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A sensitive inhibition chemiluminescence method for the determination of 6-mercaptopurine in tablet and biological fluid using the reaction of luminol–Ag(III) complex in alkaline medium

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ABSTRACT

A sensitive inhibition chemiluminescence (CL) method for the determination of 6-mercaptopurine (6-MP) is developed. The mechanism of the CL reaction between Ag(III) complex $\{[Ag(HIO_6)_2]^{5-}\}$ and luminol in alkaline solution was proposed, along with the inhibition mechanism of 6-MP on the CL emission. The inhibition degree of CL emission was proportional to the logarithm of 6-MP concentration. The effects of the reaction conditions on CL emission and inhibition were examined. Under the optimized conditions, the detection limit ($s/n=3$) was 3.7×10^{-10} g ml^{-1}. The recoveries of 6-MP were in the range of 97.7–105% with the RSD of 2.1–3.4% ($n=5$) for tablet samples, 103–106% with the RSDs of 1.1–2.1% for spiked serum sample, and 97.2–101% with the RSD of 2.0–4.5% for spiked urine sample. The accuracy of this method for the tablet analysis was examined by comparing with the pharmacopoeia method. The proposed method was used for the determination of 6-MP at clinically relevant concentrations in real urine and serum samples with satisfactory results.

1. Introduction

6-Mercaptopurine (6-MP) is an anti-cancer drug, widely used for the therapy of acute lymphoblastic leukemia [1]. The individual dosage regimens instead of standardized treatment regimens for some patients would make the drug concentration maintain at an optimal plasma level [2]. Various methodologies have been described for the determination of 6-MP, such as high-performance liquid chromatography (HPLC) [3–7], UV spectrophotometry (pharmacopoeia method) [8], spectrofluorimetry [9–12] and electrochemical methods [13].

Flow injection–chemiluminescence (CL) method using different reaction systems has been used for the analysis of many drugs because of its high sensitivity and simplicity [14,15]. Several CL systems were used for determination of 6-MP, such as tris(2,2′-bipyridine)ruthenium(II) [16,17], Cerium(IV)–RB [18], $Cu(phen)_2^{2+}$–H_2O_2 [19], luminol–H_2O_2 [20,21], potassium permanganate–formaldehyde [22], and potassium permanganate–thioacetamide–sodium hexametaphosphate system [23].

Although many methods had been established for the determination of 6-MP, the complicated procedures and poor selectivity still proscribe application of these methods to analysis of biological samples. The CL methods reported were used mostly for the determination of 6-MP in pharmaceuticals with the limit of detection ranged from 1.0×10^{-7} g ml^{-1} to 1.1×10^{-9} g ml^{-1} [16,18–22]. It is, therefore, useful to develop a new method for the determination of 6-MP in real samples.

In our previous work, a new CL reaction system with Ag(III) complex in acidic medium without luminol was developed [24]. A CL reaction of Ag(III) complex with luminol in alkaline medium was used for the determination of cortisol and 10-hydroxycamptothecin using an enhancing effect of the analyte on the CL emission [25,26]. We found that CL emission of Ag(III) complex–luminol in alkaline medium could be inhibited by 6-MP. The CL inhibition degree was linear with the logarithm of 6-MP concentration. The purpose of this work was to develop a new and sensitive inhibition CL method for determination of 6-MP in tablet and biological fluid.

2. Experimental

2.1. Instrumentation

The flow-injection system used for CL was an IFFM-E analysis system (Xi'an Remex Electronic Sci-Tech. Co. Ltd., Xi'an, China) consisting of two peristaltic pumps working at a constant flow rate (60 rpm) and a six-way injection valve with a sample loop (120 μl) automatically operated by a computer-equipped operation system of IFFM-E flow-injection analysis. An F-7000 Fluorescence spectrophotometer (Hitachi, Japan) and a TU-1900 double beam

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spectrophotometer (Beijing TAYASAF Science & Technology Co., Ltd., China) were used for studying CL mechanism. A TGL-16M centrifuge (Xiangyi Centrifuge Co., Hunan, China) was used in sample treatment.

2.2. Reagents

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).

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 [27]. The concentration of Ag(III) complex solutions was determined according to the literature [28].

Stock standard solution of 6-MP (1×10^{-3} g ml^{-1}) was prepared by dissolving 0.0500 g 6-MP in 5.0 ml of 0.2 M sodium hydroxide and diluting with deionized water to 50 ml, and stored in a refrigerator at 4 °C to keep dark. The lower concentrations were prepared immediately prior to use.

All chemicals were of analytical reagent grade, and were used without further purification. Deionized water was used throughout.

2.3. Sample treatment

6-mercaptopurine tablet was ground to a fine powder. After being homogenized, an accurately weighed portion of the power sample was dissolved with 2 ml of 0.2 M NaOH and water in a small beaker. The solution was filtered and the residue was washed with water several times, then transferred into a 25-ml calibrated flask and diluted to the volume with water. Working solutions were prepared by appropriate dilutions, so that the final concentration was in the linear range.

Urine and serum samples were provided by Hebei University Hospital. A 1.0 g aliquot of PbO_2 powder was added to 5.0 ml of blank urine, and followed by stirring for 10 min to eliminate uric acid, thiourea and ascorbic acid. After centrifugation at 4000 rpm for 15 min, the supernatant was filtered, and then the filtrate was applied to a cation exchange column (4 cm × 1.2 cm) for cleanup. The clear liquid was diluted with water to make different concentrations of 6-mercaptopurine in the linear range. The protein of a 1 ml volume of serum sample was removed by adding 4.0 ml 10% trichloroacetic acid (CCl_3COOH) in a centrifuge tube, which was shaken for 5 min, then centrifuged at 4000 rpm for 15 min. The supernatant was diluted with deionized water to make different concentrations of 6-mercaptopurine in the linear range.

2.4. Procedures

The investigation of CL intensity–time profiles was performed with the static CL analysis. In a 10 ml calibrated flask, 0.2 ml $[Ag(HIO_6)_2]^{5-}$, 0.2 ml 6-MP or 0.2 ml H_2O were mixed, then 1 ml luminol (6.72×10^{-8} M) alkaline solution was injected into the reaction tube by a quantitative injector and the CL intensity was measured without stirring.

The procedure for flow-injection analysis is shown in Fig. 1. The flow lines b and c were inserted into luminol alkaline solution and 6-MP solution, respectively, and then mixed with $[Ag(HIO_6)_2]^{5-}$ from the flow lines (a) to produce CL when the injection valve was switched to the position of injection. The peak height of signal from the CL reaction was recorded by the IFFM-E analysis system. CL spectra were recorded by using an F-7000 Fluorescence spectrophotometer.

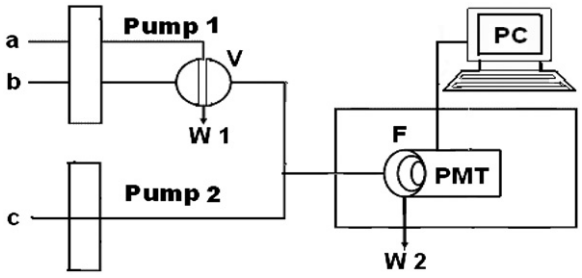

Fig. 1. Schematic diagram of flow injection CL analysis system. Pump1 and Pump2—peristaltic pumps; *V*—sampling inlet valve; *F*—flow cell: a flat spiral-coiled colorless glass tube (i.d. 1.0 mm, total diameter of the flow cell 3 cm, without gas between loops); PMT—photomultiplier tube; *W*—waste; (a) $[Ag(HIO_6)_2]^{5-}$ solution, (b) luminol alkaline solution, (c) 6-MP solution.

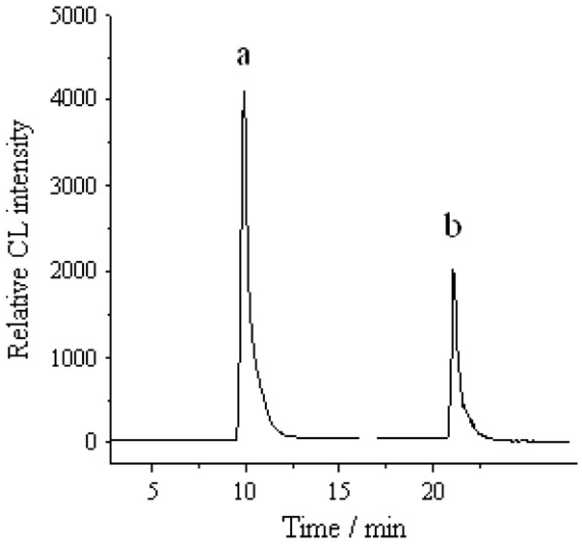

Fig. 2. CL kinetic curve and CL spectra. (a): 0.25 ml 8.4×10^{-8} g ml^{-1} luminol; 3 ml 7.667×10^{-5} M $[Ag(HIO_6)_2]^{5-}$; (b): a+0.5 ml 1.6×10^{-8} g ml^{-1} 6-MP.

3. Results and discussion

3.1. Mechanism of emission and inhibition of CL

An attempt was made to research and develop a new and sensitive CL system that could be applied for the CL determination. The CL kinetic characteristics of the reactions system were investigated in detail. The result is shown in Fig. 2. It was shown that the reaction rate in solution was very fast; from reagent mixing to peak maximum only 0.4 s was needed for $[Ag(HIO_6)_2]^{5-}$–luminol–KOH system, and it took 2 s for the signal to return to zero again. The kinetic curve also indicated that the CL could be inhibited by 6-MP.

In order to obtain more information about the enhanced and inhibition mechanism of 6-MP to CL, the CL spectra were recorded by an F-7000 fluorescence spectrophotometer (taken off lamphouse) (Fig. 3) and the UV spectra were examined (Fig. 4).

Our previous work showed that $[Ag(HIO_6)_2]^{5-}$ in H_2SO_4 medium without luminol could produce CL emission at about 490 nm, which might be caused by the excited state $(O_2)_2^*$ [24,29,30], and that $[Ag(HIO_6)_2]^{5-}$ in alkaline medium in the absence of luminol could not produce CL emission, but $[Ag(HIO_6)_2]^{5-}$ can enhance the CL emission of luminol in alkaline medium. The CL spectrum at 425 nm was observed for $[Ag(HIO_6)_2]^{5-}$–luminol in alkaline medium, which was similar to the CL spectra of H_2O_2–luminol in alkaline medium. Both spectra (Fig. 3a and b) had similar emission wavelength range and profile, which suggested that they possessed the same emitter.