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ORIGINAL PAPER

Optical Flow-Through Sensor for the Determination of Norfloxacin Based on Emission of $KMnO_4$–Na_2SO_3–Tb^{3+} System

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Abstract A simple and selective method to determine norfloxacin using an optical flow-through sensor has been developed. The present sensor was prepared by packing anionic ion exchange resin in a glass tube, followed by introducing $KMnO_4$ solution to the glass tube for immobilization on resin. The optical sensor is based on the emission intensity from the Tb(III) solution sensitized by norfloxacin. The excitation of norfloxacin occurred by the chemiluminescence from the reaction of $KMnO_4$ and Na_2SO_4 solutions. The effects of pH, concentration of Tb(III) ion, $KMnO_4$ and Na_2SO_4 solutions and flow rate of the norfloxacin solution on the chemiluminescence intensity were studied to find the optimum experimental conditions. The emission intensity increased linearly with increasing norfloxacin concentration from 1.0×10^{-3} to 1.0×10^{-8} M and the detection limit (3σ) was 8.7×10^{-9}. The applicability of the present method was demonstrated by determination of norfloxacin in various pharmaceutical preparations and serum sample.

Keywords Sensor · Optical flow-through · Chemiluminescence · Norfloxacin

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Introduction

Norfloxacin, [(1-ethyl-6-fluoro-1,4-dihydro-4-oxo-7 (1-piperazinyl)-3-quinoline carboxylic acid] (Fig. 1) is a synthetic drug [1]. It is a member of antibacterial agents named as fluoroquinolone derivatives, which are derived from nalidixic acid [2]. Norfloxacin is a, broad-spectrum antibacterial agent which exhibits high antimicrobial activity in vitro against a wide variety of Gram-negative and Gram-positive bacteria [3]. Norfloxacin features an optimum pharmacokinetics, high biological accessibility, good tolerance, and high efficiency. The mode of action for norfloxacin is the specific inhibition of the DNA gyrase, a topoisomerase II of bacterial type, which unwinds the DNA super helix before replication and transcription, an effect that terminates the reproduction of the microorganism [4].

The zwitterionic structure of norfloxacin results in rapid dissolution, absorption and tissue penetration [5]. It has been reported that clearance of norfloxacin involves both glomerular filtration and renal tubular secretion and as such, patients with severe renal failure have a prolonged half-life of the drug which requires dosage adjustments [6]. Therefore, a sensitive analytical technique is needed to monitor norfloxacin plasma concentrations in those patients. Several methods have been reported for determination of norfloxacin in pure, in dosage form and in biological fluids. Norfloxacin is official in both USP [7] and BP [8]. Various spectrophotometric methods were

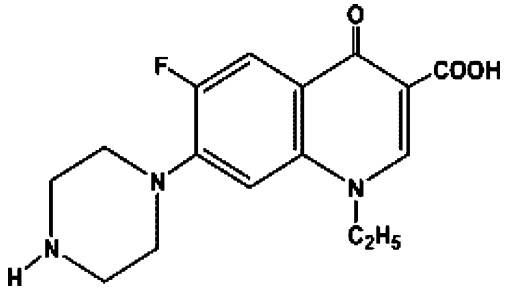

Fig. 1 Structure of norfloxacin

described for determination of norfloxacin [9, 10]. Norfloxacin has been determined by capillary electrophoresis [11, 12], polarography [13], voltammetry [14], high-performance liquid chromatography(HPLC) [15], spectrofluorimetry [16] and chemiluminescence [17]. HPLC methods generally require tedious procedures and higher analytical costs. As one of the most sensitive detection methods, chemiluminescence (CL) has been widely used in practical applications.[18–20] The simplicity, low detection limits, large calibration ranges and short analysis time of the CL method led many analysts to develop a great number of CL system in the last half century.[18–20]. However, the CL method also faced problems for example poor selectivity and pollution of waste solution when it combines with flow injection analysis. CL methods are based on the oxidation and reduction processes and need oxidant, reactant or catalyst. Generally, in order to get a good duplication for the analysis, the highly sensitive CL method is often combined with flow injection analysis. In the CL flow system, the continuous delivery of reactants into reaction zone is required, and the continuous flow of the reactants would cause reagent waste and environmental pollution, which limit the widespread application of CL. In order to overcome this problem, the CL flow-through sensors with immobilized reagents have received much attention and appeared in the literature in recent years. In most of these systems, the reagents of CL reaction, including oxidant [21–23] catalyst such as CO^{2+}, enzyme [24–27] and luminescence reagent such as luminal [28, 29] were immobilized on the suitable supports e.g. ion-exchange resin [30] and sol–gel [31]. Compared with the use of continuously delivered reagents in the conventional CL flow system, these optical flow-through sensors with immobilized reagents are advantageous not only for cost, environment, and resource considerations but also for operational convenience and instrumental simplification.

In this paper, a new type of optical flow-through sensor for determination of norfloxacin is proposed. By a very simple means, $KMnO_4$ could be immobilized on an anion-exchange resin. The detailed experiment was carried out and the application possible of the optical flow-through sensor to the practice sample was obtained. This method has been successfully applied to determine norfloxacin in pharmaceutical preparation.

Experimental

Chemicals and reagents

All experiments were performed with analytical-reagent grade chemicals and pure solvents. Ultra pure water was obtained from a Milli-Q system. Sodium sulfite ($Na_2SO_3{\cdot}7H_2O$), Terbium (III) chloride hexahydrate ($TbCl_3{\cdot}6H_2O$) were obtained from Aldrich (USA). Norfloxacin and Triton X-100 were purchased from Sigma (USA). Potassium permanganate ($KMnO_4$) was purchased from Duksan (South Korea). Tb^{3+} stock solution was prepared by dissolving an appropriate amount of terbium (III) chloride hexahydrate (0.1 M) in de-ionized water. Norfloxacin was prepared by dilution with water from stock solution of norfloxacin (1×10^{-3} M, in 0.005 M sulfuric acid) was stored in the refrigerator (4 °C). Stock $KMnO_4$ solution (0.01 M) and Na_2SO_3 solution (0.01 M) were prepared daily and diluted as required. The resins of AG 1-X8 were the product of Bio-Rad Company (USA).

Serum sample preparation: Each 1.0 ml serum sample containing the known amount of norfloxacin was deproteinized by adding 5.0 ml 20% trichloroacetic acid (CCl_3COOH) in a centrifuge tube. This mixture was centrifuge for 15 min at 4000 rpm. The centrifugate was diluted with water in order to obtain a concentration of norfloxacin in the range of linearity previously determined.

Apparatus and procedure

The schematic diagram of the flow-through sensor system is shown in Fig. 2. The CL flow-through sensor consisted of a peristaltic pump (Ismatec, Model 404 and MS-4 Reglo/6-100, Switzerland) which delivered sodium sulfite solution, Tb^{3+} solution and sample solution at a flow rate (per tube) of 10.0 ml min^{-1}. Glass column (30×2.5 mm i.d.) used as the flow cell, which was packed with a homogeneously mixed bed 0.5 g of the resin containing 1.0×10^{-4} M permanganate and plugged with glass wool at both ends to prevent loss of the resin in the column. The immobilized reagent was retained in the flow-cell in a configuration perpendicular to the optical fiber bundle. The optical fiber used was a high-grade fused silica fiber (Oriel, Stratford, CT 77527) with diameter of 3.2 mm, length of 2,000 mm and 0.56 numerical aperture. PTFE tube (0.8 mm i.d.) was used to connect all components in the flow system. The sample, Na_2SO_3, buffer solutions were mixed in the mixing coil. The distance between this mixing coil and the detector was about 10 cm. The emission signal

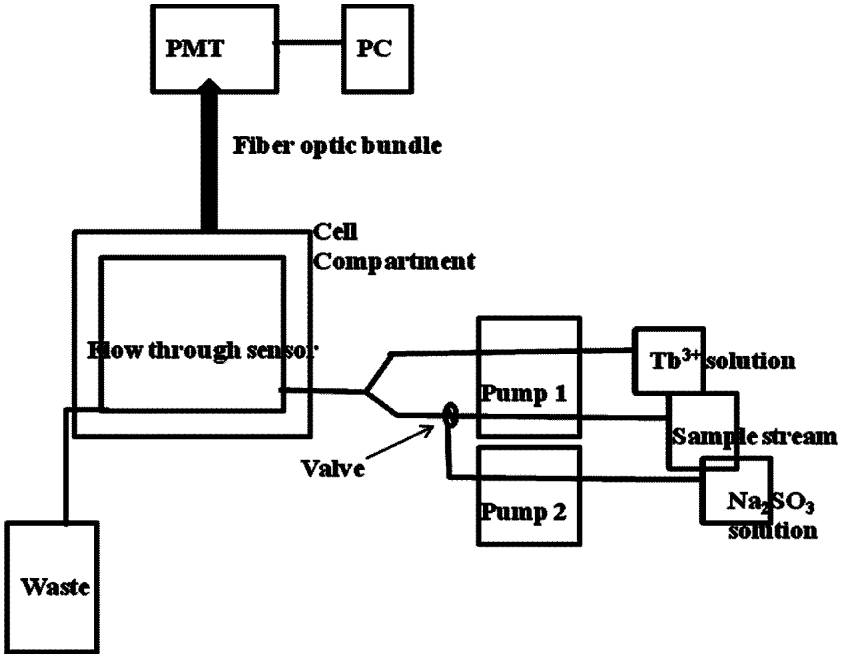

Fig. 2 Schematic diagram of the flow system to determine norfloxacin using an optical flow-through sensor

produced in flow-through sensor was passed through a monochromator, measured and transduced to an electric signal by an photomultiplier tube (Model R928, Hamamatsu, USA) placed close to the flow cell. The voltage used for the photomultiplier tube was 950 V. Emission spectra were recorded with a spectrofluorometer (Model FL111, Spex, Edison, NJ, USA). The acquisition mode used was signal (S) for the luminescence measurements. The emission intensity at 545 nm was monitored for the determination of norfloxacin. For the luminescence measurements the integration time and slit width were 1 s and 5 mm, respectively. Data treatment was performed with DM 3000 software running under Windows 95.

Immobilization of permanganate ion on the resin

To prepare a sensor, approximately 1.0×10^{-4} M potassium permanganate was added to 0.5 g of washed and ground AG 1-X8 resin, and then kept for about 10 min at room temperature to allow the adsorption process of the reagent onto the solid support matrices, then the resin was filtered, washed with water. The resulting resin absorbed potassium permanganate was packed into a glass column (30×2.5 mm i.d.), and some glass wool was inserted at both ends to prevent loss of resin.

Emission measurement

The analysis of emission measurements was performed by the use of a chemiluminescence method, where the signal is measured after a fixed time of introducing analyte into the optical flow-through sensor. The measurement was expressed as a relative emission, which is defined as the difference between the emission intensity of the Na_2SO_3–norfloxacin–Tb^{3+} and that of the immobilized reagent alone. All measurements were recorded at the specified wavelength, which produced highest difference in emission intensity of the immobilized reagent in the absence and the presence of the norfloxacin.

Results and discussion

Possible mechanism

It has been proposed that the chemiluminescence of some oxidant-sulfite systems arises from excited sulfur dioxide (SO_2*), which is one of the products in the oxidation of Na_2SO_3 [32–35]. In this case sulfite acts as a reductant to produce an excited molecule of sulfur dioxide, which emits radiation in the range of 300–550 nm. The production of SO_2* can be explain by the following reaction [34, 36]

$$HSO_3^- + MnO_4^- \rightarrow HSO_3^{\cdot} + MnO_4^{2-} \quad (1)$$

$$2HSO_3^{\cdot} \rightarrow S_2O_6^{2-} + 2H^+ \quad (2)$$

$$S_2O_6^{2-} \rightarrow +SO_4^{2-} + SO_2^* \quad (3)$$

$$SO_2^* \rightarrow SO_2 + h\nu \quad (4)$$

The chemiluminescence intensity is very weak because of the low luminescence efficiency of SO_2*. Norfloxacin and Tb^{3+} absorb radiation in the emission range of SO_2, therefore can enhance the weak CL emission through energy transfer from SO_2* to the norfloxacin. Based on this, Tb^{3+} and norfloxacin were independently added to the chemiluminescence system of $KMnO_4$–Na_2SO_3, but no significant increase in the chemiluminescence intensity was observed. However, when Tb^{3+} and norfloxacin were added together to the chemiluminescence system of $KMnO_4$–Na_2SO_3, the chemiluminescence intensity was greatly enhanced. The chemiluminescence signal can be greatly enhanced when a trivalent lanthanide ion is present, as quinolones have suitable functional groups to form stable complexes with them. The complex absorbs the energy at the characteristic wavelength of the organic ligand, and emits radiation at the characteristic wavelength of the lanthanide, due to an energy transfer (FRET) from the quinolone ligand to the emitting energy level of metal [37]. Chemically sensitized emission spectra are located at 493 and 545 nm which is the characteristic fluorescence spectrum of terbium, indicating clearly that the excited Tb^{3+} is the emitter, and there must be energy transfers in the $KMnO_4$–Na_2SO_3–Tb^{3+}–norfloxacin system.

The overall mechanism can be expressed as follow.

$$MnO_4^- + HSO_3^- \rightarrow SO_2^* \quad (1)$$

$$SO_2^* + \mathrm{Nor} \rightarrow SO_2 + \mathrm{Nor}^* \quad (2)$$

$$\mathrm{Nor}^* \rightarrow \mathrm{Nor} + h\nu \quad (3)$$

$$\mathrm{Tb(III)} + h\nu \rightarrow \mathrm{Tb(III)}^* \quad (4)$$

$$\mathrm{Tb(III)}^* \rightarrow h\nu \quad (5)$$

Step 1 is a redox reaction resulting in an excited state sulfur dioxide molecule as explained earlier in a balance reaction.

Step 2 is an excited state electron transfer reaction.

Steps 3–5 are fluorescence resonance energy transfer (FRET) process.

Fluorescence and absorption spectra

Fluorescence and absorption spectra of Tb^{3+} and norfloxacin are shown in Fig. 3 and Fig. 4 respectively. Figure 3 shows the emission peak of Tb at 545 nm (peak b) when excited at 345 nm (peak a). In order to avoid the direct excitation of Tb^{3+} [38], the disturbance of the scattered excitation light and the inner filter effect [39] we selected 345 nm rather than 259 nm. Figure 4 shows the excitation and emission peak at 336 and 429 nm respectively for norfloxacin without addition of Tb^{3+}. The emission spectrum for norfloxacin in the presence of $KMnO_4$–Na_2SO_3–Tb^{3+} system is shown in Fig. 5. As can be shown from the Fig. 5, the fluorescence intensity of Tb^{3+} ion at 545 nm can be enhanced remarkably, which indicates that norfloxacin can form a very stable complex with Tb. Therefore 545 nm was selected as the optimum wavelength for the determination of Norfloxacin.

Optimization of sensor

Effect of $KMnO_4$ concentration for immobilization

For investigating the effect of permanganate concentration on the emission intensity, the resin was treated with $KMnO_4$ solution in the range of 1.0×10^{-5} to 1.0×10^{-2} M. The result is plotted in Fig. 6. As can be seen from Fig. 6 the emission intensity was increased by decreasing the concentration of $KMnO_4$ up to 1.0×10^{-4} M. The concentration of $KMnO_4$ higher than 1.0×10^{-4} M caused a decrease in the emission intensity. $KMnO_4$ solution concentration of 1.0×10^{-4} M was used as the optimum concentration for further experiments.

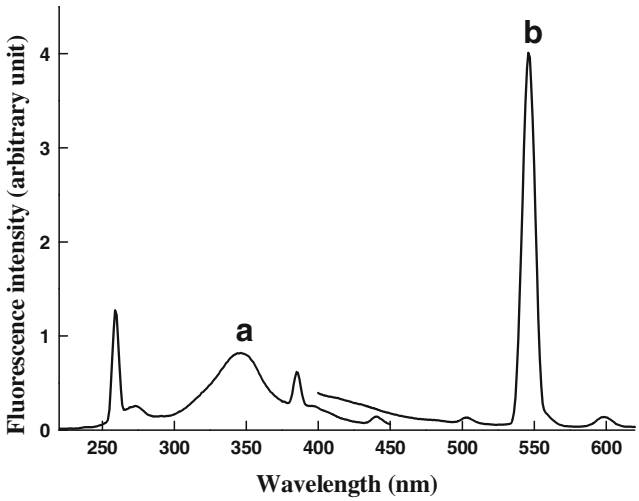

Fig. 3 Excitation (*a*) and emission (*b*) spectra of Tb^{3+}: $[Tb^{3+}]=3\times10^{-4}$ M, $\lambda_{ex}/\lambda_{em}=345/545$

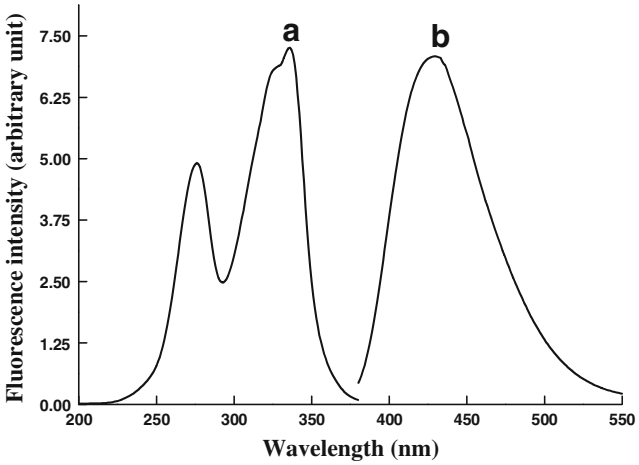

Fig. 4 Excitation (*a*) and emission (*b*) spectra of norfloxacin: [Norfloxacin]=1.0×10^{-4} M, $\lambda_{ex}/\lambda_{em}$=336/429

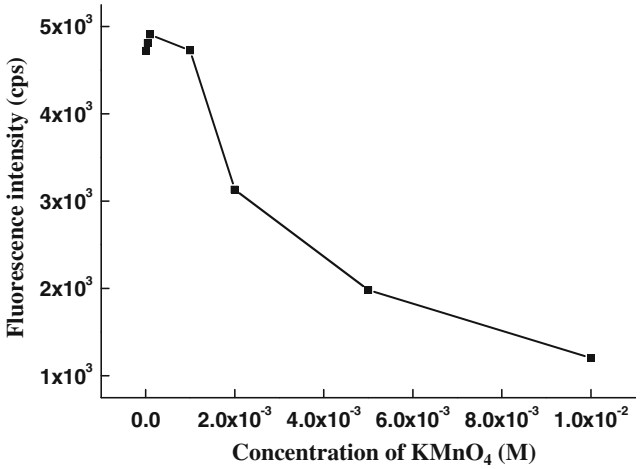

Fig. 6 Effect of $KMnO_4$ concentration to prepare an optical flow-through sensor on emission intensity for the determination of norfloxacin in aqueous solution based on the emission of $KMnO_4$–Na_2SO_3–Tb^{3+} system: [Na_2SO_3], 2.0×10^{-3} M; [norfloxacin], 1.0×10^{-5} M; [Tb^{3+}], 2.0×10^{-3} M; pH, 3.0; λ_{em}, 545 nm

Stability of the sensor and reproducibility of emission signals

The stability of each reactor was established by comparing the emission intensity of the 1.0×10^{-5} M norfloxacin concentration. From 1 to 20 times of injections, the emission intensity appeared to be steady; while more than 25 times of injection, emission intensity became decreased and fluctuated distinctly. Therefore, the optical flow-through sensor could be reused about 20 times during a period 48 h. Another indication that the reactor was losing its oxidation capacity was the color of the packing itself. The color of the packing of a new reactor was light gold. After pumping several samples into the reactor, the color of the packing at the front end of the reactor started to disappear, this meant that the KMnO4 had stripped off the resin. Moreover, it was very easy to prepare and change the reactor. The reproducibility of optical flow-through sensor is shown in Fig. 7. As Fig. 7 shows, from one to five times of injections, the emission intensity appeared to be steady.

Effect of pH on emission intensity

The effect of pH over the range of 2.0 to 3.6 for $KMnO_4$–Na_2SO_3–Tb^{3+} system emission intensity was investigated using KH Phthalate–HCl buffer system. The emission intensity increased with pH up to 3.0 and then decreased. KH Phthalate–HCl buffer solution of pH 3.0 at a concentration of 0.1 M was found to be suitable for the subsequent studies.

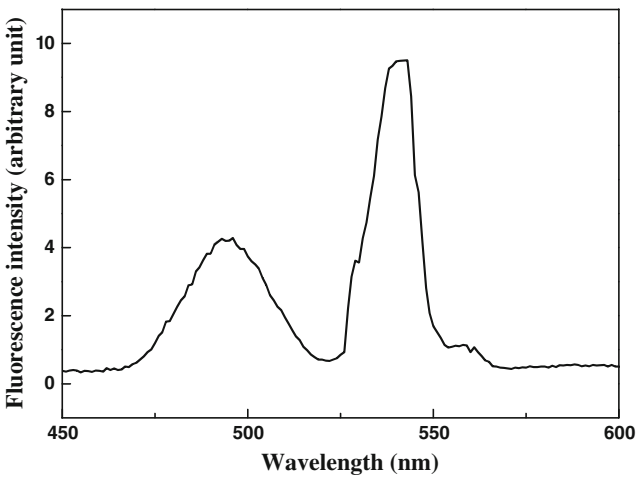

Fig. 5 Emission spectrum obtained using an optical flow-through sensor based on the emission of $KMnO_4$–Na_2SO_3–Tb(III) system: [Na_2SO_3], 2.0×10^{-3} M; [Tb^{3+}], 2.0×10^{-3} M; [norfloxacin], 1.0×10^{-4} M; pH, 3.0. λ_{em}, 545 nm

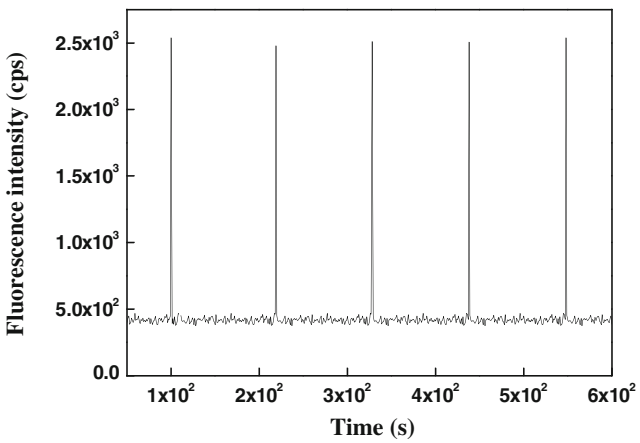

Fig. 7 Reproducibility of emission signals obtained using an optical flow-through sensor to determine of norfloxacin in aqueous solution based on the emission of $KMnO_4$–Na_2SO_3–Tb^{3+} system: [Na_2SO_3], 2.0×10^{-3} M; [norfloxacin], 1.0×10^{-5} M; [Tb^{3+}], 2.0×10^{-3} M; pH, 3.0; λ_{em}, 545 nm

Effect of flow rate

In flow injection analysis, flow rate is an important factor. Therefore the influence of the flow rate of the reagent solution on the emission response was investigated in the range of 1.2–3.8 ml/min. The results are shown in Fig. 8. The lower flow rates resulted in higher contact time for the sensing tip of the optical fiber but they were found to be unfavorable for the sensitivity because the luminescence reaction is a very fast process. The emission intensity increased with the flow rate, reaching a maximum value at 2.8 ml/min. Consequently, flow rate of 2.8 ml/min was selected in this work for fast response and high emission intensity, since higher rates resulted overpressure in the connections and excessive reagent consumption.

Effect of Tb(III) concentration

The effect of Tb^{3+} concentration on CL emission was tested over the range of 1.0×10^{-6}–5.0×10^{-3} M. The results are shown in Fig. 9. As can be seen in Fig. 9, emission signal of $KMnO_4$–Na_2SO_4–norfloxacin system highly increased in the presence of Tb^{3+} solution. Although emission signal increased with the Tb^{3+} concentration, repeatability of the measurements achieved with higher than 2.0×10^{-3} M Tb^{3+} concentration decreased remarkably, due to oscillations in the based-line and in the peak heights. Thus, 2.0×10^{-3} M Tb^{3+} solution was selected for the recommended procedure, as it leaded to the best sensitivity and precision values.

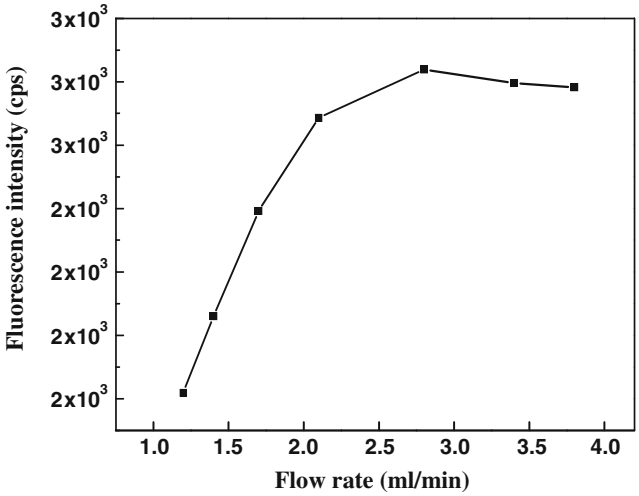

Fig. 8 Effect of flow rate for the stopped flow system on emission intensity for the determination of norfloxacin in aqueous solution using an optical flow-through sensor based on the emission of $KMnO_4$–Na_2SO_3–Tb^{3+} system: [Na_2SO_3], 2.0×10^{-3} M; [Tb^{3+}], 2.0×10^{-3} M; [norfloxacin], 1.0×10^{-5} M; [pH], 3.0; λ_{em}, 545 nm

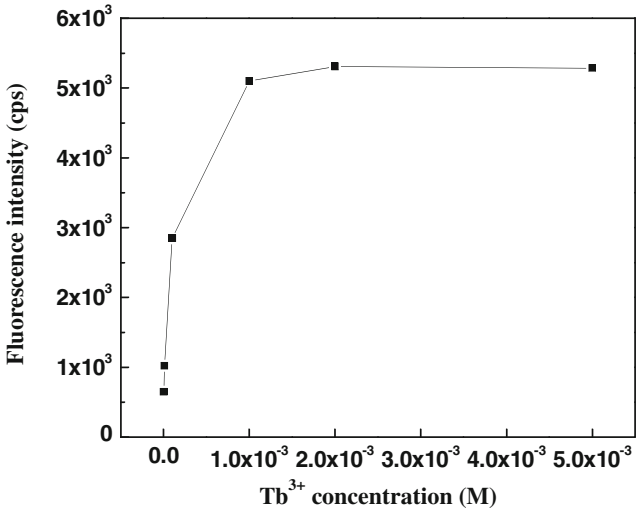

Fig. 9 Effect of Tb^{3+} ion on emission intensity for the determination of norfloxacin in aqueous solution using an optical flow-through sensor based on the emission of $KMnO_4$–Na_2SO_3–Tb^{3+} system: [Na_2SO_3], 2.0×10^{-3} M; [norfloxacin], 1.0×10^{-5} M; pH, 3.0; λ_{em}, 545 nm

Effect of Na_2SO_3 concentration

The effect of sodium sulfite concentration on the emission signal was studied in the range of 5.0×10^{-4} to 1×10^{-2} M Na_2SO_3. The maximum emission intensity was at 2.0×10^{-3} M sodium sulfite concentration. Therefore, the optimum sodium sulfite concentration was chosen to be 2.0×10^{-3} M for further studies.

Analytical application

Interference study

In order to assess the possible analytical applications of the method to the drug sample, the effect of some common excipients, such as sucrose, glucose, lactose, starch and citric acid, was studied. These chemicals were added individually to solutions containing 1.0×10^{-5} M norfloxacin so as to give 1.0×10^{-3} M of each excipient. The emission signal was measured for these solutions using an optical flow-through sensor based on the emission $KMnO_4$–Na_2SO_3–Tb^{3+} system. No significant interference was observed by adding these common excipients to the solutions, the relative errors being <1.5%.

Calibration curve of norfloxacin

The average of peak heights of three successive measurements obtained under the optimum experimental conditions for each norfloxacin standard solution was used for calibration. Figure 10 shows a typical calibration curve

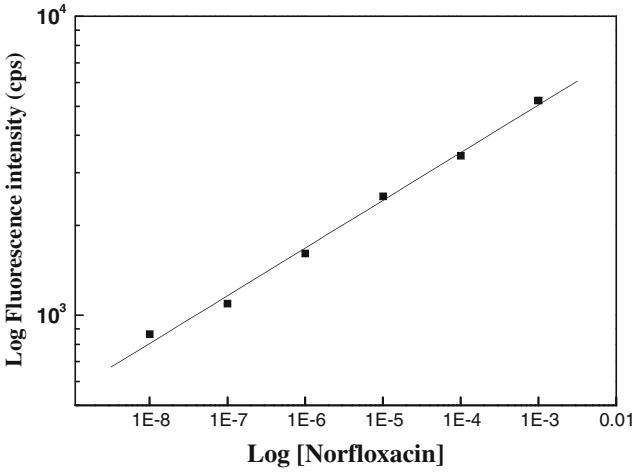

Fig. 10 Calibration curve of norfloxacin in aqueous solution obtained using an optical flow-through sensor based on the emission of $KMnO_4$–Na_2SO_3–Tb^{3+} system: [Na_2SO_3], 2.0×10^{-3} M; [Tb^{3+}], 2.0×10^{-3} M; pH, 3.0; λ_{em}, 545 nm

for different norfloxacin concentrations. For $KMnO_4$–Na_2SO_3–norfloxain–Tb^{3+} system, a linear response for norfloxacin concentration was established over the range of 1.0×10^{-3} to 1.0×10^{-8} M. The correlation coefficient in this range was 0.9928. The detection limit(S/N=3) was found to be 8.7×10^{-9} M.

Application of the investigated method

(a) Determination of norfloxacin in pharmaceutical preparations:

Calibration curve in the range of 1.0×10^{-3} to 1.0×10^{-8} M of standard norfloxacin was used for the determination of norfloxacin in pharmaceutical preparations. The investigated method was applied for the determination of norfloxacin in local commercially available pharmaceutical preparations like Urekacin tablets (Kukje pharma South Korea) and Barotham tablats (Guju pharma South Korea). The results are given in Table 1. As can be seen from the Table 1, the norfloxacin found through investigated was in close agreement with label quantities. Recovery studies were also performed for each of the analyzed sample. Standard addition method was applied for the recoveries study. The results are given in Table 1. As can be seen from Table 1 the recoveries of Urekacin tablets and Barotham tablets were found to be 100.49 and 98.74% respectively.

(b) Determination of norfloxacin in sample of serum:

The developed method was applied to the determination of norfloxacin in sample of serum. The results are shown in Table 2. For the assay of norfloxacin in serum sample the freshly prepared sample was diluted appropriately within the linear range of determination. In order to compensate the effect of the biological matrix in the measurement, standard addition method was applied to the quantification of norfloxacin in the serum sample. From the Table 2 it can be seen that the developed method can be easily performed and affords good precision and accuracy when applied to serum sample.

Over all the results showed that the method is easy, sensitive and reliable and can be applied for the determination for norfloxacin in pharmaceutical preparation and biological samples.

Table 1 Application of the investigated sensor for the determination of norfloxacin in pharmaceutical preparations

Brands of norfloxacin	Label claim/unit	Investigated method	Added (mg)	Found (mg)	Recovery (%n=5)
Urekacin tablets (Guju pharma)	200 mg per tablet	202.91 mg per tablet ±13 mg	100	100.49	100.49
Barotham tablets (Kukje pharma)	100 mg per tablet	100.99 mg per tablet ±2.095 mg	50	49.37	98.74

Conclusion

A simple and selective method to determine norfloxacin using an optical flow-through sensor has been developed. The sensor was prepared by packing anionic ion exchange resin in a glass tube, followed by introducing $KMnO_4$ solution to the glass tube. The permanganate ion was immobilized on resins, which made the method very simple. The resins could be regenerated and recycled. Comparison with liquid emission analysis, this sensor needs not to prepare large amounts of analytical reagents solution. The optical sensor is based on the emission intensity from the Tb (III) solution sensitized by norfloxacin and excited by the reaction of $KMnO_4$ and Na_2SO_4 solutions. The emission intensity at 545 nm was used for the determination of norfloxacin. The optimum flow rate, pH, Na_2SO_3 concentration and Tb^{3+} concentration, were 2.8 ml/min, 3.0, 2.0×10^{-3} and 2.0×10^{-3} M respectively. The emission intensity increased linearly with increasing norfloxacin concentration from 1.0×10^{-3} to 1.0×10^{-8} M and the detection limit (3σ) was 8.7×10^{-9}. The applicability of

Table 2 Determination of norfloxacin in serum sample

Norfloxacin sample	Added ($\times10^{-6}$ mol l^{-1})	Recovery value ($\times10^{-6}$ mol l^{-1})	Recovery (%n=5)	R.S.D (%)
Serum 1	1.50	1.54	102.66	1.65
Serum 2	2.00	2.03	101.5	0.95

the present method was demonstrated by determination of norfloxacin in various pharmaceutical preparations. For future work a better optical system, perhaps one based on a microscope with high NA objectives and a very small flow cell (capillary) may significantly improve the LOD, as well as reducing the amount of reagent used. Operating the Modified PMT detector (perhaps cooled) in photon counting mode might also improve the system.

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