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In vitro monitoring of nanogram levels of naproxen in human urine using flow injection chemiluminescence

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Abstract

Based on the synergic effect of the hydrophobicity of sodium dodecylbenzenesulfonate (SDBS) micelles and the incorporation of naproxen molecules, a simple, rapid and sensitive chemiluminescence (CL) method was described for the determination of naproxen in pharmaceuticals and urine. Under the optimum experimental conditions, the CL intensity was linear over the concentration of naproxen ranging from 1.0 to 700.0 ng mL^{-1} ($r = 0.9917$) with a detection limit as low as 0.9 ng mL^{-1} (S/N = 3). The mechanism of CL was discussed in detail based on the spectrometry. The experimental results demonstrated that the excretive amounts of naproxen reached its maximum in 4.0 h after taking 200 mg naproxen tablet; and the naproxen excretive ratio during 8.0 h was 5.46% in the body of volunteers.

Keywords: Chemiluminescence; Naproxen; Urine; Flow injection analysis

1. Introduction

Naproxen (*S*)-(+)-6-methoxy-α-methyl-2-naphthalene acetic acid, is a non-steroidal anti-inflammatory drug widely used as mild to moderate pain relief and in the treatment of osteo- and rheumatoid arthritis [1]. Its chronic or acute administration shows toxic manifestations generally characteristic of non-steriodal anti-inflammatory drugs, such as gastrointestinal erosion, bleeding and pathologic changes in the real cortex and papillae. Moreover, they are associated with other serious side effects, i.e. kidney failure, and with a number of minor side effects, such as nausea vomiting, diarrhea, constipation, decreased appetite, rash, dizziness, headache and drowsiness. Finally, they also interact with other drugs; in particular, they reduce the action of diuretics and antagonize the action of drugs used to treat hypertension.

In view of the above considerations, the development of a simple and reproducible method for the determination of naproxen in biological fluids could be very useful for toxicological and pharmaceutical purposes. Several analytical methods have been reported in the scientific literatures for the determination of naproxen in pharmaceutical preparations and biological fluids including HPLC [2], spectrofluorimetry [3,4], capillary electrophoresis [5], phosphorimetry [6], differential pulse voltammetry [7] and capillary isotachophoresis [8]. However, for such application, the chromatographic methods involve more sophisticated instrumentation and higher cost of per analysis in addition to the complexity of their separation systems. Because of a narrow linear range and serious interference from metrics, spectrofluorometric determination was usually tedious in monitoring the excretive profile of biological fluids [9]. As far as room-temperature phosphorescence, too large injection volume, temperature sensitivity and the hazards of manipulating compounds of high toxicity (as Tl(I) salts) are the main reasons of its limitation of use. Only one paper with chemiluminescence (CL) determination of naproxen was reported by Campiglio [10] based on the CL reaction with cerium (IV) in sulfuric acid medium. However, the CL method based on the reduction reaction of acidic cerium (IV) suffered from high interference from reducible substances such as cysteine and ascorbic acid [11], respectively, which were limited to the determination of analytes in pharmaceutical and biological samples. Moreover, the cerium–naproxen

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CL method mentioned above was in sulfuric acid medium and naproxen was less soluble in aqueous solution when pH value was low (<4.8), which resulted in poor sensitivity (15 ng mL^{-1}) and narrow dynamic ranges (100–1000 ng mL^{-1}). In addition, in many cases, the rates of the CL reaction in an aqueous system are very fast, bad solubility would result in imprecise measurements.

Some approaches have been reported to overcome these difficulties. Micelles have been demonstrated to influence the chemistry and photophysics of molecules by altering the microenvironment in which the molecules reside. Micelles can change microviscosity, local pH, polarity, reaction pathway or rate, etc. Sodium dodecylbenzenesulfonate (SDBS), as an anionic surfactant, having a unique structure in the hydrophobic group including a benzyl and a dodecyl group, was applied to the present method and the function of it was made clear in detail.

To the best of our knowledge, it was the first paper that reported the excretive profile of naproxen using CL method. Wang and Song [12] reported a rapid and sensitive CL method for the determination of trace puerarin in human urine with a wide linear range and a detection limit as low as 0.1 ng mL^{-1} based on the CL reaction of luminol and KIO_4 in alkaline condition. Although luminol was a good and useful CL reagent, from the results of our usual experiments it could also be easily absorbed on the tubing walls and difficult being cleaned.

In this paper, a simple, rapid and sensitive method was proposed with wide dynamic ranges. It was found that naproxen could dramatically enhance the CL intensity from sulfite in acidic medium. The enhancement of CL intensity was proportional to the concentration of naproxen ranging from 1.0 ng mL^{-1} to 0.7 μg mL^{-1} with a relative standard deviation of 1.46% for 11 repeated determination of 10 ng mL^{-1} naproxen and the detection limit was 0.9 ng mL^{-1}. The proposed method could be applied successfully in the determination of naproxen in human urine samples. The results showed that the excretive amounts of naproxen reached its maximum after taking the tablets 4.0 h, with a total excretive ratio of 5.46% in 8.0 h.

2. Experimental

2.1. Reagents

Chemicals of analytical grade were used as received. Ultrapure water was obtained from a compact ultrapure water system (18.3 MΩ cm, Barnstead, IA, USA). SDBS was purchased from Fisher Scientific Ltd. (Hong Kong). Standard solution of SDBS were prepared by further dilution of its stock solution (0.1 mol L^{-1}). Naproxen was from Sigma (Sigma–Aldrich Co., USA). A stock solution of 1.0×10^{-2} mol L^{-1} sulfite was prepared daily by dissolving 0.1260 g sodium sulfite anhydrous (Beijing Yili Fine Chemical Ltd., Beijing, China) in 100.0 mL water. Hydrochloric acid was from Beijing Chemical Industry Plant (Beijing, China).

2.2. Apparatus and procedure

A batch style BPCL luminescence analyzer (Institute of Biophysics, Chinese Academy of Science, Beijing, China) was used to obtain batch CL signals. A schematic diagram of the flow injection CL system in this work is shown in Fig. 1. Reagent solutions (R1–R3) were delivered by peristaltic pumps (P) through three flow-lines. The SDBS solution (R2) and the sulfite solution (R3) were mixed through a three-way piece, and then reacted in a mixing valve (F) with the sample solution (100 μL) which was injected into the carrier stream through the sample injection valve. The mixing coil was made by coiling a piece of glass tubing into a spiral disk shape and placed close to the photomultiplier tube. The CL emission was recorded with a flow injection CL analyzer (D, lumiflow LF-800, Microtec NITI-ON, Funabashi, Japan) controlled by a personal computer. PTFE tubing (0.8 mm i.d.) was used to connect all components in the flow system. Calibration graphs were constructed by plotting the CL intensity (peak height) versus the concentration naproxen. The fluorescence and absorption spectra were monitored using a F-2500 fluorescence spectrometer (Hitachi, Tokyo, Japan) and a Shimadzu UV-2401 UV–visible recording spectrophotometer (Shimadzu, Kyoto, Japan), respectively. The spectrofluorimeter was also adapted for the measurement of CL spectra.

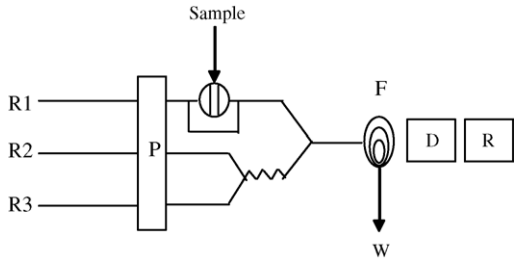

Fig. 1. Schematic diagram of the flow injection CL system for the determination of naproxen: R1: 0.060 mol L^{-1} hydrochloric acid solution; R2: 5.0×10^{-4} mol L^{-1} SDBS; R3: 1.0×10^{-5} mol L^{-1} sulfite; D: detector; F: flow cell; P: peristaltic pump; R: computer; W: waste; the flow rate: 2.5 mL min^{-1}.

The naproxen tablets were purchased from the local market. Twelve tablets were ground into fine powder and mixed. A sample equivalent to approximately 100 mg preparations was weighed accurately and dissolved with 0.10 mol L^{-1} NaOH solution and filtered. Then the solution was dissolved to 500 mL with water and diluted appropriately when determinated. Urine samples were collected from volunteers and a 1 mL aliquot of sample was mixed with 0.5 mL of acetonitrile and centrifuged for 5 min at 3000 *g*. Then the supernatant was fetched and the rest acetonitrile was blow-dried under a gentle stream of nitrogen gas. Finally, the prepared sample was diluted at 5×10^3 with distilled water and sometimes supplemented with naproxen to test the recovery of the method.

3. Results and discussion

3.1. The batch studies of CL

The CL profiles in the batch method for the mixtures of naproxen, SO_3^{2-}, SDBS and HCl solution are shown in Fig. 2. The mixing of SO_3^{2-} with SDBS in acidic solution gave weak CL emission (Fig. 2b), which was enhanced by adding a low concentration of naproxen solution. As shown in Fig. 2c, adding