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A novel spectrofluorimetric method for determination of lomefloxacin based on supramolecular inclusion complex between it and *p*-sulfonated calyx[4]arene

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Abstract

The characteristics of host–guest complexation between *p*-sulfonated calix[4]arene (SC4A) and lomefloxacin (LFLX) were investigated by fluorescence spectrometry. 1:1 stoichiometry for the complexation was established and their association constant at 25 °C was calculated by applying a deduced equation. The interaction mechanism of the inclusion complex was discussed. It was found that an appropriate amount of cationic surfactant cetyltrimethylammonium bromide (CTAB) could remarkably enhance the fluorescence intensity of the supramolecular complex system. Based on the obtained results, a novel sensitive spectrofluorimetric method for the determination of lomefloxacin was developed with a linear range of 0.01–3.0 μg ml^{-1} and a detection limit of 0.008 μg ml^{-1}. The proposed method was applied satisfactorily to determine lomefloxacin in pharmaceutical preparations.

Keywords: Spectrofluorimetry; *p*-Sulfonated calyx[4]arene; Host–guest complexation; Inclusion interaction; Lomefloxacin

1. Introduction

As the third generation of host molecules [1], calixarenes and their derivatives have been attracting more and more attention because of their unique advantages in the construction of molecular recognition systems, i.e., the variety of steric structure, and the diversity of functioning and relative availability. The sulfonation of calixarenes on their *para*-positions produces well highly water-soluble *p*-sulfonated calixarenes, which conquer the poor solubility of calixarenes in aqueous solution [2]. In the last few years, some water-soluble calix[*n*]arenes ($n = 4$–6 and 8) and resorcinarenes towards quaternary ammonium ions [3,4], trimethylammonium cations [5–7], dyes [8,9], native amino acids [10,11], and small neutral organic molecules [12] have been investigated extensively. Recently, the possible use of *p*-sulfonated calix[*n*]arenes in biological and pharmaceutical applications has occupied a current interest [13–15]. But till now, few literatures reported the quantitative determination of some substances using *p*-sulfonated calix[*n*]arenes [16,17].

Lomefloxacin (LFLX) [1-ethyl-6,8-difluoro-1,4-dihydro-7-(3-methyl-1-piperazinyl)-4-oxo-3-quinolinecarboxylix acid] (Fig. 1) is one of the synthetic antibacterial fluoroquinolone agents of the third generation, which exhibits high activity against a broad spectrum of gram-negative and gram-positive bacteria through inhibition of their DNA gyrase, and often is used as important drugs for the treatment of respiratory tract, urinary tract, skin and skin-structure infections. In consequence, it is of great importance to determine its contents in pharmaceutical preparations and in various biological samples, such as blood, urine and tissues. Up to now the most common techniques for the determination of the drug in commercial formulations and biological fluids have been performed on high performance liquid chromatography method [18,19],

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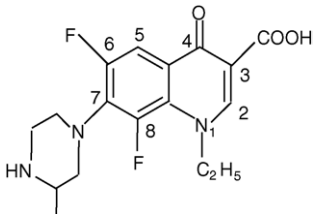

Fig. 1. Chemical structure of lomefloxacin.

microbiological assay method [20], micellar electrokinetic capillary chromatographic [21], chemiluminescent [22], spectrophotometric [23] and photochemical-fluorimetric [24] methods. However, most of them have some limitations in terms of simplicity, sensitivity, or stability.

Herein the host–guest complexation of LFLX with SC4A in aqueous solution was investigated by using fluorescence spectroscopy. When SC4A was added to the aqueous solution of LFLX, it reacted with LFLX to form an inclusion complex, an obvious decrease in fluorescence intensity and a slight and slow red shift were observed. Unexpectedly it was found that an appropriate amount of cationic surfactant cetyltrimethylammonium bromide (CTAB) could remarkably enhance the fluorescence intensity of the supramolecular complex system. So a simple, rapid, and specific spectrofluorimetric method for the determination of lomefloxacin was developed. In comparison with other fluorimetric [25,26] and HPLC methods [18,19], the present method seems to be simpler, faster, and of lower cost with better detection limit. To the best of our knowledge, it is the first example that involves the complexation of water-soluble calixarenes with fluoroquinolone agents in aqueous solution, and the first method for determination of LFLX based on supramolecular complexation.

2. Experimental

2.1. Apparatus

Fluorescence spectra and intensity measurements were made on a Hitachi F-4500 spectrofluorometer (Tokyo, Japan) with a 1.0 cm quartz cell, excitation and fluorescence emission wavelengths of 292 and 451 nm, respectively. Slit widths of both monochromators were set at 10 nm. Absorption measurements were performed with a Hitachi U-3010 spectrophotometer (Tokyo, Japan) using a 1.0 cm path length cell. All measurements were carried out at $25 \pm 0.2\,^{\circ}C$ by use of a thermostated cell holder and a thermostatically controlled water bath. A model pHS-3C (Dazhong Analytical Instruments Factory, Shanghai, China) pH meter was used for accurate adjustment of pH.

2.2. Reagents

All reagents used were of analytical-reagent grade or the best grade commercially. Doubly distilled water was used throughout. LFLX of drug standard sample was purchased from Chinese National Institute for the Control of Pharmaceutical and Biological Products (content >99.9%) and used without further purification. Stock standard solution of $1\,mg\,ml^{-1}$ was prepared by dissolving standard sample in doubly distilled water as needed. Working standard solutions were prepared by dilution of stock standard solution with doubly distilled water. SC4A was prepared according to literature [27] and identified by IR, ^1H NMR and element analysis. Stock solution of SC4A was prepared as $1 \times 10^{-3}\,mol\,l^{-1}$. β-Cyclodextrin (β-CD) was obtained from Shanghai Chemical Reagent Co., China, and $1 \times 10^{-2}\,mol\,l^{-1}$ stock solution was prepared in water. β-CD was recrystallized twice from doubly distilled water before used. $1 \times 10^{-3}\,mol\,l^{-1}$ solution of cationic surfactant cetyltrimethylammonium bromide (CTAB) was prepared. A Britton-Robinson buffer solution (pH 2.00–9.00) was prepared using $0.04\,mol\,l^{-1}$ boric acid, acetic acid and phosphoric acid, then was adjusted to accurate values by using $0.2\,mol\,l^{-1}$ sodium hydroxide.

2.3. Procedure

2.3.1. Inclusion process

A 1 ml aliquot of the stock solution ($10\,\mu g\,ml^{-1}$) of LFLX was transferred into a 10 ml volumetric flask and an appropriate amount of $10^{-4}\,mol\,l^{-1}$ SC4A was added. The mixed solution was diluted to final volume with water and stirred thoroughly, the fluorescence intensities (or absorption spectra) were determined after $25\,min^{-1}$ at $25 \pm 0.2\,^{\circ}C$.

2.3.2. Determination of LFLX

Into a 10 ml volumetric flask were placed in turn an appropriate volume of sample or working solution, 2.0 ml of $1.0 \times 10^{-4}\,mol\,l^{-1}$ SC4A solution and 1.5 ml of $1.0 \times 10^{-3}\,mol\,l^{-1}$ CTAB solution. The mixture was diluted to 10 ml with pH 6.00 Britton-Robinson buffer solution and mixed thoroughly. After incubation for 25 min at $25 \pm 0.2\,^{\circ}C$, the fluorescence intensities were measured.

2.3.3. Sample preparation

Two commercial preparations were analyzed: lomefloxacin capsules (The No. 2 Pharmaceutical Industries Co., Changzhou, China), labeled to contain 100 mg lomefloxacin per grain and lomefloxacin tablets (Searle Pharmaceutical Co., India), with a nominal content of 400 mg.

A stock solution of $1\,mg\,ml^{-1}$ LFLX was prepared as follows: the contents of 10 tablets or capsules of LFLX were pulverized carefully or evacuated. A portion of 100.0 mg of this powder was accurately weighed and dissolved in about 10 ml $0.1\,mol\,l^{-1}$ HCl solution and filtered into a 100 ml calibrated flask. The residue was washed several times with water and filtered into the flask too, and then the solution was diluted to the mark. The stock solution was diluted to appropriate concentrations for analysis.

3. Results and discussion

3.1. Fluorescence spectra and absorption spectra characteristics

Fig. 2 shows the fluorescence excitation and emission spectra of LFLX in water without using buffer solution. As can be seen this drug has strong fluorescence with excitation and emission wavelengths of 292 and 455.2 nm, respectively. When an appropriate amount of 10^{-4} mol l^{-1} SC4A was added into it, the fluorescence emission spectra decreased obviously, meanwhile a slight and slow red shift was observed (455.2 → 459.2 nm) (see Fig. 3). However, when 4-phenolsulfonate (the monomeric unit of SC4A; equiv: 1–1000) was added to LFLX, the fluorescence intensity decreased negligibly and the emission spectra had no changes. This indicates that the combination between

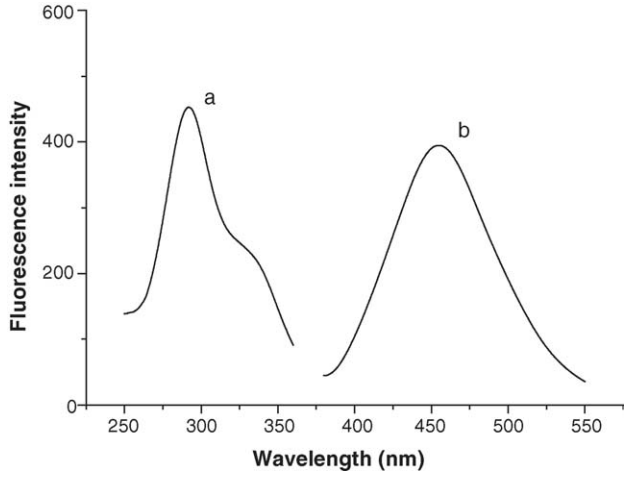

Fig. 2. Fluorescence excitation (a) and emission (b) spectra of LFLX (1 μg ml^{-1}) in aqueous solution. λ_{ex} = 292.0 nm and λ_{em} = 455.2 nm.

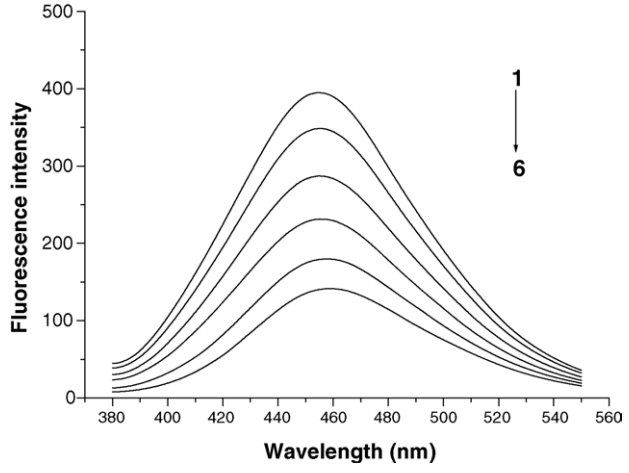

Fig. 3. Fluorescence spectra of LFLX with different concentrations of SC4A: from (1) to (6): (1) 0.0, (2) 0.2, (3) 0.5, (4)1.0 (5) 2.0, (6) 3.0×10^{-4} mol l^{-1}, C_{LFLX} = 1 μg ml^{-1}, 25 °C.

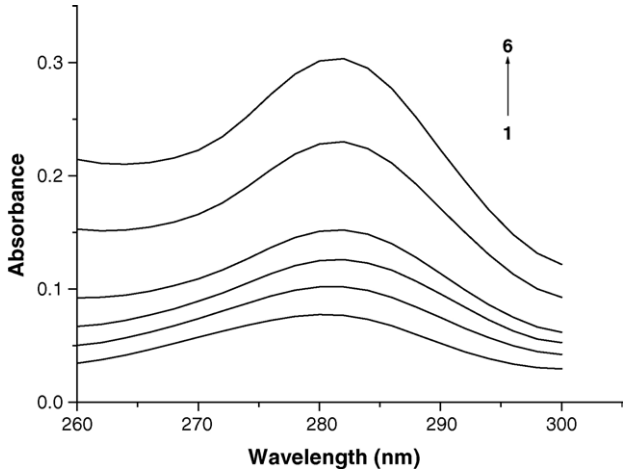

Fig. 4. Absorption spectra of LFLX with different concentrations of SC4A: from (1) to (6): (1) 0.0, (2) 0.2, (3) 0.5, (4) 1.0 (5) 2.0, (6) 3.0×10^{-4} mol l^{-1}, C_{LFLX} = 1 μg ml^{-1}, 25 °C.

4-phenolsulfonate and LFLX was very weak. Compared 4-phenolsulfonate with SC4A, the obvious difference between them is that the latter has a cavity. From the fact we can estimate that LFLX maybe was included (or partially included) into the cavity of SC4A.

The absorption spectra of LFLX in aqueous solution at various concentrations of SC4A are shown in Fig. 4. The absorption spectrum of LFLX is at 278.0 nm, the addition of SC4A to LFLX results in an increase of the absorbance of the drug and also a slight red shift (278.0 → 282.0 nm).

Further study has proved that the quenching effect of the fluorescence intensity is non-linear, which means that the quenching was not only simple static or dynamic quenching, so it can be drawn that most probably, the formation of a host–guest complex between SC4A and LFLX would be the origin of the spectral changes described above.

3.2. Discussion of Interaction mechanism

Cyclodextrins (CDs) are a well-known family of cyclic oligoscaccharides, which their structure is that of truncated cone with the hydrophilic outer surface and a hydrophobic internal cavity. Special cavity structure makes CDs able to form guest–host inclusion complexes with both organic and inorganic compounds. Since SC4A had a marked function with LFLX, we employed β-CD in order to compare the inclusion ability between it and SC4A. However, the experiments showed that the introduction of β-CD had no obvious effect on the fluorescent property of LFLX (also a similar case was found about β-CD with ciprofloxacin hydrochloride, ref. [28]). We think, one of the reasons is that the internal cavity of β-CD molecule provides a hydrophobic environment, however the LFLX molecule has a piperazine and a carboxylic group, and it exists as form of zwitterionic form with 1 N atom protonated and the carboxylic group dissociated in water solution [29]. Therefore, strong hydrophilicity makes LFLX molecule difficult to be included by the hydrophobic internal

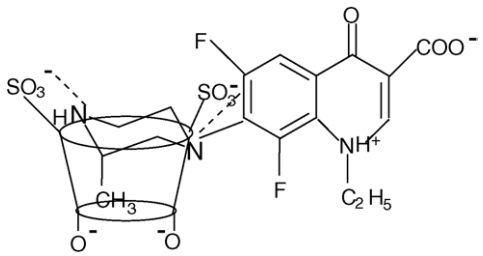

Fig. 5. The pattern of the supramolecular complex between LFLX and SC4A.

cavity of β-CD. The other important reason is that the internal cavity of β-CD is not big enough to include the whole LFLX molecule. The unexpected phenomena made us confirm that LFLX is "partially" included into SC4A since the cavity of SC4A is evidently smaller than β-CD (It is well accepted that for calix[4]arene the cavity of the "*cone*" conformation can accommodate sodium cation exactly, the pauling radius of Na^+ is 0.95 Å, in aqueous solution SC4A favorably adopts the cone conformation because of stabilization by intramolecular hydrogen-bonding interactions among OH groups [6], so the cavity of SC4A should be close to 0.95 Å too; for β-CD, its cavity is 7.8 Å). Considering the size/shape-fitting, the piperazine ring of LFLX goes into the cavity of SC4A should be the most probable pattern.

It is well known that calixarenes and their derivatives can form non-covalent inclusion complexes with various guest molecules of suitable size and characteristics with the aid of electrostatic interaction, cation–π interaction, hydrogen bonding, van de Waals, hydrophobic interaction, and so on [1]. Shinkai et al. have proposed that the main driving force of recognition of SC4A is electrostatic interaction [5]. Here we proposed the probable pattern of the inclusion manner between SC4A and LFLX: In weak acidic media (pH value of the host–guest system is about 5.5), LFLX exists in the form of zwitterionic form with 1 N atom protonated [29], while SC4A exists as form of three of OH groups of it dissociated [17]. The N atom of the piperazine ring of LFLX has a lone-pair electron, which can binding with one of the negatively charged sulphonyl groups of SC4A to form salt with the aid of electrostatic interaction, meanwhile the NH group of the piperazine ring can also combine to the other negatively charged sulphonyl group of SC4A with the help of electrostatic interaction; with the two forces the piperazine ring goes into the cavity of SC4A and form host–guest complex (Fig. 5), which makes the electron cloud density of LFLX depress. So we observed the fluorescence intensity decreased after host–guest complex formed. Whilst the CH–π interaction between the piperazine ring and the electron-rich benzene π-systems of SC4A as well as hydrophobic interaction also should be considered to contribute to the formation of the supramolecular complex according to ref. [6]. Among these forces, electrostatic interaction and structural matching effect were thought to play main roles in the formation of the supramolecular system. The results were proved by further study of the effect of CTAB and ionic strength on the host–guest complex (Sections 3.4.3 and 3.4.4)

3.3. Stoichiometry and association constant of the inclusion complex

The stoichiometry and association constant of the inclusion complex were studied under the established experimental conditions by the following method: assuming that the composition of the complex was 1:1, the following expression can be written as

$$H + G \rightleftharpoons H \cdot G \tag{1}$$

The formation constant of the complex (K) is given by

$$K = \frac{[H \cdot G]}{[H][G]} \tag{2}$$

where $[G]$, $[H]$ and $[H{\cdot}G]$ are equilibrium concentrations of guest molecule, host molecule and the host–guest complex, respectively. By using $[H] \gg [H \cdot G]$ it can be assumed that $[H]_0 = [H]$ and the following equation can be derived:

$$\frac{1}{[H \cdot G]} = \frac{1 + K[G]_0}{K[G]_0[H]_0} \tag{3}$$

where $[G]_0 = [G]+[H{\cdot}G]$ and $[H]_0 = [H]+[H{\cdot}G] \approx [H]$.

In this context the guest molecule is LFLX and the host molecule is SC4A. Since the fluorescence intensity of LFLX in the absence (F_0) and presence (F_1) of SC4A is proportional to $[G]$ and $[H{\cdot}G]$, respectively, substitution of these values into Eq. (2) gives

$$\frac{1}{F - F_0} = \frac{1}{F_1 - F_0} + \frac{1}{K[\mathrm{SC4A}](F_1 - F_0)} \tag{4}$$

Here F is the observed fluorescence intensity at each SC4A concentration tested (a similar equation was also deduced in cyclodextrin system by Mwalupindi et al. [30]).

Thus, from the data of Table 1, a linear equation of $1/F - F_0$ versus 1/[SC4A] can be acquired as: $1/(F - F_0) = -2.45 \times 10^{-3} - 3.78 \times 10^{-8}$ 1/[SC4A]. The good linear relationship supports the existence of a 1:1 complex ($R = 0.9982$). The calculated association constant (K) obtained from the ratio of the intercept to the slope is $K = 6.48 \times 10^4\ \mathrm{l\,mol^{-1}}$. The large association constant indicates the strong interaction of the host and the guest molecules.

Table 1
Data of the inclusion complex

$1/(F - F_0)$	1/[SC4A] ($\mathrm{l\,mol^{-1}}$)
-2.16×10^{-2}	5.00×10^5
-9.29×10^{-3}	2.00×10^5
-6.12×10^{-3}	1.00×10^5
-4.65×10^{-3}	5.00×10^4
-3.95×10^{-3}	3.33×10^4

$C_{\mathrm{LFLX}} = 1\ \mu\mathrm{g\,ml^{-1}}$.

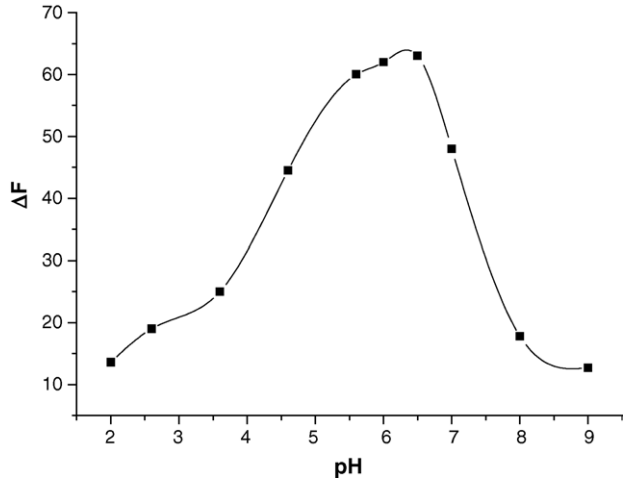

Fig. 6. Influence of pH value on the fluorescence intensity of LFLX–SC4A complex; $C_{LFLX} = 1\ \mu g\ ml^{-1}$, $C_{SC4A} = 2.0 \times 10^{-5}\ mol\ l^{-1}$.

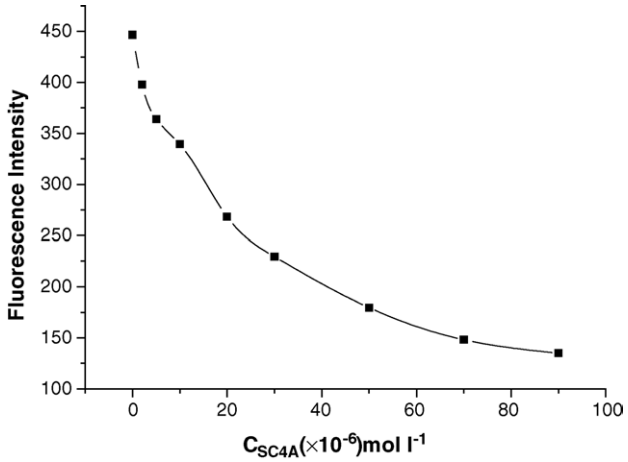

Fig. 7. Influence of SC4A concentration on the fluorescence intensity of LFLX, $C_{LFLX} = 1\ \mu g\ ml^{-1}$.

3.4. Optimization of experimental variables

3.4.1. Influence of pH

The fluorescence intensity of LFLX in acid media is stronger than that in basic media, and the fluorescence intensity is relatively stable and strong in the range of pH value from 2.00 to 7.00, this is in agreement with ref. [28]. It was found that among various buffer solutions Britton-Robinson solution was the most suitable, so we choose it in subsequent experiments. The pH dependence of the system was studied over the range 2.00–9.00. The experimental results (Fig. 6) show the effect of pH value on the fluorescence quenching intensity ($\triangle F$), where $\triangle F$ is the fluorescence intensity difference between the absence and the presence of buffer solution of various pH values. As can be seen that $\triangle F$ is high and almost remained constant over the pH range between 5.60 and 6.50. Therefore, a pH of 6.00 was chosen for further studies.

We found that more volume of the buffer solution had a better effect on the stability of fluorescence intensity, so we employed pH 6.00 Britton-Robinson buffer solution to dilute the mixed solution to final volume.

3.4.2. Influence of SC4A

The effect of SC4A concentration on the fluorescence intensity of LFLX and LFLX–CTAB system was examined, respectively. Fig. 7 shows the effect on the fluorescence intensity of addition of various SC4A concentrations. As can be seen from the figure, when the concentration of SC4A is in the range of 2.0×10^{-6} to $9.0 \times 10^{-5}\ mol\ l^{-1}$, remarkable quenching effect of LFLX was observed. This means that in this concentration range of SC4A, inclusion interaction between the host and guest is strong, and stable host–guest complex has formed. Fig. 8 shows the effect on the fluorescence increasing intensity of LFLX–CTAB system. SC4A concentration of $2.0 \times 10^{-5}\ mol\ l^{-1}$ was used in subsequent experiments.

3.4.3. Influence of ionic strength

To probe the driving force for inclusion of LFLX by SC4A, we examined the effect of NaCl ionic strength on the inclusion process. If the addition of NaCl solution had no obvious effect on the inclusion process, the main driving force should be a hydrophobic interaction, either hydrogen bonding, or any other weak forces, not an electrostatic interaction between LFLX and the electron rich aromatic ring of SC4A. Experimental results of the effect of NaCl solution on the LFLX–SC4A system are shown in Fig. 9. As can be seen that when the NaCl concentration is $0.01\ mol\ l^{-1}$, the fluorescence intensity of the LFLX–SC4A system had no remarkable and orderly changes when SC4A was added, reveals that there is no inclusion between LFLX and SC4A. These results demonstrate that NaCl ionic strength has strong effect on the inclusion process, means that the main driving force for inclusion of LFLX by SC4A is electrostatic interaction between the positively charged LFLX and the negatively

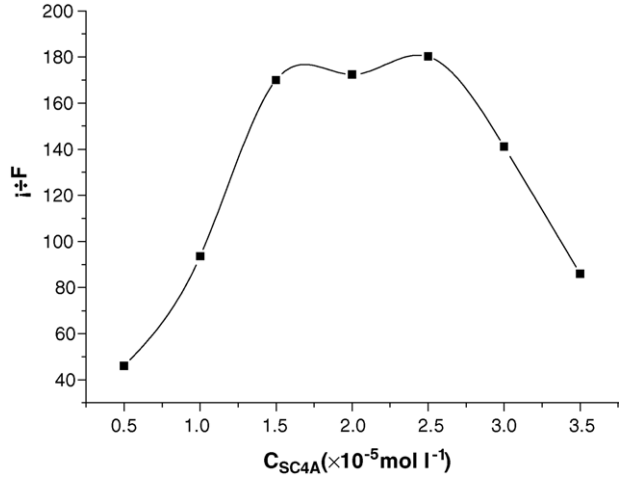

Fig. 8. Influence of SC4A concentration on the fluorescence intensity of LFLX–CTAB system, $C_{LFLX} = 1\ \mu g\ ml^{-1}$, $C_{CTAB} = 1.5 \times 10^{-4}\ mol\ l^{-1}$, pH 6.00.

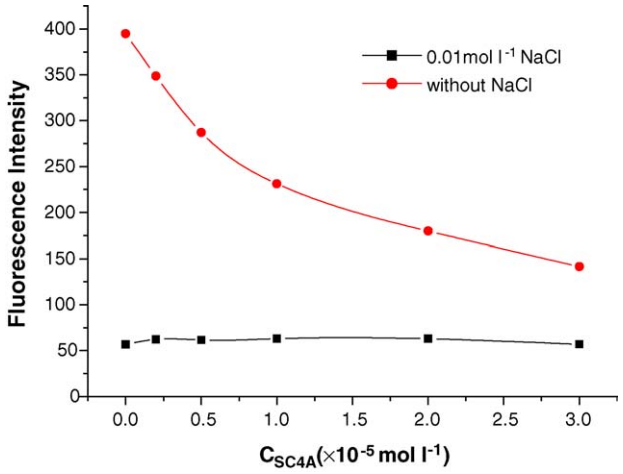

Fig. 9. Influence of NaCl solution on the fluorescence intensity of LFLX–SC4A system, $C_{LFLX} = 1\ \mu g\ ml^{-1}$.

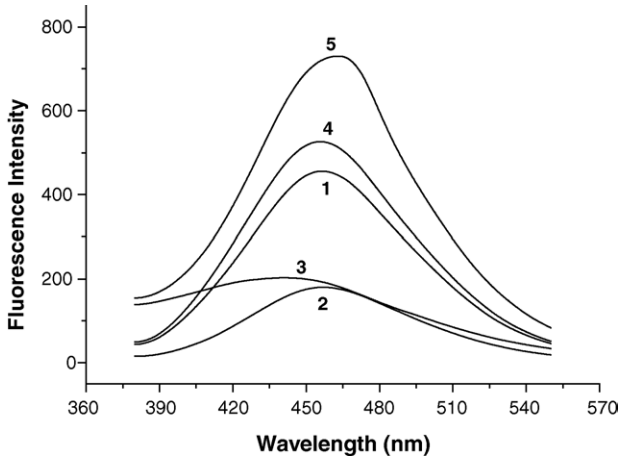

Fig. 11. Fluorescence emission spectra: (1) LFLX; (2) LFLX + SC4A; (3) CTAB + SC4A; (4) CTAB + LFLX; (5) CTAB + LFLX + SC4A; C_{LFLX} = $1\ \mu g\ ml^{-1}$, $C_{SC4A} = 2.0 \times 10^{-5}\ mol\ l^{-1}$, $C_{CTAB} = 1.5 \times 10^{-4}\ mol\ l^{-1}$.

charged sulphonyl groups of SC4A [31], this is in agreement with our conjecture in Section 3.2.

3.4.4. Influence of CTAB

The effect of cationic surfactant cetyltrimethylammonium bromide (CTAB) on the fluorescence increasing intensity of LFLX–SC4A complex was studied.

It is found that cationic surfactant CTAB with positive charge has a little increasing effect on the fluorescence intensity of LFLX itself in aqueous solution, but when SC4A also exist, the fluorescence intensity increased sharply with the addition of CTAB and then kept constant (Figs. 10 and 11). This also illustrated that SC4A and LFLX had formed host–guest complex. The reason was given as follows: In acidic media, the protonated 1 N atom of LFLX and the negatively charged sulphonyl group of SC4A have strong electrostatic interaction, and the electrostatic interaction was sensitive to external circumstances, since trimethylammonium cations can be included into the cavity of water-soluble calix[4]arenes with the aid of electrostatic interaction and cation–π interaction [5–7,32], when the concentration of CTAB increased, the cationic surfactant has a binding site competition to the negatively charged SC4A with LFLX, which leads to the LFLX–SC4A complex partially dissociated, and the released LFLX was quickly solubilized into the micelle of CTAB, which brought the fluorescence intensity of the system enhance markedly. While kept on increasing CTAB concentration, the competition arrived at its equilibrium, meanwhile fluorescence intensity achieved maximum and then hardly changed [33].

According to the results attained above, 1.5×10^{-4} $mol\ l^{-1}$ of the final CTAB concentration was selected in the following experiments.

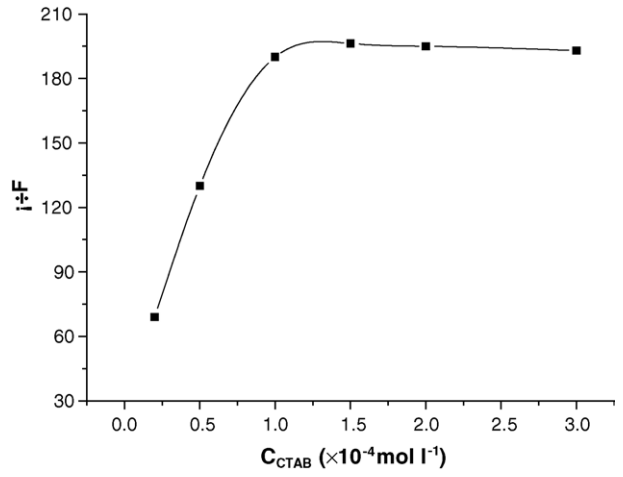

Fig. 10. Influence of CTAB concentration on the fluorescence intensity of LFLX–SC4A, $C_{LFLX} = 1\ \mu g\ ml^{-1}$, $C_{SC4A} = 2.0 \times 10^{-5}\ mol\ l^{-1}$, pH 6.00.

3.4.5. Effect of reaction time

The effect of reaction time was studied, the results (Table 2) shows that the fluorescence intensity reached a maximum after the reagents had been added for 25 min and remained at least 2 h. Hence, after the reaction was carried out for 25 min, the subsequent fluorescence measurements were made at room temperature (25.0 °C) within 2 h.

Table 2
Effect of reaction time on fluorescence intensity of the complex. C_{LFLX} = $1\ \mu g\ ml^{-1}$, $C_{SC4A} = 2.0 \times 10^{-5}\ mol\ l^{-1}$, $C_{CTAB} = 1.5 \times 10^{-4}\ mol\ l^{-1}$, pH 6.00

Time (min)	Fluorescence intensity
5	510
10	617
25	730
50	731
70	730
90	730
110	729

Table 3
Determination of lomefloxacin in pharmaceutical formulations ($P = 0.95$)

Content (mg per grain)	Present method (found + S.D.[a])	Reference method [25] (found + S.D.[a])	Experimental t-value[b]
100	99 ± 2	97 ± 3	1.2
400	401 ± 3	402 ± 2	0.9

[a] Standard deviation (average of five determinations).
[b] The tabulated values of t at the 95% confidence limit is 2.78.

Table 4
Determination of LFLX in pharmaceutical formulations

Drug	Sample content ($\mu g\,ml^{-1}$)	LFLX added ($\mu g\,ml^{-1}$)	LFLX found ($\mu g\,ml^{-1}$)	R.S.D. (%)	Recovery (%) + S.D. ($n = 6$)
Sample 1	0.02	0.03	0.0515	2.1	103 ± 1.5
Sample 1	0.05	0.05	0.0995	0.4	99.5 ± 0.4
Sample 2	0.01	0.04	0.0496	0.7	99.1 ± 0.9
Sample 2	0.10	0.10	0.2060	2.1	103 ± 1.8

3.4.6. Effect of the addition order of reagents

The effect sequence of adding reagents on the fluorescence recovery was studied, and the order: LFLX, SC4A, CTAB and buffer solution was proved to be the best suitable.

3.5. Calibration curve

According to the proposed method, a calibration curve was constructed under the optimal conditions. The increasing fluorescence intensity was proportional to LFLX concentration in the range 0.01–3.0 $\mu g\,ml^{-1}$. The concentration of LFLX was calculated from the linear regression equation:

$$F = 13.31 + 403.2C \quad (R = 0.9995)$$

where F was the fluorescence increasing intensity and C was the concentration of LFLX ($\mu g\,ml^{-1}$). The relative standard deviation ($n = 10$) was obtained from a series of 10 standards each containing 1.0 $\mu g\,ml^{-1}$ LFLX. The limit of detection of 0.008 $\mu g\,ml^{-1}$ LFLX was calculated with the signal to noise ratio (S/N) value of 3. Relative standard deviation (1.09%) was obtained from a series of 10 standards each containing 1.0 $\mu g\,ml^{-1}$ LFLX.

3.6. Analysis of pharmaceutical formulations

LFLX was satisfactorily determined in two pharmaceutical preparations (lomefloxacin capsules from The No. 2 Pharmaceutical Industries Co., sample 1; and lomefloxacin tablets from Searle Pharmaceutical Co., sample 2) following the method described in Section 2. According to the spectral characteristics obtained from these preparations, there is no interference from the excipients (Table 3). As can be seen, results obtained by the proposed method agree with the reference method. In the t-test, no significant differences were found between the two methods because the calculated t-values of the two methods were all less than the corresponding theoretical one (95% confidence).

Moreover, to check the validity of the proposed methods, the standard addition method was applied by adding LFLX to the previously analyzed tablets or capsules. The recovery of each drug was calculated by comparing the concentration obtained from the (spiked) mixtures with those of the pure drugs. Table 4 shows the results of analysis of the commercial capsule and tablet and the recovery study (standard addition method) of studied drugs.

3.7. Effect of interfering substances

A study of some potential interfering substances in the spectrofluorimetric determination of LFLX was performed by selecting them as the excipients often used in table formulations. Samples containing a fixed amount of the LFLX (0.05 $\mu g\,ml^{-1}$) and variable concentrations of excipients were measured. Lactose, sucrose, glucose and fructose do not cause interference at weight ratios of excipient/LFLX < 800. This fact indicates good selectivity of the method for determination of the studied drugs in raw material and in their dosage forms.

4. Conclusion

The inclusion interaction between lomefloxacin and *p*-sulfonated calix[4]arene in aqueous solution was investigated by fluorescence spectrometry. 1:1 stoichiometry for the complexation was established and their association constant at 25 °C was calculated by a deduced equation. The interaction mechanism of the host–guest complex was also discussed. Competitive binding of CTAB to the lomefloxacin–*p*-sulfonated calix[4]arene complex remarkably enhanced the fluorescence intensity of lomefloxacin. According to these results, a novel sensitive spectrofluorimetric method for determination of lomefloxacin based on supramolecular inclusion complex was developed and was applied successfully to determination of lomefloxacin in pharmaceutical formulations. Undoubtedly, the research results provide very interesting and useful information for pharmaceutical and biomedical analysis by employing water-soluble calixarenes as reagents, which always have been neglected.

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