Methyl-β-Cyclodextrin /CTAB Sensitized Fluorescence Method for the Determination of Levofloxacin

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Abstract

In this work, the method of methyl-β-cyclodextrin (methyl-β-CD) and cetyltrimethyl ammonium bromide (CTAB) cooperatively sensitized fluorescence analysis to determine levofloxacin (LVFX) was developed. The results were shown that, in the system of methyl-β-cyclodextrin-CTAB, the fluorescence intensity of LVFX increased a lot than in H2O medium. Under the conditions of λexc = 330/507 nm and pH 4.5, the linear range and the detection limit for LVFX were found to be 0.04 ~ 4.0 μg/mL and 0.316 ng/mL, respectively. The mechanism of sensitized fluorescence method was discussed by the Benesi-Hildebrand method and the fluorescence quantum yield of fluorescence. The proposed method has been applied for the determination of LVFX in eye drops real samples with satisfactory recovery.

Keywords: Levofloxacin; Methyl-β-cyclodextrin; CTAB; Fluorescence spectrometry

Introduction

Levofloxacin (LVFX, Figure 1) is a broad-spectrum antimicrobial racemic fluoroquinolone agent. It is the active L-isomer of the ofloxacin, and its antibacterial activity is about two times of ofloxacin. Levofloxacin is one of the third generation of fluoroquinolones antimicrobial drug which exhibits activity against both Gram-positive and Gram-negative bacteria through inhibition of their DNA gyrase [1]. It is used to treat the infections of respiratory tract, urinary tract, intestinal tract, skin soft tissue and other systems. It has features of wide antibacterial spectrum, strong antibacterial effect and no cross resistance between a variety of drugs. Hence, extensive efforts have been devoted for the detection of LVFX including high-performance liquid chromatography (HPLC) [2], spectrophotometry [3], capillary electrophoresis (CE) [4], molecularly imprinted polymer (MIP) [5] and so on.

Cyclodextrins (CDs) are natural and neutral oligosaccharides. They have the truncated conical shape with an open cavity that is relatively hydrophobic and is able to host molecules with appropriate size and polarity, thus affecting the chemical, physical or biological properties of the guest molecule [6]. It is documented that even partial addition of guest molecules into the cavity and thus changing the distribution interval of materials in the medium, thus increasing the solubility. At the same time, the formation of micro environment of this medium has a protective effect on excited electrons of the fluorescent agent, reducing non-radiative processes of fluorescent substance, thus improving the fluorescence intensity enhancement. Based on this, CDs have been used to determinate the drug, pesticide and metal ions [7-12], organic molecules [20,21] and other substances. Enhancement mechanism of CTAB in fluorescence spectroscopy is the same with UV-Vis spectroscopy. The formation of aggregates in solution can increase the enrichment degree increases and the distribution coefficient increases, so the sensitivity was significantly improved. In the analysis of molecular fluorescence spectrum, CTAB was also reported for the analysis of metal ions [18,19], drugs [17] and other substances. Enhancement mechanism of CTAB in fluorescence spectroscopy is the same with UV-Vis spectroscopy. The formation of aggregates in solution can increase the distribution coefficient of materials in the medium, thus increasing its solubility. At the same time, the formation of micro environment of this medium has a protective effect on excited electrons of the fluorescent agent, reducing non-radiative processes of fluorescent substance, thus improving the fluorescence quantum yield.

Figure 1: schematic structure of levofloxacin.

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Fluorescence spectroscopy has the advantages of high sensitivity, good selectivity and fast analyzing, so it plays an important role in pharmaceutical analysis. However, due to the low concentration or the weak fluorescence intensity, we often add spectral sensitizing agent to improve the sensitivity of fluorescence spectra, and the synergistic method is one of the commonly used means in fluorescence spectrum analysis. Jiang has used room temperature ionic liquid and surfactant synergic fluorescence method for the determination of iron ion [22]. Zhu has used β-CD and CTAB microemulsion synergic fluorescence method for the determination of trace Bismuth [12]. But the study of methyl-β-CD and CTAB synergic fluorescence method for the determination of LVFX has not been reported.

Based on the fact that methyl-β-CD and CTAB have a strong sensitizing effect on fluorescence intensity of LVFX, a new method for the analysis of LVFX is proposed. The interaction mechanism was also investigated by the Benesi-Hildebrand method and the fluorescence quantum yield of fluorescence. The proposed method has been applied for the determination of LVFX in real samples and human serum with satisfactory recovery.

**Experimental**

**Reagents and apparatus**

Reagents: 50.0 μg/mL (1.35×10^-3 mol/L) levofloxacin standard solution (obtained from Dalian Meilun Biotech Co., Ltd) was prepared by dissolving 0.05g of LVFX in 100 mL with distilled water and kept in the darkness. 0.01 mol/L methyl-β-CD solution was prepared by dissolving 1.31g of methyl-β-CD in 100 mL with distilled water. 1% CTAB solution (0.0274 mol/L) was prepared by dissolving 1g of CTAB in 100 mL with distilled water. pH 4.5 B-R buffer solution was prepared by dissolving 0.05g of LVFX in 100 mL with distilled water and kept in the darkness. 0.01 mol/L methyl-β-CD solution was prepared by dissolving 1.31g of methyl-β-CD in 100 mL with distilled water. pH 4.5 B-R buffer solution was prepared by dissolving 0.05g of LVFX in 100 mL with distilled water and kept in the darkness. 0.01 mol/L methyl-β-CD solution was prepared by dissolving 1.31g of methyl-β-CD in 100 mL with distilled water. 1% CTAB solution (0.0274 mol/L) was prepared by dissolving 1g of CTAB in 100 mL with distilled water. pH 4.5 B-R buffer solution was prepared by dissolving 0.05g of LVFX in 100 mL with distilled water and kept in the darkness. 0.01 mol/L methyl-β-CD solution was prepared by dissolving 1.31g of methyl-β-CD in 100 mL with distilled water. pH 4.5 B-R buffer solution was prepared by dissolving 0.05g of LVFX in 100 mL with distilled water and kept in the darkness. 0.01 mol/L methyl-β-CD solution was prepared by dissolving 1.31g of methyl-β-CD in 100 mL with distilled water. pH 4.5 B-R buffer solution was prepared by dissolving 0.05g of LVFX in 100 mL with distilled water and kept in the darkness. 0.01 mol/L methyl-β-CD solution was prepared by dissolving 1.31g of methyl-β-CD in 100 mL with distilled water. pH 4.5 B-R buffer solution was prepared by dissolving 0.05g of LVFX in 100 mL with distilled water and kept in the darkness. 0.01 mol/L methyl-β-CD solution was prepared by dissolving 1.31g of methyl-β-CD in 100 mL with distilled water. pH 4.5 B-R buffer solution was prepared by dissolving 0.05g of LVFX in 100 mL with distilled water and kept in the darkness. 0.01 mol/L methyl-β-CD solution was prepared by dissolving 1.31g of methyl-β-CD in 100 mL with distilled water. pH 4.5 B-R buffer solution was prepared by dissolving 0.05g of LVFX in 100 mL with distilled water and kept in the darkness. 0.01 mol/L methyl-β-CD solution was prepared by dissolving 1.31g of methyl-β-CD in 100 mL with distilled water. pH 4.5 B-R buffer solution was prepared by dissolving 0.05g of LVFX in 100 mL with distilled water and kept in the darkness. 0.01 mol/L methyl-β-CD solution was prepared by dissolving 1.31g of methyl-β-CD in 100 mL with distilled water. pH 4.5 B-R buffer solution was prepared by dissolving 0.05g of LVFX in 100 mL with distilled water and kept in the darkness. 0.01 mol/L methyl-β-CD solution was prepared by dissolving 1.31g of methyl-β-CD in 100 mL with distilled water. pH 4.5 B-R buffer solution was prepared by dissolving 0.05g of LVFX in 100 mL with distilled water and kept in the darkness. 0.01 mol/L methyl-β-CD solution was prepared by dissolving 1.31g of methyl-β-CD in 100 mL with distilled water. pH 4.5 B-R buffer solution was prepared by dissolving 0.05g of LVFX in 100 mL with distilled water and kept in the darkness. 0.01 mol/L methyl-β-CD solution was prepared by dissolving 1.31g of methyl-β-CD in 100 mL with distilled water. pH 4.5 B-R buffer solution was prepared by dissolving 0.05g of LVFX in 100 mL with distilled water and kept in the darkness.

Apparatus: A F-4500 spectrophotofluorimeter (Hitachi, Japan) was used for all fluorescence measurement. The pH was measured on a pH S-25 pH meter (Shanghai, China). All absorption spectral recordings and absorbance measurements were performed on a UV 2501 spectrophotometer (Shimadzu, Japan).

Fluorescence measurements: In volumetric flask (5.0 mL), 0.35 mL 0.01 mol/L methyl-β-CD solution, 0.25 mL 0.0274 mol/L CTAB solution, 1.0 mL of B-R buffer solution (pH=4.5) and adequate LVFX standard solution or sample solution were added; the solution was diluted to the mark with distilled water. Then fluorescence spectra were recorded in the range of 300-650 nm upon excitation at 330 nm.

**Determination of relative fluorescence quantum yield:**

Fluorescence quantum yields of levofloxacin with or without methyl-β-CD were measured using 1.0×10^-3 g/mL quinine sulfate as reference material.

Under the same apparatus conditions, according to the equation \( \frac{F - F_0}{F_0} = \frac{Y - Y_0}{Y_0} \) [15], the quantum yield of the analyte was calculated. In brief, \( Y \) and \( Y_0 \) correspond to measurement-needed fluorescence quantum yield (\( Y_0 = 0.55, 25^\circ C \)), \( F_0 \) and \( F \) are the integral areas of two calibration fluorescence emission curves, \( A_0 \) and \( A \) are the absorbance (\( A_{\text{absorbance}} = \lambda_{\text{emission}} \)) of the standard and measurement-needed materials.

**Result and Discussion**

**Fluorescence Spectra of LVFX**

The fluorescence spectra of LVFX (F) in water (curve 1), methyl-β-CD (curve 2), CTAB (curve 3) and methyl-β-CD-CTAB medium (curve 4) are shown in Figure 2. It could be seen that the fluorescence intensity of LVFX was enhanced in the medium of methyl-β-CD-CTAB. And the fluorescence intensity of LVFX had the largest increase in the methyl-β-CD-CTAB medium.

**Effect of pH**

Effect of pH on fluorescence intensity (F) is shown in Figure 3. Different pH values were tuned by adding different pH B-R buffer solutions. It could be seen from Figure 3 that the fluorescence intensity of LVFX in the methyl-β-CD-CTAB medium was increased with pH.
increasing before pH 4.5, and reached a maximum value at pH 4.5. After pH 4.5, F was decreased. Thus, 1.0 mL B-R buffer solution of pH 4.5 was chosen for the determination.

Effect of methyl-β-CD and CTAB amount

The effect of 0.01 mol/L methyl-β-CD amount on fluorescence intensity (F₁) and the effect of 0.0274 mol/L CTAB amount on fluorescence intensity (F₂) was studied in the range of 0.10–0.45 mL (Figure 4). In the curve 1, the maximum fluorescence intensity was obtained with 0.35 mL of the reagent. After that, F₁ was decreased with increasing amount of methyl-β-CD. In the curve 2, F₂ gradually reached a maximum when the amount of CTAB was 0.25 mL, but it was decreased when the amount was more than 0.25 mL. Thus, 0.35 mL of methyl-β-CD and 0.25 mL of CTAB were chosen as an optimum amount for all measurements. The mass ratio of methyl-β-CD and CTAB is 1:8:1, and the molar ratio is 1:2.

Effect of reaction time

The effect of reaction time on the fluorescence intensity (F) was studied at room temperature. It was observed that fluorescence intensity (F) increased a little at 20 min and then got stabilized and remained stable at least for about 2 h. Therefore, 30 min was chosen.

Effect of temperature

The effects of temperature on fluorescence intensity (F) were tested. It was found that F was steady ranging from 15 to 25°C, and decreased gradually after 25°C. Therefore, 20°C (room temperature) was chosen for this work.

Effect of foreign substances

The effects of the different foreign substances were discussed in the determination of the 1.00 μg/mL of LVFX. The level of tolerated concentrations of foreign substances was considered as the maximum concentration found to cause a change in signal, less than ± 5%, compared with the signal without foreign substances. The tolerance limits for various foreign ions were in Table 1 (tolerance ratio in mass).

Analytical application

Under the optimum conditions, the linear regression equation was determined to be: F=29.064+624.60c (μg/mL), R=0.9985. A linear relationship was observed over the range of 0.04 ~ 4.00 μg/mL. The detection limit estimated (S/N = 3) was 0.316 ng/mL.

Sample analysis

The proposed method was applied to determine LVFX in levofloxacin eye drops and in spiked human serum. The data are listed in Table 2. Obtained values of the recoveries ranged from 96.1%-100.0% and 98.8%-100.4% for levofloxacin eye drops and human serum; The result of levofloxacin eye drops obtained by the proposed method (2.97 mg/mL) was in good agreement with the label value (3 mg/mL).

Discussion of sensitizing effect mechanism

Inclusion interaction of levofloxacin and methyl-β-CD: Methyl-β-CDs have the property of forming an inclusion complex with various guest molecules with suitable polarity and dimension and then effect the fluorescence intensity of the guest compounds [6].

According to the Benesi-Hildebrand method, it was found that the double reciprocal plot had good linear relationships (Figure 6), which could support the formation of a 1:1 complex. The inclusion constant Kᵢ for methyl-β-CD and LVFX in the absence of CTAB was 1.18×10⁵ L/mol, and Kᵢ in the presence of CTAB was 1.74×10⁵ L/mol. The value of Kᵢ was much larger than Kᵢ', which suggest that the inclusion complex is more stable when CTAB was present. The possible reason is that the addition of surfactant CTAB causes part hydrophobic groups of surfactant to get into the cavity of the Methyl-β-CD, and replace the H₂O molecules in it [24], the Methyl-β-CD and LVFX inclusion function is more stable, so that the fluorescence enhanced.

Fluorescence quantum yield: The fluorescence quantum yield was one of the mostly basic and significant parameters for fluorescence substance, which represented the ability of translating absorption energy to fluorescence and was tightly related to chemical structure and microenvironment of the system. The fluorescence quantum hat the fluorescence intensity of the system has enhanced.

Conclusion
In this paper, a new fluorimetric method was developed for the determination of LVFX, which based on the fact that the fluorescence intensity of LVFX could be enhanced dramatically by methyl-β-CD and CTAB. The results show that under certain conditions, methyl-β-CD and LVFX can form a stable inclusion complex, which make LVFX better adapt to the less polar and more rigid micro environment, so the fluorescence intensity of LVFX increases; CTAB can change the micro environment, improve the fluorescence quantum yield, and fluorescence intensity increased further. The present method has been applied for the determination of LVFX in real samples with satisfactory recovery. Therefore, it is concluded that the proposed method is simple, sensitive and rapid for the determination of LVFX.

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