CHINESE JOURNAL OF ANALYTICAL CHEMISTRY
Volume 43, Issue 9, September 2015
Online English edition of the Chinese language journal

Available online at www.sciencedirect.com
ScienceDirect

Cite this article as: Chin J Anal Chem, 2015, 43(9), 1322–1328.

RESEARCH PAPER

Flow Injection-Chemiluminescence Method for Determination of Hydrocortisone in Human Serum by Using Trivalent Silver Complex

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Abstract: A flow-injection chemiluminescence (FI-CL) reaction system with diperiodatoargentate (III) (DPA) was developed for determination of hydrocortisone in human serum. The weak chemiluminescence signal from the reaction between DPA and sulfuric acid system could be greatly enhanced in the presence of hydrocortisone. The optimal conditions of the CL system were: 1.0 M H_2SO_4, 2.5×10^{-4} M DPA，and flow rate of 4.20 mL min^{-1}. Under the optimal conditions, the CL intensity was linear to the concentration of hydrocortisone from 3.0×10^{-10} g mL^{-1} to 1.0×10^{-7} g mL^{-1} with a detection limit of 2.2×10^{-10} g mL^{-1} (3σ). The relative standard deviation (RSD) was 0.63% (n = 11) for 5.0×10^{-8} g mL^{-1} hydrocortisone solution. The proposed method was successfully applied to the determination of hydrocortisone in serum samples with the recoveries of 93.0%–110.0%, and the RSDs were 0.7%–2.5%. The possible chemiluminescence mechanism was discussed by use of fluorescence spectra and UV-vis absorption spectra.

Key Words: Chemiluminescence; Flow-injection; Hydrocortisone; Diperiodatoargentate (III); Serum

1 Introduction

Hydrocortisone, the most potent glucocorticoid produced by human adrenals, plays a vital role in physiological processes in human body (Fig.1). Hydrocortisone is mainly involved in metabolic and immunological processes. Hydrocortisone is an anti-inflammatory and immunosuppressive factor[1], and might affect the production of immunoglobulin G (IgG). In metabolic actions, it is primarily involved in intermediary metabolism (usually catabolism) such as decreasing glucose uptake by most of the tissues and increasing gluconeogenesis by promoting the synthesis of the gluconeogenic enzymes in the liver[2]. The concentration of blood hydrocortisone generally indicates the hyperirritability level of the body because it is responsible for several stress-related disorders (e.g. pain and mental problems), and is secreted in higher levels during the body's 'fight or flight' response[3]. Hydrocortisone can be used in clinical diagnosis of human diseases characterized by deficiency of adrenal steroid excretion in Addison's disease or overproduction in Cushing's syndrome (CS). Furthermore, high serum levels of hydrocortisone are also found in individuals with stress responses, such as psychiatric diseases, obesity, diabetes, alcoholism, and pregnant woman. Low levels of cortisol are found in patients with rare adrenal enzyme defects or under long-lasting stress[4].

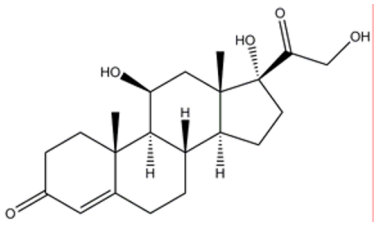

Fig.1 Structure of hydrocortisone

Received 21 July 2015; accepted 6 August 2015
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This work was supported by the National Natural Science Foundation of China (Nos. 21475153, 21107008, 91232703), and the Specialized Research Fund for the Doctoral Program of Higher Education of China (No. 201207110001).

DOI: 10.1016/S1872-2040(15)60858-1

Quantitative determination of hydrocortisone can be accomplished by a variety of methods, including immunological techniques[5,6], high performance liquid chromatography (HPLC)[7,8], gas chromatography-mass spectrometry (GC-MS)[9], liquid chromatography-mass spectrometry (LC-MS or LC-MS/MS)[10–12], electrokinetic chromatography[13] , etc. However, these methods often suffer from some disadvantages. For example, MS and LC require expensive instruments and strict experimental conditions; cross-reaction of antibody with other compounds often occurs in immunoassay. Therefore, development of a simple detection method for hydrocortisone with good sensitivity and high accuracy remains a challenge.

Chemiluminescence (CL) is an attractive and powerful detection technique with high sensitivity, fast response time, wide linear range and very low detection limit. The combination of a flow injection (FI) technique with CL detection provides a simple, inexpensive and reproducible means of screening a wide variety of analysts, which has been extensively applied in different fields of analytical chemistry[14–17]. Complexes with transition metals in the higher oxidation state can exist stably in alkaline media by suitable multi-tooth ligand complexing generally[18–20], among which diperiodatoargentate (III) (as short DPA) is a powerful and stable oxidizing agent and has been widely used in the study of reaction kinetics and oxidation mechanism of inorganic and organic chemical reaction [21]. The application of DPA in analytical chemistry has attracted much attention for its strong oxidizability and catalytic properties. DPA-based CL methods have been reported for the determination of hydrocortisone based on the reaction of DPA with luminol[22]. However, the involvement of chemiluminescence reagents might affect the selectivity and sensitivity of the method.

The goal of the present work was to develop a FI-CL method free of chemiluminescence reagents for sensitive and selective analysis of hydrocortisone. The weak chemiluminescence of DPA in acid condition can be greatly enhanced in the presence of hydrocortisone, no CL reagent was required and the selectivity could be also enhanced. Based on this principle, a novel chemiluminescence method was established for determination of hydrocortisone by combining with flow-injection technique. The method was successfully applied for determination of hydrocortisone in human serum. The possible CL reaction mechanism was also investigated through fluorescence and UV-visible spectroscopy characterization.

2 Experimental

2.1 Instruments and reagents

As shown in Fig.2, two peristaltic pumps were used to deliver all reagents. Injection was achieved by a six-port injection valve (Hanzhou, China) equipped with a 100-μL loop. PTFE tube (0.8 mm i.d.) was used to connect all components of the flow system. The CL signal was monitored by a BPCL ultra-weak luminescence analyzer (Institute of Biophysics, Chinese Academy of Science, Beijing, China) consisting of a flat coil glass flow cell facing the window of the photomultiplier tube (PMT). Data acquisition and treatment were performed by BPCL software. Fluorescence spectra were obtained on LS-45 Luminescence Spectrometer (Perkin Elmer, USA). UV-vis absorption spectrum was recorded with the TU1901 UV-vis spectrophotometer (Beijing Purkinje General Instrument Co. Ltd., China).

Hydrocortisone was obtained from Dr Ehrenstorfer (Germany). $AgNO_3$, KIO_4, $K_2S_2O_8$ and KOH were obtained from the First Chemical Reagent Factory of Shanghai (Shanghai, China). All the reagents above were of analytical grade and used as received. Double-distilled water was used throughout. A stock solution of 5.0×10^{-4} M hydrocortisone was prepared by dissolving a suitable amount of hydrocortisone with a small volume of methanol, and then diluted into flask with double-distilled water. The solution was stored at 4 °C. The diluted working solutions were prepared freshly.

2.2 Experimental procedure

2.2.1 Synthesis of DPA

DPA was synthesized according to the reported method[23]. In brief, KIO_4 (3.24 g), $AgNO_3$ (1.36 g), $K_2S_2O_8$ (3.00 g), KOH (8.00 g) were added in 200 mL water. The mixture was heated to boiling and refluxed for about 40 min under constant stirring, then cooled down and filtrated. The obtained DPA was stored in refrigerator at 0–4 °C under dark. UV-vis spectrum was used to characterize the complex, which exhibited two absorbance peaks at 362 nm and 253 nm. The concentration of the prepared DPA complex solution was determined by the absorbance at 362 nm ($A = \varepsilon CL$, the molar extinction coefficient $\varepsilon = 1.26 \times 10^{-4}$ L mol^{-1} cm^{-1}).

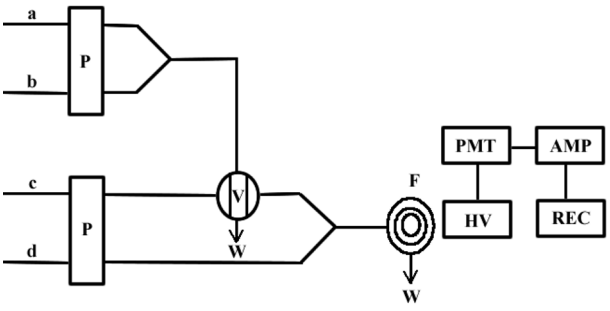

Fig.2 Schematic diagram of the flow injection-chemiluminescence (FI-CL) system

P: Peristaltic pump; V: Valve; F; Flow Cell; PMT; Photomultiplier Tube; AMP: Amplifier; HV: High Voltage; REC: Recorder; W: Waste; HV; –950 V. a: H_2SO_4; b: Hydrocortisone; c: Water; d: diperiodatoargentate (III) (DPA)