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A novel chemiluminescence assay of mitoxantrone based on diperiodatocuprate(III) oxidation

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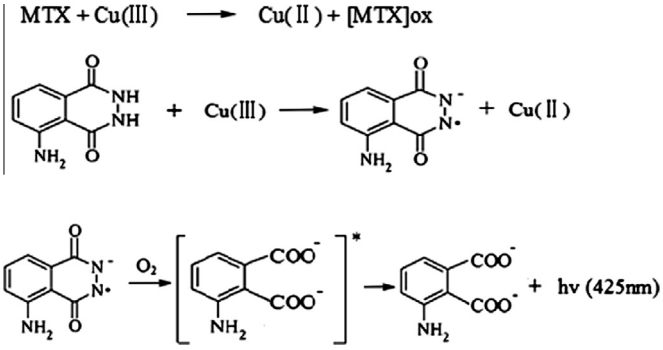
HIGHLIGHTS

- A novel diperiodatocuprate(III) oxidant with luminol system was developed.
- This new CL system was used for analyzing mitoxantrone (MTX) for the first time.
- The developed method can allow a lower detection limit of 1.1×10^{-9} g/ml (3σ).
- It has a potential application for ultrasensitive and selective analysis of MTX.

GRAPHICAL ABSTRACT



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ABSTRACT

A novel and strong chemiluminescence (CL) of luminol with diperiodatocuprate ($K_5[Cu(HIO_6)_2]$) was observed in alkaline medium. After the addition of mitoxantrone (MTX) into this system, the CL intensity could be greatly inhibited by MTX. Based on the phenomenon, a sensitive CL method was established for analysis of MTX combining with flow injection technology. Under optimum experimental conditions, the CL intensity was linearly related to the logarithm concentration of MTX from 5.0×10^{-9}–1.0×10^{-7} g/ml with the detection limit of 1.1×10^{-9} g/ml ($S/N = 3$). The relative standard deviation was 1.2% for 5.0×10^{-8} g/ml of MTX. The proposed method was successfully applied for determination of MTX in pharmaceutical preparations and biological fluids. The possible CL reaction mechanism was also discussed briefly.

Introduction

Mitoxantrone (MTX) belongs to the second generation anthracycline antibiotics (Fig. 1), which shows high efficacy in breast cancer, acute leukemia and non-Hodgkin's lymphoma [1,2]. It is used more widely due to their higher efficacy and lower side effect. Therefore, it requires the development of highly sensitive and precise methods for clinical assay.

Several methods have been described for the analysis of MTX. Resonance Rayleigh scattering (RRS) [3], electrochemical assays [4–7], high performance liquid chromatography (HPLC) [8–10], capillary electrophoresis (CE) [11] and chemiluminescence (CL) [12]. Chemiluminescence is an attractive detection means for trace analysis due to some features that make it superior to the other methods to those involving light (absorption and fluorescence spectroscopy). On account of flow-injection CL excellent sensitivity, wide linear dynamic range, short analysis time, inexpensive apparatus and easy operation, the FI-CL method has received much attention in various fields [13–17].

In recent years, there has been renewed interest in the development of new oxidant reagents, which can widen the application of CL analysis. Some transition metals in highest oxidation, such as trivalent copper complex, can be stabilized by chelating with suitable polydentate ligands. Diperiodatocuprate ($K_5[Cu(HIO_6)_2]$, DPC) is a weak oxidizing agent in alkaline medium with the

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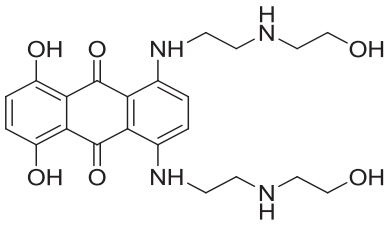

Fig. 1. Chemical structure of mitoxantrone.

reduction potential 0.42 V (vs SCE) [18]. DPC is a versatile one-electron oxidant for the oxidation of various organic compounds in alkaline medium and have been used as an oxidant in luminol-type CL reactions [19–21].

To our knowledge, there is no report using the oxidation of DPC for MTX analysis by CL method. In this paper, it was found that the reaction of DPC and luminol can emit strong CL with a low concentration of luminol, however, the CL is hardly observed when luminol with the same concentration reacts with other oxidants. In the experiments, it was found that MTX could significantly inhibit the CL from the DPC–luminol reaction in alkaline medium. Based on this finding, a highly sensitive CL detection for MTX was developed. The method has been successfully applied to determination of MTX in pharmaceutical preparations and biological fluids.

Experimental

Reagents and solutions

Sodium persulfate ($Na_2S_2O_8$), potassium periodate (KIO_4), cupric sulfate ($CuSO_4{\cdot}5H_2O$) and potassium hydroxide (KOH) were obtained from Kermel Chemical Reagent Company (Tianjin, China). Luminol was purchased from Solarbio Company (China). Mitoxantrone (MTX) was obtained from Yikangsida Medical Science and Technology Company (Beijing, China). All the other chemicals used in this work were of analytical grade.

MTX stock solution (1.0×10^{-4} g/ml) was prepared by dissolving 10.03 mg MTX with deionized water to 100 mL in a brown volumetric flask to avoid exposure to light and air. The working standards were prepared by diluting with deionized water as required. Luminol stock solution (1.0×10^{-2} mol/L) was prepared by dissolving 0.1772 g luminol in 100 mL of 0.1 mol/L NaOH and stored in the refrigeration at 4 °C. It was allowed to stand for approximately 24 h before use. The working solution of luminol was prepared by diluting luminol with 0.1 mol/L KOH solution.

The DPC stock solution (0.01 mol/L) was prepared by oxidizing Cu(II) in the alkaline medium according the known method [22]. In brief, KIO_4 (0.23 g), $CuSO_4{\cdot}5H_2O$ (0.125 g), $Na_2S_2O_8$ (0.14 g) and KOH (0.8 g) were added to 30 mL water. The mixture was heated to boiling for about 20 min on a hot plate with constant stirring. The boiling mixture turned intensely red and the boiling was continued for another 20 min for the completion of the reaction. The dark red product solution was then cooled to room temperature and diluted to 50 mL with distilled water. The stock solution obtained was stored in the refrigeration at 4 °C and was found to be fairly stable for several months. DPC working solutions were freshly prepared before use. The concentration of DPC solution was determined by the absorbency at 415 nm with a molar absorptivity (ε) of 6230 ± 100 L^3 mol^{-1} cm^{-1} [20].

Apparatus

A schematic diagram of the flow system employed in this work is shown in Fig. 2. Two pumps of Luminescence Analyzer (IFFM-E, Remax Analytical Instrument Limited Co., Xi'an, China) were used to deliver flow streams. Polytrafluoroethylere (PTFE) tubing (0.8 mm i.d.) was used to connect all components in the flow system. The flow cell was a 10 cm length of spiral glass tubing (0.5 mm i.d.) and the distance between injection valve and flow cell was about 10 cm. The CL signal was detected by the photomultiplier tube (PMT) voltage of 800 V placed near the flow cell and recorded with a computer. UV absorption spectra were measured on UV-2550 spectrophotometer (Shimadzu, Japan). Fluorescence spectra were recorded with a RF-5301 fluorospectrophotometer (Shimadzu, Japan) to study the luminescence characteristics.

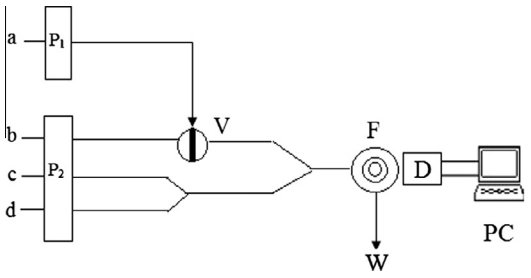

Fig. 2. Schematic diagram of the flow system for determination of MTX: a, DPC (2.0×10^{-4} mol/L); b, water carrier; c, luminol (2.0×10^{-7} mol/L) prepared in 0.1 mol/L KOH; d, MTX; P, peristaltic pump; V, injection valve; F, flow cell; W, waste; D, detector; PC, luminescence analyzer controlled by personal computer.

Procedures

Using the flow system schematically shown in Fig. 2, DPC solution was injected into the carrier stream (deionized water) by use of an six-way injection valve, merged at a T-piece with the mixture of luminol and MTX, then reached the flow cell, producing CL emission. The CL signal was measured by a CL analyzer equipped with PMT, which was connected to data processing system. The quantitation of MTX was carried out by measuring the relative CL intensity $\Delta I = I_o - I_s$, where I_o and I_s are the CL intensities of blank solution and sample solution, respectively.

Sample preparation

Without any pretreatment, five branches of MTX injections (2 mg/2 mL, Shenghe Pharmaceutical Co., Ltd., Sichuan) were mixed. Pipetting 1.0 mL MTX injection and diluted to 100 mL. It was diluted appropriately with water prior to measurement so that the concentration of MTX was in its linear response range.

The human serum samples were obtained from the Red Cross Blood Centre in Henan Province (China). Three different known amounts of standard solution of MTX (1.0×10^{-4} g/ml) were added to 200 μL of serum samples, respectively, and then were treated with triple acetonitrile and thoroughly vortex-mixed for 3 min. After centrifugation at 12,000 rpm for 15 min, the supernatants were collected and dried with an nitrogen stream. The residue diluted with water appropriately so that the final concentration was in the linear range of determination. A blank solution was prepared by treating the drug-free serum in the same way.

Three mice were purchased from the Henan Laboratory Animal Centre (Zhengzhou, China) weighing 20–40 g, each mouse was administered an injection of 0.1 mg (4 mg/kg) MTX via caudal vein. Blood samples were obtained from the orbital vein after 10 min. After centrifugation at 3000 rpm for 5 min at 4 °C, the plasma was separated immediately. 100 μL of the plasma samples was taken and pretreated as described as the human serum.