.5 \times 10^{-5}$ mol/L; $H_2SO_4$: 0.02 mol/L; Cefazolin sodium: $8.0 \times 10^{-8}$ g/mL

The interfering effects from foreign species were investigated. The tolerated ratios of foreign substances to $4 \times 10^{-7}$ g/mL analyte were 100-fold for starch and $Ca^{2+}$; 50-fold for lactose[4] and D-galactose; 40-fold for glucose, dextrin, $Zn^{2+}$, $Mg^{2+}$ and $Fe^{3+}$; 30-fold for sucrose; 2-fold for $Cu^{2+}$ and equal for amounts of ascorbic acid, citric acid and sodium benzoate. Since samples had been pretreated by deposition and cation exchange, there was no interference in the analysis of real samples.

Under the optimized conditions, the linearity was evaluated for cefazolin sodium by using the proposed systems. The regression equations of the calibration curves in the range of $4.0 \times 10^{-8}$-$4.0 \times 10^{-6}$ g/mL can be shown as follows: LgI = 0.0018 C + 1.9848, with a correlation coefficient ($R^2$) of 0.9902. It can be seen that the linearity is acceptable based on the criteria ($R^2 \geq 0.98$) described by Green[32].

The limit of detection (LOD) was determined as the sample concentration that produces a peak with a height three times of the level of baseline noise. The LOD was $1.73 \times 10^{-8}$ g/mL. The RSD was found to be 2.3 % for 11 determinations of $8.0 \times 10^{-8}$ g/mL. The LOD value of the method is lower than that of spectrometry[3-5], fluorometry[6], CE[7], LC[9,11-16] and chemiluminescence method[19,20]. It is indicated that the proposed enhanced chemiluminescence system has satisfactory linearity, sensitivity and precision.

**Sample analysis:** During the first 3 h after injection, a mean concentration of cefazolin sodium in urine was $7.320 \pm 1.498 \times 10^{-5}$ g/mL[33]. This drug can be administered perorally or intravenously and they are excreted mainly into the urine. Based on these informations and the LOD of this method it was suggested that the effective detection for the drugs in patient urines after the administration could be achieved.

Calibration curves were prepared for cefazolin sodium. 0.5 g cefazolin sodium powder injections taken from five bottles (0.1 g per a bottle) with same batch number was dissolved with water to 25 mL for analysis. The proposed method and UV-method[34] were applied for the determination of cefazolin sodium in the powder injections. The obtained results were 96.8 % with the relative standard deviation (RSD) of 2.1 % (n = 7) for this method and 97.0 % for UV method, without obvious difference.

In order to evaluate the validity of the proposed method, recovery studies were carried out on real samples to which known amounts of cefazolin sodium were added at three concentration levels. The result is given in Table-1.

TABLE-1
DETERMINATION OF CEFAZOLIN SODIUM (n = 5)

| Sample | Content ($10^{-8}$ g/mL) | Added ($10^{-8}$ g/mL) | Detected ($10^{-8}$ g/mL) | Recovery (%) | RSD (%) |
|---|---|---|---|---|---|
| Injection | 6.5 | 3.2 | 9.44 | 91.9 | 2.1 |
| | | 6.4 | 12.8 | 98.4 | 1.8 |
| | | 25 | 31.3 | 99.2 | 1.6 |
| Urine* | 5.8 | 3.2 | 8.97 | 99.1 | 1.6 |
| | | 5.1 | 10.4 | 90.2 | 1.9 |
| | | 20 | 25.6 | 99.0 | 1.5 |

*Diluted urine sample.

For the injection solution made with the cefazolin sodium powder, the concentration of the drug was $6.5 \times 10^{-8}$ g/mL and the recoveries were in the range of 91.9-99.2 % with the RSDs of 1.6-2.1 %. The proposed method was applied for the determination of cefazolin sodium in patient urine taken at 7 h after the administration of 2.0 g cefazolin sodium. The urine sample was treated using the procedure described above and then analyzed by standard curve method. For the diluted urine sample, the concentration of the drug was $5.8 \times 10^{-8}$ g/mL and the recoveries were in the range of 90.2-99.1 % with the RSDs of 1.5-1.9 %. Considering dilution factor, the concentration of the drug in the patient urines was $3.625 \times 10^{-4}$ g/mL.

## Conclusion

A novel chemiluminescence method was developed based on the reaction of $[Ag(HIO_6)_2]^{5-}$-Rh6G-cefazolin sodium system in sulphuric acid medium. The excited forms of both Rh6G* and Rh6G oxide* are the iluminants, which de-excited to its ground state, producing the chemiluminescence emission at about 550 and 420 nm, respectively. The proposed chemiluminescence method is rapid and sensitive enough and suitable to perform determination of cefazolin sodium in urine.

## ACKNOWLEDGEMENTS

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