Gatifloxacin (GFLX) is a fourth-generation synthetic broad-spectrum 8-methoxy fluoroquinolone antibacterial drug derivative, 1-cyclopropyl-6-fluoro-8-methoxy-7-(3-methylpiperazin-1-yl)-4-oxo-1,4-dihydro-quinoline-3-carboxylic acid. It offers several advantages over previous generation antibiotics [1] featuring in vitro activity against clinically important pathogens and resistant strains (especially penicillin-resistant Streptococcus pneumonia) with better pharmacokinetics [2]. Gatifloxacin is prescribed for the treatment of acute bacterial exacerbation of chronic bronchitis, acute sinusitis, community-acquired pneumonia, uncomplicated urinary tract infections (cystitis) and complicated urinary tract infections. It acts intravenously by inhibiting topoisomerase II (DNA gyrase) or topoisomerase IV [3]. Therefore, it is important to determine its contents in various biological fluids and tissues.

Various methods were reported in literature for the determination of gatifloxacin in pharmaceutical formulations and biological fluids including spectrophotometry [4–6], fluorimetry [7–9], HPLC [10–12], capillary electrophoresis (CEC) [13], voltammetry [14] and mass-spectrometry [15]. Although these methods are relatively sensitive, they suffer from the disadvantages common to the use of complicated equipment and high cost.

Chemiluminescence (CL) methods provide many advantages for pharmaceutical determinations such as high sensitivity, high selectivity, simple equipment, and low background signal. However, the CL intensity of many systems is very weak, so additional fluorescent substances are often used as sensitizers. Lanthanide ions terbium and europium in the form of chelates are characterized by unique spectroscopic properties such as line emission spectra, large Stokes shift and long luminescence lifetime. These complexes emit characteristic lanthanide ion line emission spectra, when excited at wavelengths absorbed by the organic ligand [16]. This is due to an intramolecular energy transfer through the excited triplet state of the ligand to the emitting level of the lanthanide [17, 18].

In this work, a weak CL emission produced by KMnO₄–SO₃⁻–GFLX was observed, but this method often suffered from interferences of some organic species in biological fluids when used for the direct determination of GFLX in biological samples, due to the low sensitivity obtained with this system. While GFLX formed a complex with trivalent terbium ion, the complex emitted CL intensively via intramolecular energy transfer from the ligand to Tb(III), giving a characteristic peak at 545 nm. Based on this, a rapid and sensitive flow injection CL method was proposed for the determination of gatifloxacin. The proposed method was proved to be simple, rapid, sensitive and suitable for continuous analysis, and the CL emission intensity depended on the concentration of the analyte in the
flow injection (FI) system. The method was applied to the determination of gatifloxacin in pharmaceutical preparations and biological fluids. Moreover, the possible energy transfer mechanism was carefully discussed in this work.

EXPERIMENTAL

Chemicals. All chemicals were of analytical-reagent grade and doubly distilled water was used throughout. Stock standard solution (1.0 \times 10^{-3} M) of GFLX (Institute of Medicinal Biotechnology, Beijing, China) was prepared by dissolving an accurately measured amount of GFLX in water. During the experiments, this solution was found to be stable for several weeks when kept in the dark at room temperature. Working standard was prepared daily by dilution of stock standard solution with water. Chengdu Hengrui Pharmaceuticals co. ltd. (Sichuan, China, Approval number: H20052429) provided GFLX capsules while Nanjing Sanhome Pharmaceutical Co. Ltd. (Jiangsu, China, Approval number: H20041086) provided GFLX tablets.

Standard stock solution of the Tb(III) (0.01 M) was prepared by dissolving 934.5 mg Tb_4O_7 in 15.0 mL HCl (11.6 M) at 95°C, evaporating the solution to be almost dry, and then diluting it to 500 mL with water. Stock KMnO_4 (0.01 M) and Na_2SO_3 solution (0.01 M) were prepared daily and diluted as required. Urine and serum samples were obtained from several healthy volunteers.

Apparatus. The CL-FI system used for the determination of GFLX was schematically shown in Fig. 1. FI-2100 flow injection analysis system (Beijing Haiguang Instrument Company, China) was used. Polytetrafluoroethylene (PTFE) tubings (0.8 mm i.d.) were used to connect all components in the flow system. GFLX was injected into the carrier stream (Na_2SO_3) by a six-way injection valve with a sample loop (120 \mu L) which was then merged with Tb(III) and KMnO_4 sequentially in the flow cell with an optimum flow rate of 1.5 mL/min. The sample zone was carried through the reaction coil where the CL reaction took place. The CL signal was measured with a photomultiplier tube of a GD-1 weak luminescence analyzer (Xi’an RuiKe Electronic Equipments Company, China). Fluorescence and chemiluminescence spectra were recorded with a RF-5301PC spectrofluorimeter (Shimadzu, Japan) with the excitation slit shuttered.

Dosage forms preparation. Ten tablets were weighed and ground into homogenized powder. An accurately weighted amount of the powder equivalent to 200 mg corresponding to one tablet was transferred into a small beaker, in which 0.2 M HCl was added to dissolve the powder. The solution was filtered and the residue was washed with water several times. The solution was transferred into a small calibrated flask and diluted to desired concentration with water. The same treatment was done with GFLX capsules.

Urine and serum sample preparation. The proposed methods were applied to the determination of gatifloxacin in human urine and serum, kindly provided by healthy volunteers.

The serum samples were deproteinized by using trichloroacetic acid (CCl_3COOH). First, 4.0 mL of 10% (w/w) trichloroacetic acid were added to 1.0 mL of serum sample in a plastic centrifuge tube, then the mixture was rotated for 15 min at a rate of 2000 rpm, and the upper clear solution was taken and diluted properly for the determination of GFLX. No further pretreatment was required for urine samples.

Procedure. The flow injection configuration used for the determination of the studied gatifloxacin was designed to provide different reaction conditions for magnifying the CL signal. As shown in Fig. 1, every 10 s 120 \mu L sample was injected into the stream of sulfite by the six-way injection valve and further mixed with the solutions of Tb(III) and KMnO_4 in the CL cell, which gave rise to an intense CL signal. The relative CL intensity \Delta I (the difference of CL intensity between GFLX solution and the reagent blank without GFLX) was proportional to the concentration of GFLX.

RESULTS AND DISCUSSION

Optimization of experimental conditions. In the chemiluminescence system of KMnO_4—SO_3^{2-}—Tb(III)—GFLX, KMnO_4 was the oxidant, Na_2SO_3 was the carrier stream and reductant, and Tb(III) acted as sensitizer. A series of experiments was conducted to establish the optimum analytical conditions. These factors include: type of acid, acidity, reagent concentrations and flow injection system parameters.

Effect of acid. The nature and concentration of the acid used in the reaction has a very significant influence on the CL emission. The influence of different acids, such as HCl, H_2SO_4 and HNO_3, was studied by