Pharmaceutica Analytica Acta
ISSN: 2153-2435
Ren and Zhu, Pharm Anal Acta 2015, 6:6
DOI: 10.4172/2153-2435.1000376

Research Article
Open Access

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 H_2O medium. Under the conditions of $\lambda_{ex/em}$=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 binding to the CD cavity is accompanied by noticeable changes in the photophysical properties of the guest compound such as fluorescence intensity enhancement. Based on this, CDs have been used to determinate the drug, pesticide and metal ions [7-12]. Native β-CD is very poorly soluble in water (1.85 g / 100mL,25°C) [13]. CDs modified with hydrophilic substituents have enhanced solubility in water compared with their native forms [14]. Modified CDs were found to exhibit stronger inclusive ability than native CDs. The strong inclusion ability probably because the substitutents of methyl or hydroxyl propyl groups increase the openness of cyclodextrins, and make it easier for guest molecules to get into the cavity, and therefore provide a greater inclusion constant [6]. M.L.Polo et al. have investigated the formation of inclusion complex between melatonin and methyl-β-cyclodextrin (methyl-β-CD) spectrophotometrically and observed the enhancement in fluorescence intensity of melatonin [15]. This simple method was satisfactorily applied to the determination of melatonin in pharmaceutical preparations and urine samples.

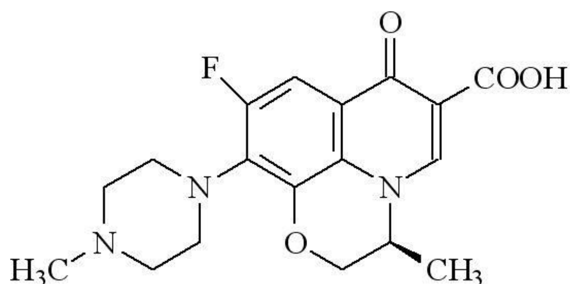

Figure 1: schemical structure of levofloxacin.

Research shows that surfactant has sensitizing effect on spectral analysis. Water-soluble surfactant aggregates can change the interface state of solution system, so as to improve the sensitivity of material spectral analysis. As a kind of common surfactants, cetyltrimethyl ammonium bromide (CTAB) is widely used in the analysis of UV and fluorescence spectra. In the analysis of UV-Vis spectrum, micellar and microemulsio are the most common sensitizers in surfactants. Song et al. have used CTAB as a sensitizer to determinate metal ions [16], drugs [17] and other substances. Enhancement mechanism of CTAB in UV-Vis spectroscopy can be considered from the distribution coefficient. By adding surfactant medium, solubility of analyte in the aggregates increases, 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], 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 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.

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Received May 16, 2015; **Accepted** May 28, 2015; **Published** June 04, 2015

Citation: Qiuye Ren, Xiashi Zhu (2015) Methyl-B-Cyclodextrin /CTAB Sensitized Fluorescence Method for the Determination of Levofloxacin. Pharm Anal Acta 6: 376. doi:10.4172/21532435.1000376

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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 0.04mol/L boric acid, acetic acid, phosphoric acid and 0.02 mol/L NaOH.

Apparatus: A F-4500 spectrofluorimeter (Hitachi, Japan) was used for all the fluorescence measurement, with excitation and emission slits at 5.0 nm, λex=330nm and 1-cm quartz cell. 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).

Experiment method

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^{-6} g/mL quinine sulfate as reference material.

Under the same apparatus conditions, according to the equation $Y_u = Y_s \times \frac{F_u}{F_s} \times \frac{A_s}{A_u}$ [15], the quantum yield of the analyte was calculated. In brief, Y_s and Y_u correspond to measurement-needed fluorescence quantum yield (Y_s=0.55, 25°C), F_s and F_u are the integral areas of two calibration fluorescence emission curves, A_s and A_u are the absorbance ($\lambda_{absorbance} = \lambda_{emission}$) of the standard and measurement-needed materials.

Determination of inclusion constant by direct fluorescence technique: In a 5.0 mL volumetric flask, an aliquot of LVFX (1.0 μg/mL), 1.0 mL of B-R buffer solution (pH=4.5) and various amounts of 0.01 mol/L methyl-β-CD were added, and then diluted to the mark with deionized water and mixed thoroughly. After that, the fluorescence intensity of mixture was measured in a 1-cm quartz cell. The inclusion constant K was calculated according the modified Benesi-Hildebr and equation (double reciprocal plot) [23]:

$$\frac{1}{\Delta F} = \frac{1}{K \cdot k \cdot Q \cdot C_0 \cdot C_D} + \frac{1}{k \cdot Q \cdot C_0}$$

Briefly, ΔF=F-F_0, F and F_0 were the fluorescence intensity of LVFX with or without methyl-β-CD, k was the instrument constant, Q was fluorescence quantum yield, C_0 was the concentration of LVFX, C_{CD} represented the concentration of methyl-β-CD. K was the inclusion constant. The calibration graph was obtained by plotting $\frac{1}{F-F_0}$ versus $\frac{1}{C_D}$. The intercept divided by the slope of calibration graph was K.

Results 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 or 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

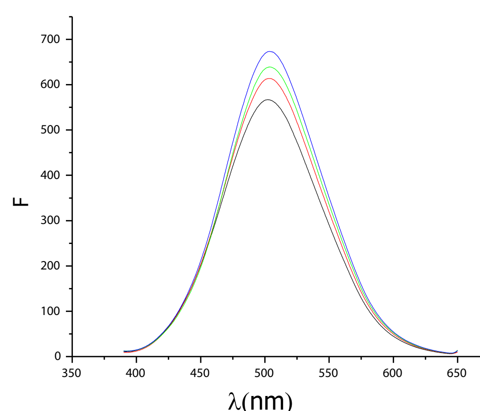

Figure 2: Emission spectra of LVFX

(LVFX: 2.7×10^{-5} mol/L, methyl-β-CD: 7×10^{-4} mol/L, CTAB: 1.37×10^{-3} mol/L)

1-LVFX in water, 2-LVFX in methyl-β-CD, 3-LVKX in CTAB, 4-LVFX in methyl-β-CD-CTAB.

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increasing before pH 4.5, and reached a maximum value at pH4.5. 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 (F_1) and the effect of 0.0274 mol/L CTAB amount on fluorescence intensity (F_2) was studied in the range of 0.10–0.45mL (Figure 4). In the curve 1, the maximum fluorescence intensity was obtained with 0.35 mL of the reagent. After that, F_1 was decreased with increasing amount of methyl-β-CD. In the curve 2, F_2 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.

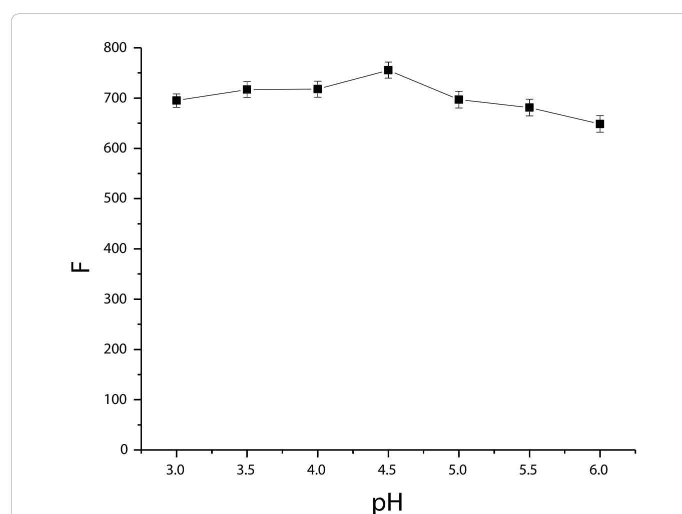

Figure 3: Effect of pH on LVFX fluorescence intensity
(LVFX: 2.7×10^{-5} mol/L, methyl-β-CD: 7×10^{-4} mol/L, CTAB: 1.37×10^{-3} mol/L).

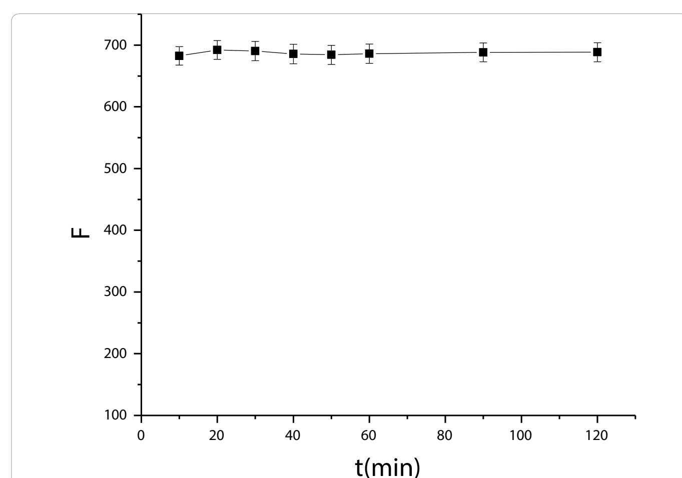

Figure 4: Effect of methyl-β-CD and CTAB amount on LVFX fluorescence intensity
(LVFX: 2.7×10^{-5} mol/L, 1-effect of methyl-β-CD amount on LVFX fluorescence intensity, 2-effect of CTAB amount on LVFX fluorescence intensity)

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 (2.97mg/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 K_1 for methyl-β-CD and LVFX in the absence of CTAB was 1.18×10^3 L/mol, and K_2 in the presence of CTAB was 1.74×10^5 L/mol. The value of K_2 was much larger than K_1, 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 H_2O 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 quantu 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 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

Tested substances	Tested substances to analyte ratio (w/w)
K^+	16000
Na^+	4000
Al^{3+}	1
Mg^{2+}	50
Zn^{2+}	10
Fe^{3+}	2
Cu^{2+}	0.5
Cl^-	6000
NO_3^-	50
Glucose	1500
Sucrose	2000
Glycine	500

Table 1: Tolerance limits of interfering substances.

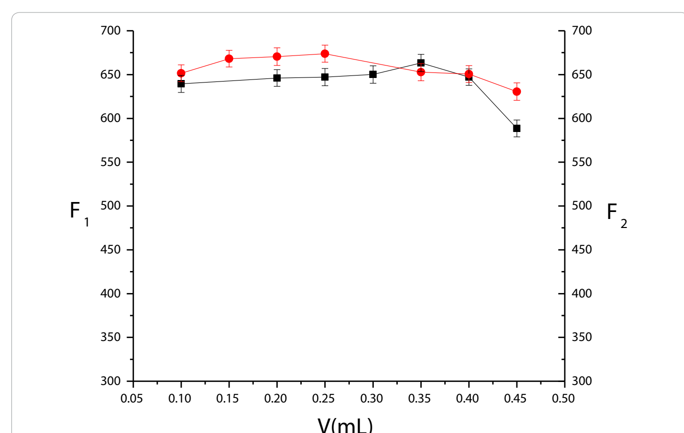

Figure 5: Effect of reaction time on LVFX fluorescence intensity
(LVFX: 2.7×10^{-5} mol/L, methyl-β-CD: 7×10^{-4} mol/L, CTAB: 1.37×10^{-3} mol/L).

Sample	Added (μg/mL) LVFX	Found μg/mLμ	Recovery (%)
levofloxacin eye drops	0.0	0.89	-
	0.5	1.39	100.0
	1.0	1.87	98.3
	2.0	2.79	96.1
human serum	0.0	ND	-
	0.5	0.49	99.4
	2.0	1.98	98.8
	3.0	3.01	100.4

Table 2: Results of sample determination and recovery.

medium	Y_u
H_2O	0.151
Methyl-β-CD	0.201
CTAB	0.294
Methyl-β-CD-CTAB	0.375

Table 3: The fluorescence quantum yield.

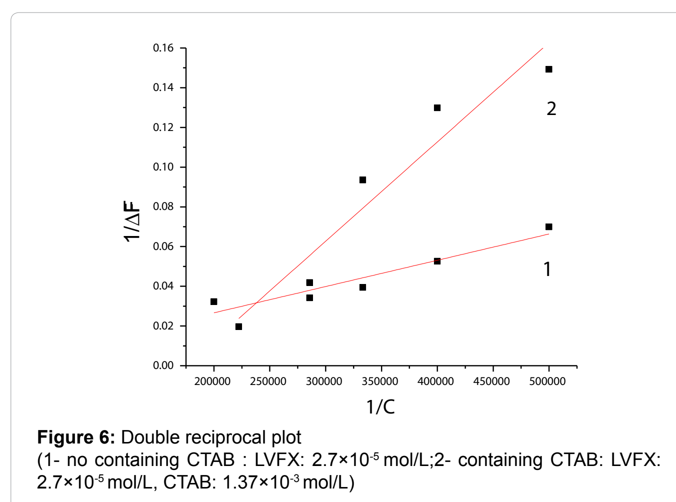

Figure 6: Double reciprocal plot
(1- no containing CTAB : LVFX: 2.7×10^{-5} mol/L;2- containing CTAB: LVFX: 2.7×10^{-5} mol/L, CTAB: 1.37×10^{-3} mol/L)

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.

Acknowledgements

The authors acknowledge the financial support from the National Natural Science Foundation of China (21375117) and a project funded by the Priority Academic Program Development of Jiangsu Higher Education Institutions.

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