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ORIGINAL ARTICLE

Investigation on the inclusion behavior of Norfloxacin with 2-methyl-β-cyclodextrin

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Abstract The formation of the complexes of Norfloxacin with 2-methyl-β-cyclodextrin (Me-β-CD) was studied by UV-Vis absorption spectroscopy, fluorescence and nuclear magnetic resonance spectroscopy (NMR). Experimental conditions including Me-β-CD concentration and media acidity were investigated in detail at room temperature. The results suggest that Norfloxacin exists in three molecular forms in aqueous solution at different pH values, namely, the acidic form, the neutral form and the alkaline form). Me-β-CD was more suitable for inclusion of Norfloxacin in the acidic medium. The binding constant (*K*) of the inclusion complex was determined by fluorescence measurement, and the complexation ratio was determined as 1:1 in the concentration range used in this study. A mechanism was proposed to explain the inclusion process based on the experimental NMR data.

Keywords Cyclodextrin · Norfloxacin · Inclusion complex · UV-Vis absorption · Fluorescence · NMR

Introduction

Investigations of molecular recognition have attracted much attention in supramolecular chemistry involving natural and artificial host-guest systems [1, 2]. Cyclodextrins (CDs) are the most widely used host molecules in supramolecular chemistry. They are polysaccharides made up of six to eight D-glucose monomers connected at the 1 and 4 carbon atoms. With a hydrophobic internal cavity and a hydrophilic external surface, CDs can form inclusion complexes with various guest molecules which possess suitable polarity and dimension [3–7]. This ability has been widely used in pharmaceutical industries, and has also been used for various other analytical purposes [8–10]. Furthermore, CDs have been used as models for proteins and enzymes because they interacted with many substances in a manner similar to of that proteins and enzymes [11]. The inclusion process of pharmaceutical molecules with CDs usually results in a modulation of the physicochemical and pharmaceutical properties of guest molecules [12, 13], such as increased solubility, improved chemical stability and bioavailability, reduced toxicity controlled-rate release and so on [14]. Therefore, it would be of great importance to comprehensively understand the inclusion behavior of molecules of pharmaceutical interests with CDs.

When the fluorescent guests are included in the CD cavity, the non-radiative decay processes of luminophores are significantly attenuated and hence fluorescence emission increased [15–17]. Due to its high sensitivity, selectivity and instrumental simplicity, fluorescence method has been used to investigate the phenomena of inclusion complexes and determine the association constants of complexes [17–19]. Nuclear magnetic resonance spectroscopy (NMR) is also a powerful tool to study the CDs complexes [20–25] that can provide not only quantitative information, but also detailed information on geometry of the complex.

Fluoroquinolones (FQs) are an important group of synthetic, broad-spectrum antimicrobial agents, which have been widely used to treat infection in many parts of the body by killing the harmful bacteria or preventing their growth. Being the first FQ drug approved for human use, Norfloxacin (Fig. 1) was found to exhibit broader activity against Gram (−) and Gram (+) bacteria, less protein binding, higher drug tolerance, lower toxicity and longer

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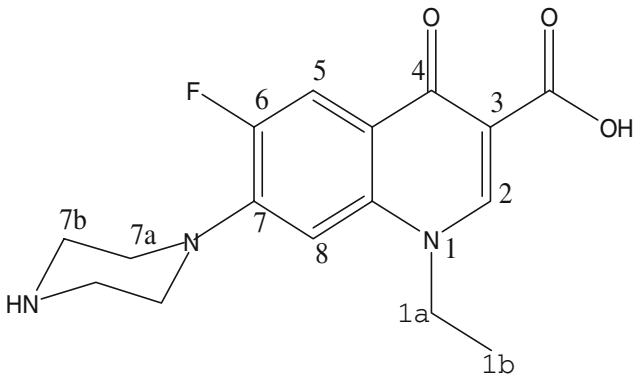

Fig. 1 The molecular structure of Norfloxacin

half-life [26]. So, it is necessary to study the inclusion complex of this drug with CDs to suit the pharmaceutical and clinic applications.

As the relatively low solubility and molecular binding abilities of natural CDs limit their further uses [27], modification of CDs has become one of the "hot" topics in recent host–guest supramolecular chemistry [28, 29]. Here we selected Me-β-CD as host molecule, which is unharmful to human body [30–32], to investigate the interaction between FQ and Me-β-CD. The inclusion process was characterized by fluorescence and ^{1}H NMR, and the experimental data will hopefully provide information for the development of new cyclodextrin drugs-carrier system.

Experimental

Apparatus

UV-757CRT spectrophotometer (Shanghai Precision £ Scientific Instrument Co. LTD.); RF-540 fluorescence spectrophotometer (Shimadzu); Advance DRX 300 MHz superconducting NMR spectrometer (Bruker). Excitation and emission band width were both set at 2 nm. All experiments were carried out at room temperature (20 ± 1 °C).

Reagents

A stock solution of 1×10^{-4} mol/L Norfloxacin was prepared by directly dissolving commercial Norfloxacin (99.9%, purchased from Yunnan Gourmet Factory) in water. The concentration of the Me-β-CD (purchased from Nanjing Tcm Institute of Materia Medica,The degree of substitution DS is 12.5) stock solution was 1×10^{-2} mol/L. Phosphate buffer solution was used to adjust the pH-value of the media. D_2O was used as the solvent in the NMR measurements. All other reagents were of analytical-reagent grade and were used without further purification. Doubly distilled water was used throughout.

Procedure

A 1 mL aliquot of the Norfloxacin stock solution was transferred into a 10 mL volumetric burette, then an appropriate amount of 1×10^{-2} mol/L Me-β-CD and 2 mL of 0.2 mol/L phosphate buffer solution were added. The solution was diluted to a final of 10 mL with distilled water. The final mixture solution was dissolved thoroughly under ultrasonic for 30 min, and then equilibrated for 30 min at 20 ± 1 °C. The working solution was transferred into a 1 cm × 1 cm quartz cell to record absorption and fluorescence spectra. The fluorescence emission was monitored at 420 nm and the excitation wavelength was 270 nm. Both the excitation and emission slits were set at 5 nm. All measurements of absorption and fluorescence were made against a blank solution treated in the same way but without Norfloxacin in a 1.0 cm quartz cell at different pH.

NMR measurements

1×10^{-4} mol/L Norfloxacin and 1×10^{-4} mol/L Me-β-CD solutions with a ratio of 1:1 was mixed thoroughly. With D_2O as solvent, ^{1}H NMR and ROESY spectra were obtained at 300.13 MHz with 10 μs as 90° pulse width. All experiments were performed at 20 ± 1 °C.

Results and discussion

Absorption study

Because Norfloxacin itself has definite acidity in specifically solution [33], different pH may affect the inclusion process. So the inclusion process of Norfloxacin with Me-β-CD was studied at different pH was studied. Figure 2 shows the absorption spectra of Norfloxacin in the absence and presence of Me-β-CD at different pH at room temperature. The maximum absorption wavelength of Norfloxacin is 270 nm. The absorbance of Norfloxacin increased with increasing concentration of Me-β-CD and the absorption peak was slightly shifted, suggesting that a stable complex was formed between Me-β-CD and Norfloxacin.

Fluorescence study

Figure 3 shows that adding Me-β-CD to Norfloxacin solution at pH 3.05 resulted in a significant enhancement of

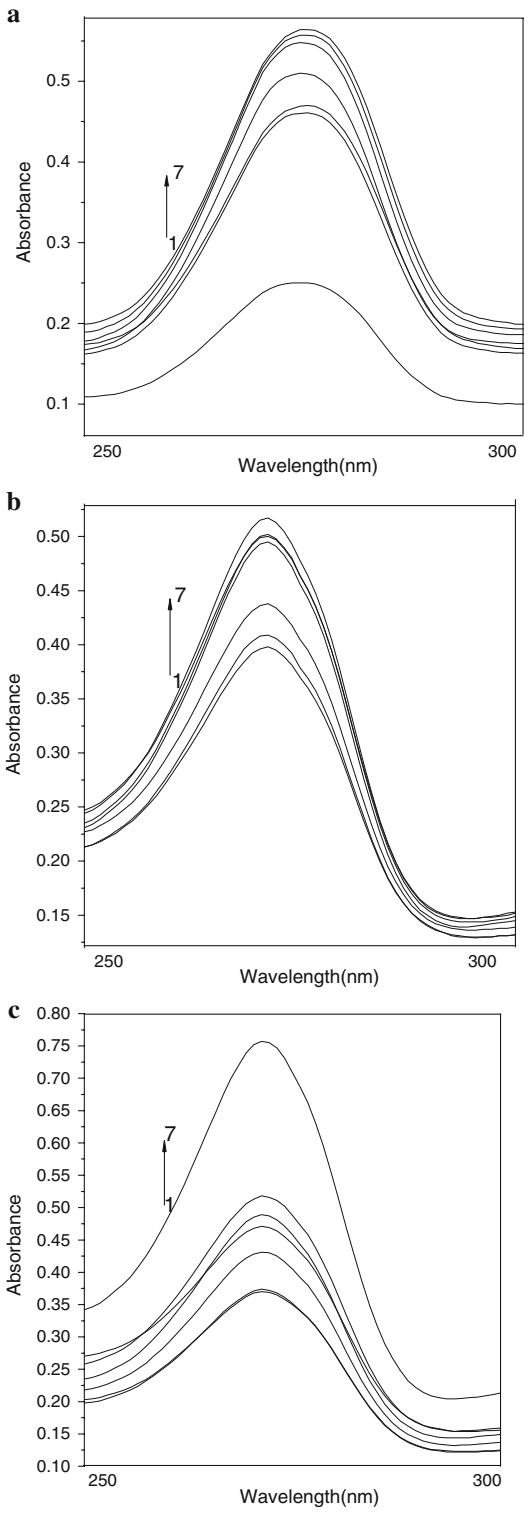

Fig. 2 The absorption spectra of Norfloxacin in the presence of Me-β-CD at (**a**) pH 3.05, (**b**) pH 6.53 and (**c**) pH10.53. From 1 to 7: the concentration of Me-β-CD is 0–6 $\times$ 10^{-3} M

the fluorescence signal. The experiment was also performed at pH 6.53 and 10.53. Very similar results were obtained. Only the emission wavelength was pH dependent, being 420 nm at pH 3.05, 417 nm at pH 6.53, and 416 nm at pH 10.53. These results suggest that the inclusion complex was formed between Me-β-CD and Norfloxacin. The Me-β-CD cavity provided an apolar environment for the Norfloxacin molecule and the motion of the Norfloxacin molecule in the cavity was largely confined. Thus, the enhanced rigidity of the Norfloxacin molecule resulted in an increase of its fluorescence quantum yield.

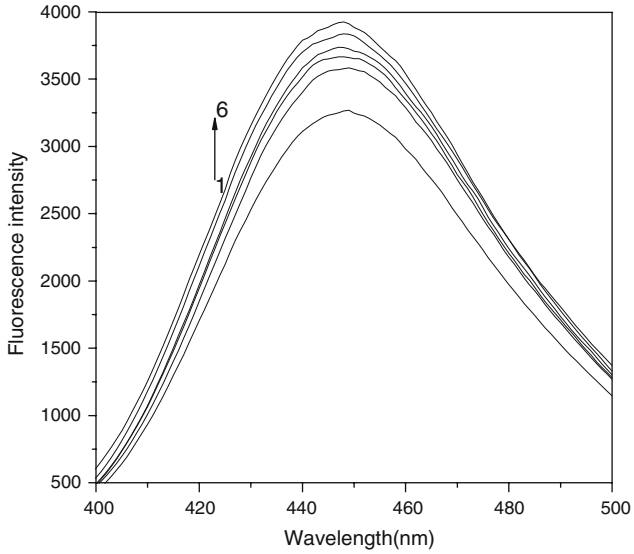

Fig. 3 Fluorescence spectra of Norfloxacin in the absence and presence of Me-β-CD at pH 3.05. The concentration of Me-β-CD is 0–5 $\times$ 10^{-3} M from 1 to 6

Formation constants of Norfloxacin-Me-β-CD complex

The inclusion formation constant (K) is a measure of the complexing power of CD. The formation constant and the ratio of the complex can be obtained from fluorescence data using the modified Benesi-Hildebrand equation (1) [34, 35].

$$1/(F - F_0) = 1/([\text{Me-}\beta\text{-CD}]K\alpha) + 1/\alpha \quad (1)$$

where, F is observed fluorescence intensity of the Norfloxacin solution at each Me-β-CD concentration tested, F_0 is the presents fluorescence intensity of Norfloxacin solution in the absence of Me-β-CD, K is the formation constant of the complex, α is an instrumental constant. The good linear relationship obtained when $1/(F - F_0)$ is plotted against 1/[Me-β-CD] supports the existence of a 1:1 complex. The results were consistent with the observed absorption results. The calculated formation constants at different pH and the corresponding correlation coefficients were listed in Table 1.

As shown in Table 1, the formation constant values were very sensitive to pH and increased in the order $K_{3.05} > K_{6.53} > K_{10.53}$. It can thus be concluded that Me-β-CD is more suitable for inclusion of the Norfloxacin molecule in acidic media. In basic media, the negatively charged Norfloxacin with more hydrophilic character was predominant, leading to the weaker interaction with Me-β-CD.

^{1}H NMR study

The formation of inclusion complex can be proved from the changes of chemical shift in ^{1}H NMR spectra. Figure 4 illustrated the ^{1}H NMR spectra of Norfloxacin and the complex of Me-β-CD with Norfloxacin.

The chemical shifts for the protons of Norfloxacin both in the absence and presence of Me-β-CD are summarized in Table 2. As can be seen in Table 2, H-1a, H-1b, H-5 and H-8 of Norfloxacin experienced larger shifts because of the diminished freedom of rotation caused by the penetration of Norfloxacin molecule into the Me-β-CD cavity. In contrast, H-7a and H-7b experienced smaller changes in their chemical shifts, implying that part of the piperazine ring might be outside the Me-β-CD cavitiy. Thus, it can be inferred that the matrix structure of Norfloxacin entered into the cavity of Me-β-CD.

2D NMR data

The ^{1}H NMR results suggest a possible orientation of the Norfloxacin molecule in the Me-β-CD cavity. In order to prove the geometry of the inclusion complex of Norfloxacin with Me-β-CD, two-dimensional ROESY studies were carried out to examine the configuration of Norfloxacin in the Me-β-CD cavity. Figure 5a shows a partial contour plot of NOESY spectra of the Norfloxacin complex with
Me-β-CD. There appear several intermolecular cross-peaks between H-2, H-5 and H-8 of Norfloxacin and H-3 and H-5 of Me-β-CD, which might indicate that matrix construction of the guest molecule was in the center of Me-β-CD. As shown in Fig. 5b, only weak intermolecular cross-peaks between Methyl group of Norfloxacin and H-3, H-5 of Me-β-CD were observed. Moreover, the

Table 1 The formation constants and the correlation coefficients of Me-β-CD with Norfloxacin were calculated by fluorescence measurement in different pH values

pH	Linear equation	K (M^{-1})	R
3.05	$y = 2 \times 10^{-6}x + 0.0415$	2.075×10^4	0.967
6.53	$y = 1 \times 10^{-5}x + 0.1315$	1.315×10^4	0.9991
10.53	$y = 2\times10^{-5}x + 0.0285$	1.425×10^3	0.9667

Table 2 Variation of H chemical shifts before and after inclusion

H	1a	1b	2	5	7a	7b	8
Norfoxacin	4.573	1.147	8.152	7.499	3.405	3.172	6.721
Inclusion	4.510	1.188	8.186	7.584	3.410	3.175	6.781
$\Delta\delta$	−0.063	0.041	0.034	0.085	0.005	0.003	0.060

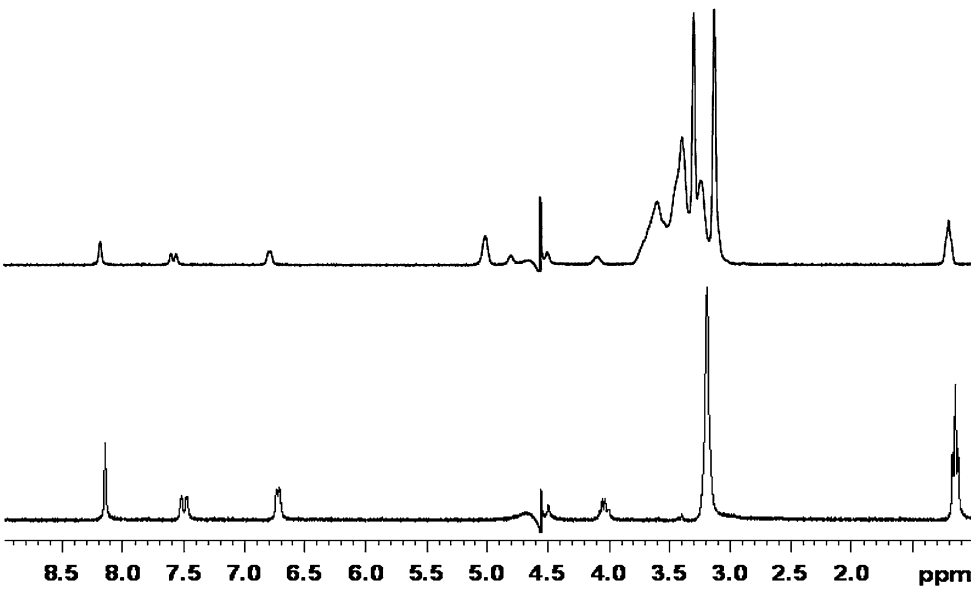

Fig. 4 ^{1}H NMR spectra: top is the inclusion complex of Norfloxacin and Me-β-CD; underside is the Norfloxacin without Me-β-CD

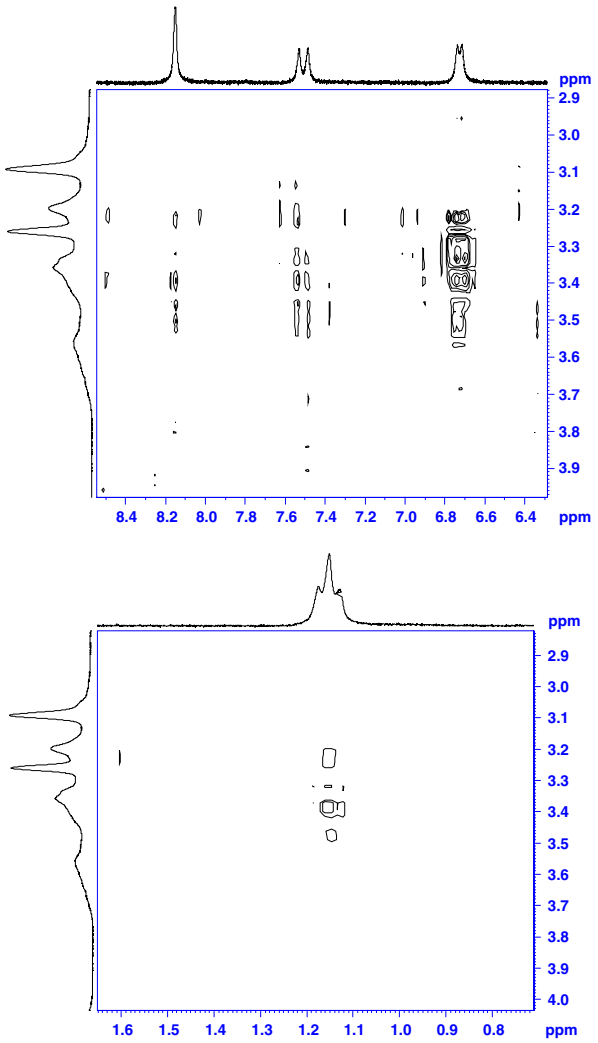

Fig. 5 2D NMR spectra of the Me-β-CD inclusion complex with Norfloxacin

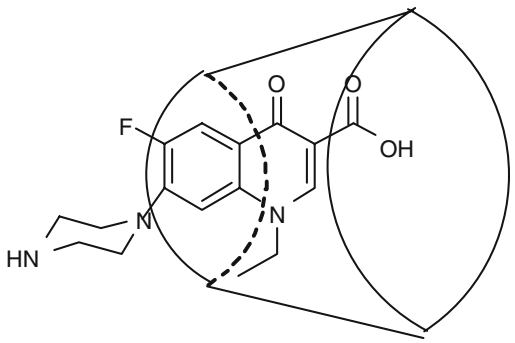

Fig. 6 The molecular model of the inclusion of Norfloxacin with Me-β-CD

interaction observed for H-5 was greater than for H-3 of Me-β-CD. These results indicated that matrix of the guest molecule enters into the cavity of Me-β-CD from the small ring-edge side of Me-β-CD, leaving the piperazine ring out of the Me-β-CD cavity (shown in Fig. 6). Combining fluorescence spectra with NMR data, it can be concluded that Norfloxacin is closely included into inner cavity of Me-β-CD to form a supramolecular system.

The related inclusion mechanism

The fluorescence characteristic of Norfloxacin was sensitive to the pH value in media. As shown in the following equilibrium equation, different forms of Norfloxacin are known to exist as a function of pH, namely anionic, protonated and neutral species.

$$H_2L^+ \rightleftharpoons HL \rightleftharpoons L^-$$

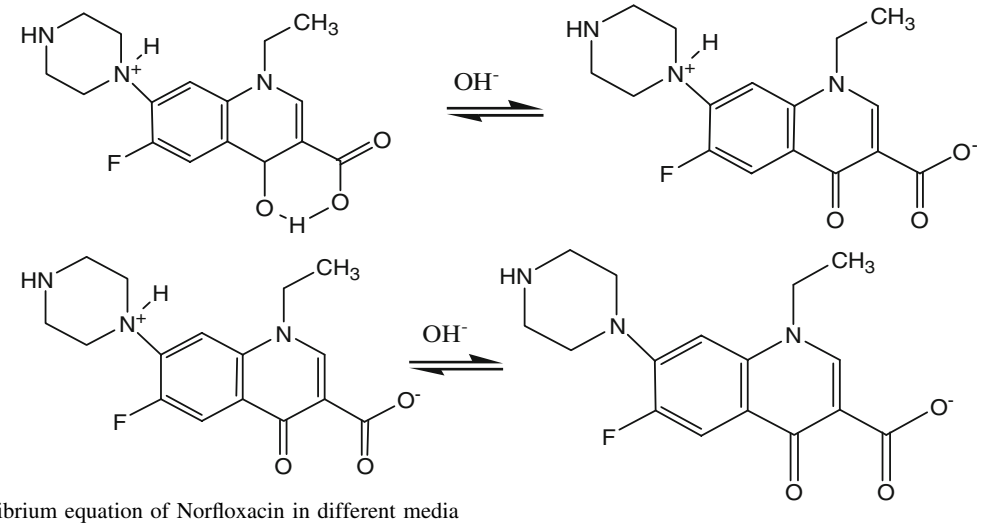

Fig. 7 The equilibrium equation of Norfloxacin in different media

Figure 7 shows that in acidic and neutral media, the positively charged and the neutral form of Norfloxacin are predominant, respectively, while in basic media, Norfloxacin exists mainly as the negative charged form.

The inclusion interactions are based on the simultaneous cooperation of several weak interactions between host (Me-β-CD) and guest (Norfloxacin), including dipole–dipole, electrostatic, van der waals, hydrogen bonding, and hydrophobic interaction [36, 37]. The Me-β-CD is not charged ($2 < pH < 11$) and the major inclusion interactions here include hydrophobic interactions between the guest and the Me-β-CD cavity, and hydrogen bonding of the guest to –OH groups on the Me-β-CD ring.

Conclusions

Absorption and fluorescence measurements have demonstrated the inclusion complexation interaction between Norfloxacin and Me-β-CD. The major factors affecting molecular recognition is size matching, between Me-β-CD and guest, and the hydrophobic property of the guest molecule. The obtained inclusion complex was more stable under the acidic conditions, which can be attributed to the hydrophobic effect. The fluorescence results showed that Norfloxacin formed a stoichiometric 1:1 complex with Me-β-CD over the concentration range evaluated. In the present study we demonstrated that Me-β-CD can be used as guest complexing agent, which acted as substrate reservoir in a dosage-controlled manner. And the inclusion process has been characterized, demonstrating that Me-β-CD can be used as a drugs carrier system.

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