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Spectrofluorimetric study of the interaction between europium(III) and moxifloxacin in micellar solution and its analytical application

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ABSTRACT

A sensitive spectrofluorimetric method has been developed for the determination of moxifloxacin (MOX) using europium(III)–MOX complex as a fluorescence probe in the presence of an anionic surfactant, sodium dodecyl benzene sulfonate (SDBS). The fluorescence (FL) intensity of Eu^{3+} was enhanced by complexation with MOX at 614 nm after excitation at 373 nm. The FL intensity of the Eu^{3+}–MOX complex was significantly intensified in the presence of SDBS. Under the optimum conditions, it was found that the enhanced FL intensity of the system showed a good linear relationship with the concentration of MOX over the range of 1.8×10^{-11}–7.3×10^{-9} g mL^{-1} with a correlation coefficient of 0.9998. The limit of detection of MOX was found to be 2.8×10^{-12} g mL^{-1} with relative standard deviation (RSD) of 1.25% for 5 replicate determination of 1.5×10^{-8} g mL^{-1} MOX. The proposed method is simple, offers higher sensitivity with wide linear range and can be successfully applied to determine MOX in pharmaceutical and biological samples with good reproducibility. The luminescence mechanism is also discussed in detail with ultraviolet absorption spectra.

1. Introduction

Moxifloxacin (MOX) (1*S*,6*S*)-1-cyclopropyl-7-[2,8-diazobicyclo(4.3.0)non-8-yl]-6-fluoro-8-methoxy-4-oxo-1,4-dihydroquinolone-3-carboxylic acid, is a fourth generation new 8-methoxyquinolone derivate of fluoroquinolones with enhanced activity in vitro against Gram positive bacteria and maintenance of activity against Gram negative bacteria [1–3]. The bactericidal activity of MOX is mediated by the inhibition of DNA gyrase (topoisomerase II) and topoisomerase IV, essential enzymes involved in bacterial DNA replication, transcription, repair and recombination [4]. MOX is prescribed for the bacterial infections of the respiratory tract including sinusitis, community acquired pneumonia and acute exacerbations of chronic bronchitis [5]. The drug is rapidly absorbed, reaching maximum plasma concentrations between 1 and 4 h after oral administration; its half-life of 11–15 h allows a daily administration [3]. MOX is administered to patients in 400 mg daily doses, being that the final concentrations in serum and urine for the treated patients are of 2.00–5.00 and 30.00–60.00 μg mL^{-1}, respectively [6]. Bayer AG developed the drug and it is marketed worldwide (as the hydrochloride) under the brand name Avelox for oral treatment. It achieves good tissue penetration and has a convenient once-daily administration schedule, as well as being available in both intravenous and oral formulations in some markets. Due to clinical advantages of MOX, it is still meaningful to develop a simple and sensitive analytical method for the determination of MOX.

Several methods have been reported for the quantification of MOX including spectrofluorimetry [7], voltametry [8], squere-wave adsorptive voltammetry [9], square-wave voltammetry using Cu(II) [10], high-performance liquid chromatography (HPLC) with fluorescence [11–13], HPLC with UV detection [14], high-performance thin layer chromatography (HPTLC) [15], capillary electrophoresis with laser-induced fluorescence [16], liquid chromatography [2] and chemiluminescence coupled with flow-injection (CL-FIA) [17]. However, most of these methods are lacking in sensitivity, selectivity or require sophisticated instruments. Spectrofluorimetry is a simple and highly sensitive method for the assay of a large number of drugs and metals and permits the selective and sensitive determination of low concentrations of an analyte.

Europium complex has been used widely as a fluorescence probe for the determination of some biomolecules including,

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metacycline-europium to determine lysozyme [18], Eu^{3+}-doxycycline complex to determine NADP [19], tetracycline-Eu^{3+} to determine bilirubin [20], europium-thenoyltrifluoroacetone and doxycycline–europium to determine human serum albumin [21,22], and oxytetracycline–Eu^{3+} to determine nucleic acid [23]. Europium complex has been used as fluorescence probe because of its high fluorescence quantum yield, large Strokes' shift, narrow emission bands, and a long fluorescence lifetime and hence to avoid potential background fluorescent emission interferences from the biological matrix [24]. There was no report about spectrofluorimetric method for the determination of MOX using Eu^{3+} as fluorescence probe. MOX having carboxylic and keto-oxygen atoms are involved in complexation with Eu^{3+} which shows a large Stokes' shift and narrow emission bands.

In the present paper, a fluorescence system, Eu^{3+}–MOX–sodium dodecyl benzene sulfonate (SDBS) has been proposed. In our study, the experimental results indicate that MOX could form a complex with Eu^{3+} and emit characteristic fluorescence of Eu^{3+} at 591 and 614 nm corresponding to the $^5D_0$–$^7F_1$ and $^5D_0$–$^7F_2$ transition of Eu^{3+} ion respectively. It was also observed that the fluorescence intensity of Eu^{3+}–MOX was enhanced significantly in the presence of SDBS and the enhanced intensity was proportional to the concentration of MOX added. Therefore, a sensitive method for the determination of MOX based on the fluorescence enhancement effect by the Eu^{3+}–MOX complex in the presence of SDBS was proposed. The characteristics of the fluorescence spectrum of the Eu^{3+}–MOX–SDBS system and the effect of different experimental conditions on the fluorescence intensity were studied systematically. The proposed method is easily carried out, affords good precision and accuracy and has been successfully applied to the determination of MOX in pharmaceutical preparations and biological fluids with satisfactory results which signifies the importance of this work.

2. Materials and methods

2.1. Reagents

All chemicals were of analytical reagent grade and were used without further purification. Distilled deionised (DI) water (Millpore, MilliQ Water System, USA) was used throughout. MOX was purchased from Sigma–Aldrich (St. Louis, USA). A stock solution (1.0×10^{-3} mol L^{-1}) of MOX was prepared in DI water. Working solutions of desired concentrations were freshly prepared by appropriate dilution of each stock solution with DI water. Stock standard solution of Eu^{3+} (1.0×10^{-3} mol L^{-1}) was prepared by dissolving Eu_2O_3 (purity, 99.99%) in 1:1 HCl and evaporating the solution to almost dryness before diluting to 100 mL with DI water. The stock standard solution was kept in the refrigerator at 4 °C. The working standard solutions were prepared by appropriate dilution with water. Sodium dodecyl benzene sulfonate (SDBS) (1×10^{-3} mol L^{-1}) was prepared by dissolving 0.017 g in a 50 mL volumetric flask using DI water and preserved at 4 °C. Tris–HCl buffer was prepared by dissolving appropriate amount of tris(hydroxy methyl) aminomethane in 500 mL DI water and pH was adjusted using 0.1 M HCl. Working solutions were prepared daily from the stock solution by appropriate dilution immediately before used.

2.2. Apparatus

All the spectrofluorimetric measurements were conducted with a spectrofluorimeter (Model F-4500, Hitachi, Japan). The spectrofluorimeter was equipped with a 450-W Xenon lamp (Model XBO 450 W/1, Osram, Germany) as the excitation light source and a photomultiplier tube (Model R928, Hamamatsu, Japan) powered at 950 V as the detector. The excitation and emission slits were set to 10 nm to measure all fluorescence spectra. pH was adjusted using a pH meter (Model Orion, 520A, USA). All the UV–visible spectra were measured in UV-1800 (Shimadzu, Japan).

2.3. Analytical procedure

Apparent fluorescence (FL) excitation and emission spectra were measured at room temperature and optimum excitation and emission wavelengths were found from these spectra. To a 10 mL volumetric flask, solutions were added in the following order: 1 mL of Eu^{3+} ion solution, 1 mL of buffer solution, certain amount of MOX, and 1 mL of SDBS. The mixture was diluted to 10 mL with doubly DI water, mixed thoroughly, and stood for 20 min. The solution was then put into the 1 cm quartz cell for measuring FL spectra and intensities. The FL intensity was measured with a 1 cm quartz cell with an excitation wavelength of 373 nm and an emission wavelength of 614 nm.

2.4. Sample preparation

Commercially available 10 tablets of Avolex (each contains 400 mg MOX) were weighed and grounded to fine powder by pestle in a mortar. The powder was transferred into a 1 L calibrated dark flasks containing 500 mL of DI water and dissolved in ultrasonic bath for 20 min and diluted to the mark with DI water. The dissolved sample was filtered through a Millipore membrane filter paper and diluted with DI water to obtain the appropriate concentration for analysis of MOX.

The proposed procedure was applied to determine MOX in spiked human urine and serum samples. Urine and serum samples were spiked with appropriate amounts of MOX stock solution. The serum sample was deproteinized by adding 5 mL of 20% trichloroacetic acid (CCl_3COOH) in a centrifuge tube and centrifuged for 15 min at 8000 rpm. 0.1 mL of the prepared serum sample was mixed with the standard solutions of MOX and diluted appropriately within the linear range of determination. For the urine samples, further pretreatment was not required except proper dilution in order to make the concentrations of MOX within the working range.

3. Results and discussion

3.1. Spectral characteristics of fluorescence

The FL emission and excitation spectra of (1) Eu^{3+}; (2) MOX; (3) Eu^{3+}–MOX; (4) Eu^{3+}–MOX–SDBS are shown in Fig. 1. It can be observed from Fig. 1 that only Eu^{3+} ion solution exhibited weak fluorescence signal because of weak absorption of the metal ion itself (Fig. 1a, curve 1). When MOX was added into the Eu^{3+} ion solution, the characteristic FL peaks appeared at 591 and 614 nm (Fig. 1a, curve 3) corresponding to the $^5D_0$–$^7F_1$ and $^5D_0$–$^7F_2$ transition of Eu^{3+}, respectively and maximum emission peak was obtained at 614 nm. It is reported that luminescence intensities of the $^5D_0$–$^7F_1$ and $^5D_0$–$^7F_2$ emissions are very sensitive to the nature of the ligand environment. But the $^5D_0$–$^7F_1$ transition retains its magnetic dipole character in low symmetry systems, and its radiative transition probability is not much affected by the ligand environment. On the other hand, the $^5D_0$–$^7F_2$ is predominantly associated with electric dipole, and their radiative transition probabilities are very sensitive to the nature of the ligand environment [25]. Thus, the FL intensity was enhanced significantly at 614 nm after the addition of MOX (Fig. 1a, curve 3). The results indicated that MOX can form a binary complex with Eu^{3+} which can emit the characteristic fluorescence of Eu^{3+}. It is hypothesized that MOX can absorb the