J Incl Phenom Macrocycl Chem (2011) 69:173–179
DOI 10.1007/s10847-010-9828-y

ORIGINAL ARTICLE

Preparation and characterization of the inclusion complex of Ofloxacin with β-CD and HP-β-CD

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Received: 22 April 2010 / Accepted: 7 July 2010 / Published online: 15 July 2010

Abstract The formation of the inclusion complexes of Ofloxacin with cyclodextrins (CDs) including β-cyclodextrin (β-CD), and hydroxypropyl-β-cyclodextrin (HP-β-CD) were studied by Fluorescence, UV–Vis absorption spectroscopy and nuclear magnetic resonance spectroscopy (NMR) in solution. Experimental conditions including the concentration of various CDs and media acidity were investigated in detail at room temperature. The results suggested that in different pH solutions, CDs have different inclusive capacity to different forms Ofloxacin. β-CD was most suitable for inclusion of neutral form and HP-β-CD was suitable for acidic form. 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 Fluorescence · UV–Vis absorption · NMR · Ofloxacin · Cyclodextrin · Inclusion complex

Introduction

Cyclodextrins (CDs) are polysaccharides made up of six to eight D-glucose monomers connected at the 1 and 4 carbon atoms. They have the property of forming inclusion complex with various guest molecules with suitable polarity and dimension because of their special molecular structure—hydrophobic internal cavity and hydrophilic external surface [1–7]. This ability has been widely used in pharmaceutical industries, and has also been used for analytical purposes [8–10]. Furthermore, the CDs have been used as models for proteins and enzymes because they interact with many substances in a manner similar to proteins and enzymes [11]. In addition, especially in pharmaceutical industries, since the inclusion process of pharmaceutical molecules with CDs led to important modifications of pharmaceutical properties of guest molecules [12, 13], the pharmaceutical interest in CDs extends to enhance solubility, chemical stability and bioavailability of poorly soluble drugs, to reduce toxicity and to control the rate of release so on and so forth [14]. Therefore, it is essential to comprehensively understand the effects of inclusion about pharmaceutical molecules.

Since many guest compounds present fluorescent properties, it is interesting to analyze the changes produced in such properties when these compounds form inclusion complexes. The non-radioactive decay process of analyst is often significantly attenuate as the fluorescence emission increases [15–17]. Due to its sensitivity, selectivity and instrumental simplicity, fluorimetric method can be used as a resource to improve the performance of analytical methods and to determine the association constants of complexes [18–20].

High resolution Nuclear Magnetic Resonance is a powerful tool for studying CD complexes [21–26]. This is because NMR techniques can provide not only quantitative information but also detailed information about the geometry of the complex. Two-dimensional Nuclear Overhauls Effect Spectroscopy (NOESY) is one of many NMR tools which have proven to be a powerful technique

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for investigating intermolecular interaction. The NOESY technique appears to be a potential method for determining the structure of CD complexes.

There are few literature references concerning the inclusion of Ofloxacin (Fig. 1) sodium with CDs. Furthermore, the results of our research would provide a theoretical basis for developing new drug carriers and new drug forms.

Experimental section

Materials

β-Cyclodextrin and HP-β-CD were purchased from a commercial manufacturer (Yun Nan, China), and wee purified by recrystallization. Ofloxacin sodium was kindly provided by Shanxi Pharmaceutical Industry (China). All other reagents were of analytical-reagent grade and were used without further purification. Doubly distilled water was used throughout.

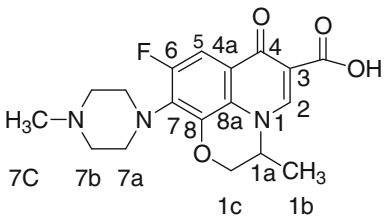

Fig. 1 The molecular structure of Ofloxacin

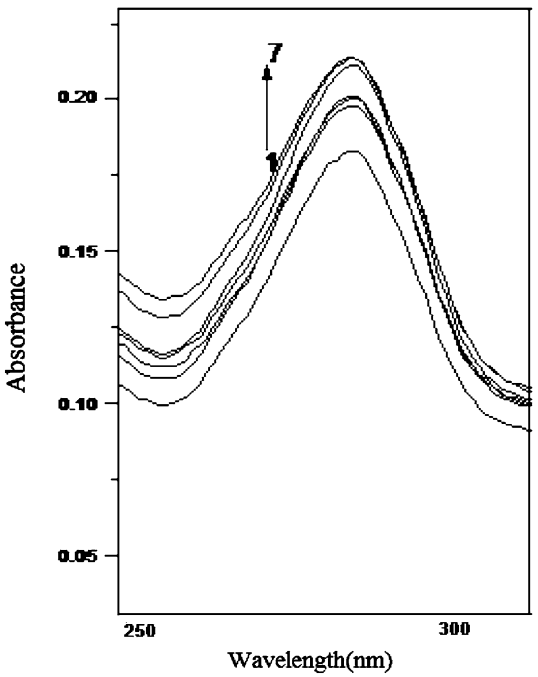

Fig. 2 The absorption spectra of Ofloxcain in the presence of HP-β-CD in pH3.0 values and the concentration of HP-β-CD is $1 \sim 6 \times 10^{-3}$ M

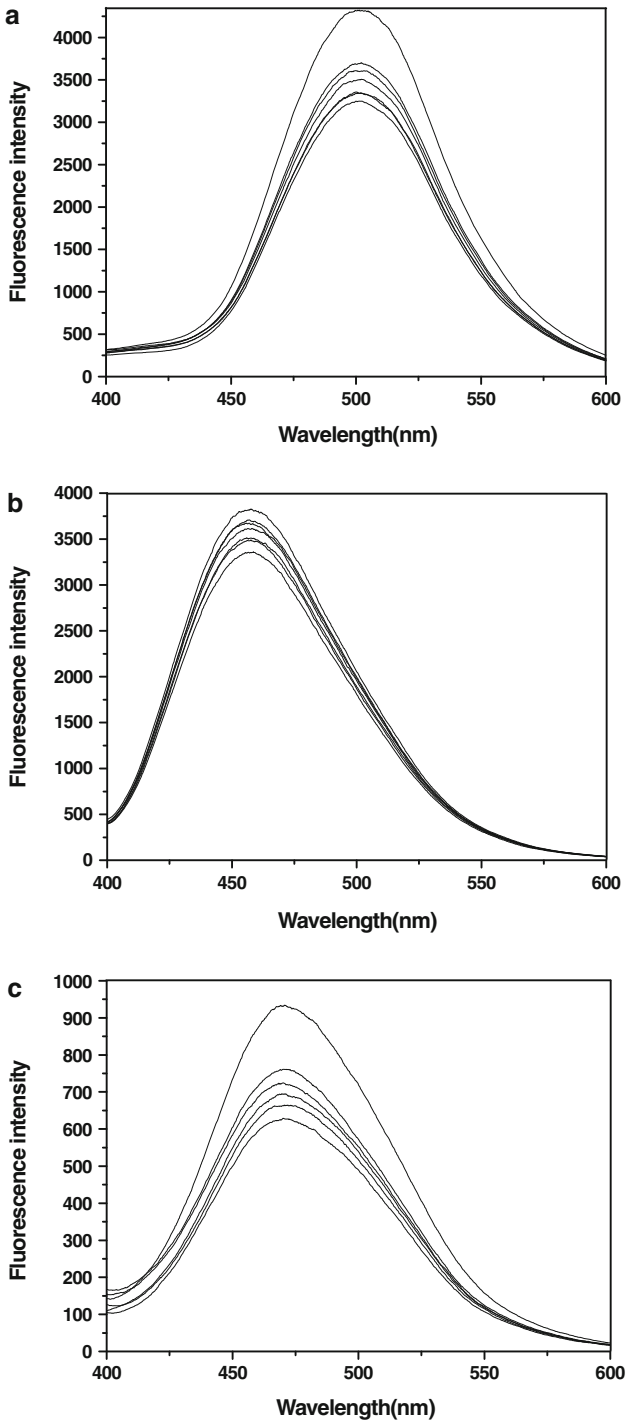

Fig. 3 Fluorescence spectra of Ofloxcain in the presence of HP-β-CD in different pH values and the concentration of HP-β-CD is $1 \sim 6 \times 10^{-3}$ M, **a** pH = 3.05; **b** pH = 7.53; **c** pH = 10.53

Table 1 The constants of inclusion complex between Ofloxacin with CDs

PH	Ofloxacin/HP-β-CD		Ofloxacin/β-CD	
	K	R^2	K	R^2
3.05	1300	0.9991	357	0.9814
7.53	500	0.9834	1640	0.9990
10.53	1171	0.9929	1370	0.9855

Apparatus

UV-757CRT spectrophotometer (Shanghai Precision £ Scientific Instrument Co. LTD), F-4500 fluorescence spectrophotometer (Hitachi), and 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).

Procedure

The concentration of Ofloxacin stock solution is 1.0×10^{-4}mol/L. A volume of 0.1 mL Ofloxacin solution was added into 10 mL volumetric burette, then, CDs (β-CD and HP-β-CD) solution of 1.0×10^{-3}mol/L from 2.00 to 6.00 ml was added into burette one by one and diluted to 10 mL with deionized water. 2 mL of 0.2 mol/L phosphate buffer solution was used to control the pH value of the media. The final mixture solution was dissolved thoroughly

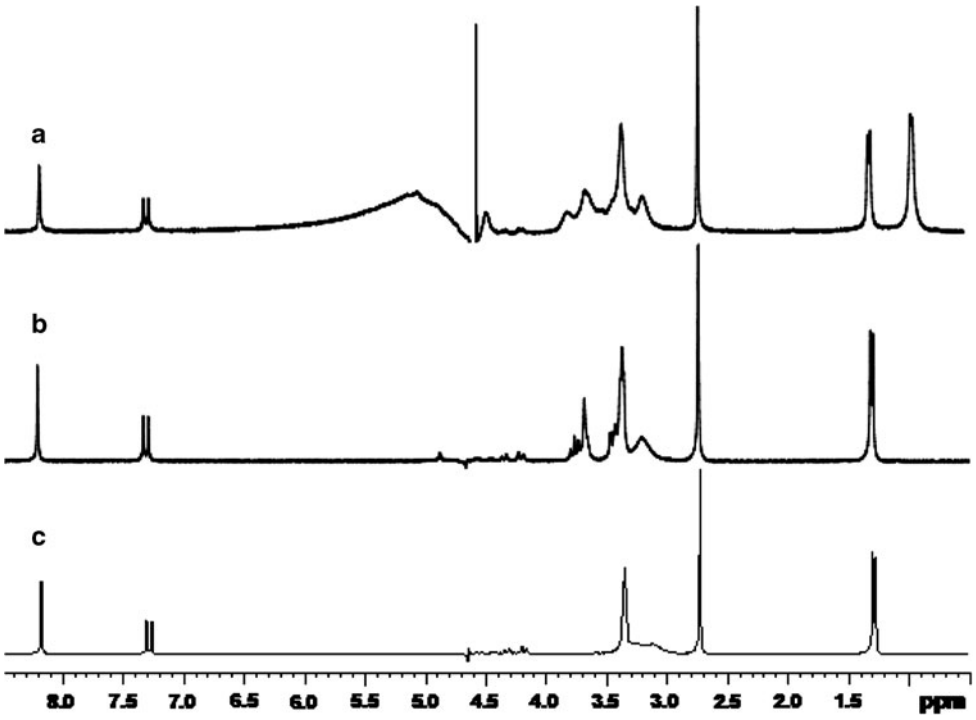

Fig. 4 The ^{1}H NMR spectra of inclusion complexes. **a** HP-β-CD +ofloxcian;**b** β-CD +ofloxcian;**c** ofloxcian

Table 2 The ^{1}H NMR chemical shifts corresponding to Ofloxacin in the absence and presence of CDs in D_2O

Ofloxacin (H)	Ofloxacin (δ0)	β-CD/ofloxacin (δ1)	$\Delta\delta$1	HP-β-CD/ofloxacin (δ2)	$\Delta\delta$2
H-2	8.176	8.191	0.015	8.190	0.014
H-5	7.341	7.330	−0.011	7.331	−0.010
H-1a	4.444	4.455	0.010	4.456	0.012
H-1c	4.197	4.210	0.013	4.208	0.011
H-7a	3.353	3.359	0.006	3.351	−0.002
H-7b	3.172	3.175	0.003	3.170	−0.002
H-7c	3.720	2.719	−0.001	2.719	−0.001
H-1b	1.301	1.306	0.005	1.301	0

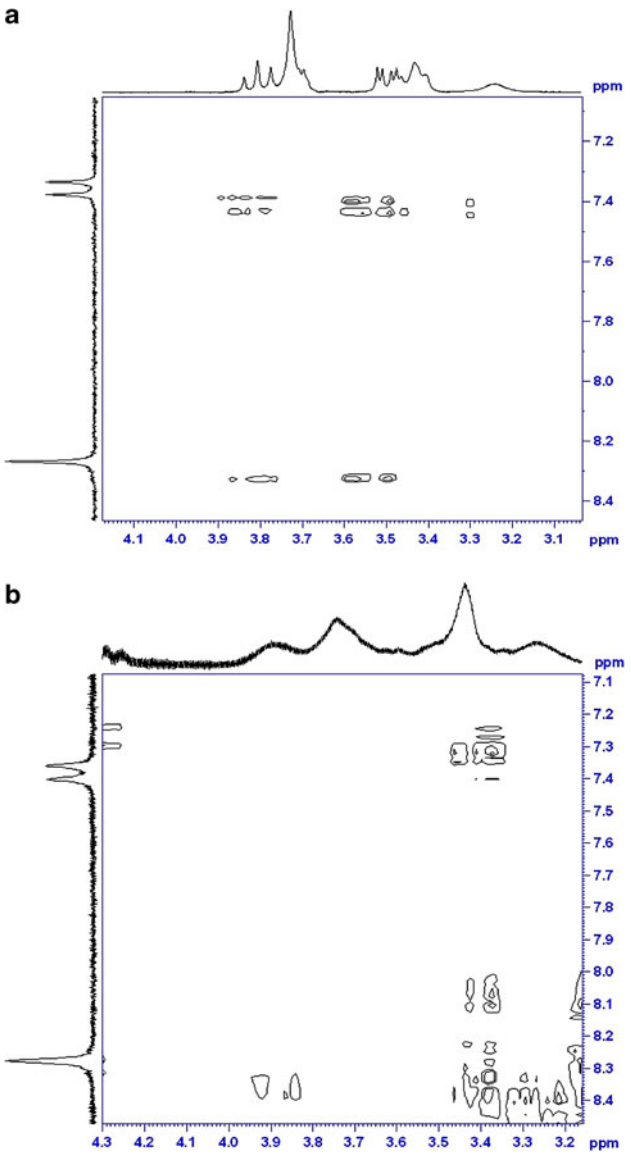

Fig. 5 2D-NMR spectra of the inclusion complexes of Ofloxcain with CDs. **a** β-CD + ofloxcian; **b** HP-β-CD + ofloxcian

under ultrasonic for 30 min, and then equilibrated for 30 min at 20 ± 1 °C. The fluorescence emission was monitored at about 460 nm and the excitation wavelength was 270 nm. Both the excitation and emission slits were set at 2 nm. All measurement of absorption and fluorescence were made against a blank solution treated in the same way but without Ofloxacin in a 1.0 cm quartz cell at different pH.

NMR measurements

1×10^{-4}mol/L Ofloxacin and 1×10^{-4}mol/L CD solutions (β-CD and HP-β-CD) with a volume ratio of 1:1 were mixed thoroughly. With D_2O as solvent, ^1H 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 spectra study

Ofloxacin itself has definite acidity in specifically solution [27], so different pH may affect the inclusion process. Such, the inclusion process of Ofloxacin with CDs were studied at different pH was studied. Figure 2 shows the absorption spectra of Ofloxacin in the absence and presence of HP-β-CD at pH 3.05 at room temperature. The experiments were also performed at pH 6.53 and 10.53. Very similar results were obtained. The maximum absorption wavelength of Ofloxacin is 270 nm. The absorbance of Ofloxacin increased with increasing concentration of HP-β-CD and the absorption peak was slightly shifted, suggesting that a complex was formed between HP-β-CD and Ofloxacin. Similar phenomena were observed for the β-CD.

Fluorescence study

Figure 3 shows that adding HP-β-CD to Ofloxacin solution at different pH resulted in a significant enhancement of the fluorescence signal. These results suggest that the inclusion complex was formed between HP-β-CD and Ofloxacin. The CD cavity provided an apolar environment for the Ofloxacin molecule and the motion of the Ofloxacin molecule in the cavity was largely confined. Thus, the enhanced rigidity of the Ofloxacin molecule and the formation of inclusion complex that reduces the aggregation of Ofloxacin molecules in the solution resulted in an increase of its fluorescence quantum yield. Similar phenomena were observed for the β-CD.

The formation constant (K) and the ratio of the complex (shown in Table 1) were calculated from these data according to the modified Benesi-Hildebrand equation Eq. 1 [28, 29]

$$1/(F - F_0) = 1/([CDs]K\alpha) + 1/\alpha \quad (1)$$

Here, F is observed fluorescence intensity of Ofloxacin sodium solution at each CDs concentration, F_0 represents fluorescence intensity of Ofloxacin sodium solution in the absence of CDs, K is formation constant, α is a constant.

It can be concluded from the Table 1 that HP-β-CD was suitable for the inclusion with Ofloxacin in acidic, and β-CD was suitable for the inclusion in neutral media. That was associated to the molecule dissociation in different pH values. Besides, the good linear relationship obtained when

$1/(F-F_0)$ were plotted against 1/[CDs] supports the existence of a 1:1 complex.

^{1}H-NMR studies

The formation of inclusion complex can be proved from the changes of chemical shift in ^{1}H NMR spectra. Figure 4 illustrated the change of hydrogen atom of Ofloxacin and CDs before and after forming the inclusion complexes. The chemical shifts for the protons of Ofloxacin both in the absence and presence of CDs (β-CD and HP-β-CD) are summarized in Table 2. As can be seen in Table 2, H-1a, H-2 and H-5 of Ofloxacin experienced larger shifts because of the diminished freedom of rotation caused by the penetration of Ofloxacin molecule into the CDs cavity. In contrast, H-7a, H-7b, H-7c and H-1b experienced smaller changes in their chemical shifts, implying that part of the piperazine ring might be outside the CDs cavity. Thus, it can be inferred that the matrix structure of Ofloxacin entered into the cavity of CDs.

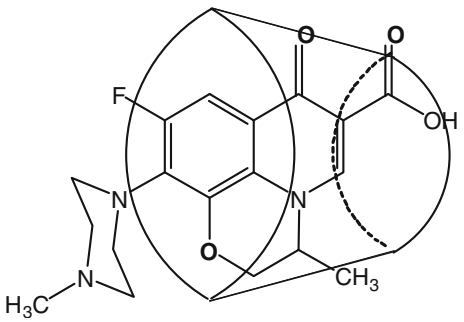

Fig. 6 The molecular model of the inclusion of ofloxcain with CDs

2-D NMR study

In ^{1}H NMR study, a possible orientation of Ofloxacin in cavity is got, but it is not quite definitive. In order to prove the geometry of inclusion compound of Ofloxacin with CDs, a 2D NOESY NMR experiment was used to examine the configuration of Ofloxacin in the CDs cavity. Figure 5 shows a partial contour plot of NOESY spectra of the inclusion complex of Ofloxacin and CDs. There are several intermolecular cross-peaks between H-2, H-5 and H-1a of Ofloxacin and H-3 and H-5 of CDs, which might indicate that matrix construction of the guest molecule was in the center of CDs. Moreover, the interaction observed for H-3 was greater than for H-5 of CDs. These results indicated that matrix of the guest molecule enters into the cavity of CDs from the big ring-edge side of CDs, leaving the piperazine ring out of the CDs cavity (shown in Fig. 6). Combining fluorescence spectra with ^{1}H NMR data, it can be concluded that Ofloxacin is closely included into inner cavity of CDs to form a supramolecular system.

The related inclusion mechanism

The fluorescence character of Ofloxacin was related to the pH value in media. There were three Ofloxacin molecular forms in different pH solution, and the equilibrium of different forms is as follow:

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

Figure 7 shows that in acidic media, the positively charged form of Ofloxacin is predominant; in neutral media, the neutral form is predominant; while in basic media, the negative charged is predominant. The inclusion interactions were based on the cooperation of several weak

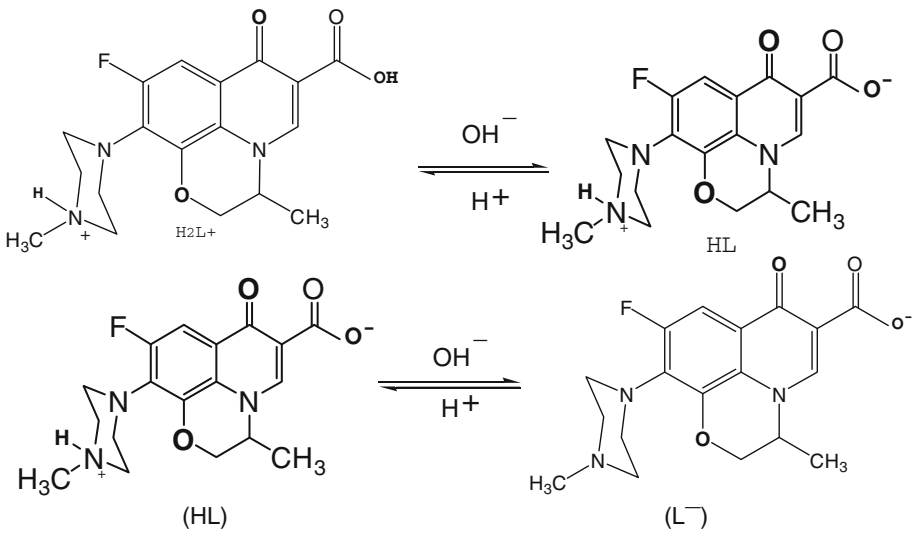

Fig. 7 The equilibrium equation of Ofloxcain in different media

forces working between receptor (CDs) and substrate (Ofloxacin), including dipole–dipole, electrostatic, van der waals, hydrogen bonding, and hydrophobic interaction [30, 31]. The CDs are not charged ($2 < PH < 11$) and the major inclusion interactions are hydrophobic interactions between the guest and the CDs cavity and hydrogen bonding of guest to introduced groups on the CDs ring.

Conclusion

The present study shows that Ofloxcian sodium interacts with CDs and forms a complex. The major factors affecting molecular recognition is size matching, between CDs and guest, and the hydrophobic property of the guest molecule. The binding constants of Ofloxcian sodium in the presence of CDs suggest that in acidic media the HP-β-CD exhibited the strongest inclusion property; in neutral media the β-CD exhibited the strongest inclusion property. The fluorescence results showed that Ofloxacin formed a stoichiometric 1:1 complex with CDs over the concentration range evaluated. In addition, the NMR data suggest proposed structure of the CDs and inclusion complex. In the present study we demonstrated that CDs can be used as guest complexing agent, which acted as substrate reservoir in a dosage-controlled manner.

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