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Preparation and study on the solid inclusion complex of sparfloxacin with β-cyclodextrin

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Abstract

The interaction of sparfloxacin with β-cyclodextrin (β-CD) has been studied by several analytical techniques, including ^{1}H-NMR, ^{13}C-NMR, fluorescence spectroscopy, infrared spectroscopy, thermal analysis, and scanning electron microscope. In this paper, solid inclusion complex of sparfloxacin with β-CD was synthesized by the coprecipitation method. In addition, the characterization of the inclusion complex has been proved by fluorimetry, Infrared, differential scanning calorimetry and 1D, 2D NMR. The experimental results confirmed the existence of 1:1 inclusion complex of sparfloxacin with β-CD. The formation constant of complex was determined by fluorescence method and ^{1}H-NMR. Spacial configuration of complex has been proposed on 2D NMR techniques.

Keywords: Sparfloxacin; β-CD; Inclusion complex; 1D NMR; 2D NMR

1. Introduction

Cyclodextrins (CDs) are polysaccharides made up of six to eight D-glucose monomers connected at the one and four 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. [1–5]. This ability has been widely used in pharmaceutical industries, and has also been used for analytical purposes [6–8]. 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 [9]. 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 [10,11], 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 [12]. 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-radiative decay process of analyte is often significantly attenuate as the fluorescence emission increases [13–15]. 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 [16–18].

High resolution Nuclear Magnetic Resonance is a powerful tool for studying CD complexes [19–24]. This is because NMR techniques can provide not only quantitative information but also detailed information about the geometry of the complex. 2D nuclear overhause effect spectroscopy (NOESY) is one of many NMR tools which has proven to be a powerful technique for investigating intermolecular interaction. The NOESY technique appears to be a potential method for determining the structure of CD complexes.

About solid inclusion complex, common used methods include infrared spectra (IR), differential scanning calorimetry (DSC), X-ray. Many inclusion complexes of guests with CDs were studied by these methods [25,26].

In this work, we not only studied the character of sparfloxacin-β-CD complex by fluorescence, but also prepared its solid complex by coprecipitation method and determining its formation by means of fluorescence, IR, DTC,

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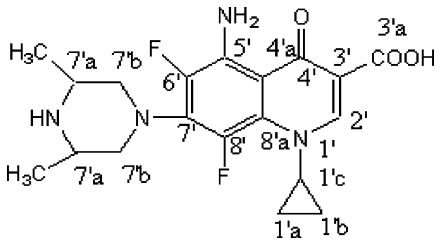

Scheme 1. Structure of sparfloxacin.

scanning electron microscope, ^{1}H-NMR. The formation constant of complex was obtained according to data of fluorescence and ^{1}H-NMR, using modified Benesi–Hildebrand equation. A detailed spacial configuration of the complex has been proposed based on 2D NMR spectra.

According to our examination, we have found one report about inclusion between sparfloxacin and CDs [27]. The contribution of our research lies in providing theoretical bases for developing new drug carriers and new drug forms. We have studied the solid inclusion complex of ciprofloxacin with β-CD [28], β-CD interact with ciprofloxacin on the piperazine ring. However, the similar compound, sparfloxacin interact with β-CD not on the piperazine ring, but on the opposite side (Scheme 1).

2. Experimental section

2.1. Materials

β-CD was purchased from a commercial manufacturer(Yun Nan, China), and was purified by recrystallization in water. Sparfloxacin was kindly provided by Shanxi Pharmaceutical Industry (China), with pharmaceutical grade, D_2O was from Aldrich Chemical Inc.

2.2. Apparatus

Bruker Avance DRX 300 MHz superconducting NMR spectrometer; Shimadzu RF-540 fluorescence spectrophotometer; Shimadzu DT-40 thermal Analyzer; PE FT-1730 Infrared; Hitachis-570 Scanning Electron Microscope.

2.3. Procedure

2.3.1. Fluorescence measurement

Fluorescence measurements were performed by an RF-540 spectrofluorimeter using 1 cm quartz cell, slit width was 10 nm, excitation at 310 nm and fluorescence emission obtained at 420 nm. All experiments were carried out at 25 °C.

2.3.2. NMR measurements

^{1}H-NMR and 2D ROESY NMR of the samples were performed in D_2O, ^{13}C-NMR in DMSO-d_6.

2.3.3. Infrared spectroscopy

Infrared spectra were obtained with FT-1730 Infrared spectroscopy using KBr pelleting. The range of spectra was from 650 to 4000 cm^{-1}.

2.3.4. Differential scanning calorimetry

DSC analyses were carried out in the temperature range from 25 to 400 °C in a stream of nitrogen atmosphere on DT-40 thermal analysis. During experiments, aluminium crucibles were used. Sample weighing (sparfloxacin, β-CD, α-Al_2O_3) were 5 mg. The heating rate was 15 °C min^{-1}, the flow rate of nitrogen atmosphere was 25 ml min^{-1}. α-Al_2O_3 was used as reference.

2.3.5. Microscopic morphological structure measurement

Microscopic morphological structure measurement were performed with S-570 scanning microscope.

2.4. Preparation of solid complex of sparfloxacin with β-CD

Accurately put 0.28375 g β-CD into 30 ml distilled water in 50 ml conical flask and then oscillated this solution enough. After that, 0.098 g sparfloxacin was put into 20 ml distilled water, stirred by electromagnetic stirrer until it was solved. Then slowly poured β-CD solution into stirred sparfloxacin solution, continuously stirred for 12 h at room temperature. The reaction mixture was put into refrigerator for 24 h. At this time, we observed that white crystal precipitated. The precipitate was filtered by G4 sand filter funnel, and washed with distilled water. After dried in oven at 80 °C, for 12 h, the white powder product was obtained. This is inclusion complex of sparfloxacin with β-CD.

3. Results and discussion

3.1. Fluorescence study

Fig. 1 shows that the addition of β-CD to sparfloxacin solution results in a significant enhancement of the fluorescence signal. From the figure, we can see fluorescence signals are enhanced with increasing concentration of β-CD. These data suggest that a stable complex is formed between β-CD and sparfloxacin. The CD cavity provides an apolar environment for the sparfloxacin molecule and thus increases the quantum yield of the fluorescence of sparfloxacin. The formation constant and the ratio of the complex were calculated from these data by use of the modified Benesi–Hildebrand equation (Eq. (1)) [29,30].

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

Here, F is observed fluorescence intensity of sparfloxacin solution at each β-CD concentration, F_0 presents fluorescence intensity of sparfloxacin solution in the absence of β-CD, K is forming constant, α is a constant. By making

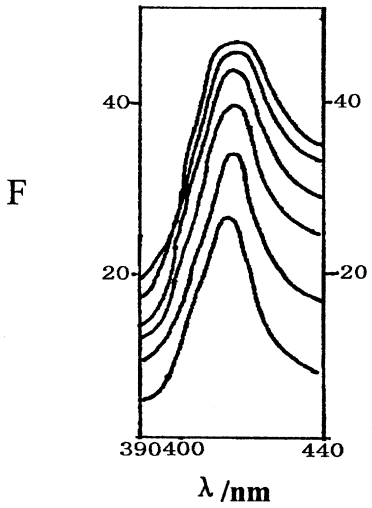

Fig. 1. The fluorescence spectrum of inclusion complex.

a plot of double reciprocal curve of $1/(F-F_0)$ against 1/[β-CD] (see Fig. 2), the calculated formation constant is 0.5×10^2 $(mol/l)^{-1}$, and according to the linear fit of double reciprocal plot, the ratio of the complex is 1:1.

3.2. ^{1}H-NMR studies

Fig. 3 illustrated that most changes of H atoms in chemical shift due to the presence of β-CD.

3.3. ^{13}C-NMR studies

From data of Table 1, we can see that 1′a-C and 2′-C of sparfloxacin have significant upfield changes in the ^{13}C-NMR chemical shift signal in contrast other carbons.

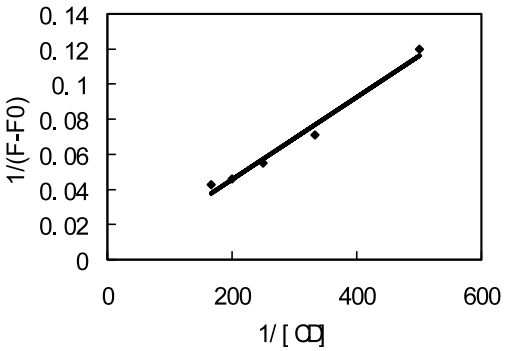

Fig. 2. Double reciprocal curve of 1:1 inclusion complex of sparfloxacin with β-CD.

Such results can be explained by the interaction of sparfloxacin of β-CD. This is consistent with fluorescence study.

3.4. Infrared spectra studies

Compared IR spectra of sparfloxacin, β-CD, and complex of β-CD with sparfloxacin, just as Fig. 4 shows that absorption intensity of C–N group appearing in 1625 cm^{-1} gave rise to changes, the absorption intensity of CN in inclusion complex was weaker than in sparfloxacin, and had a red shift about 8 cm^{-1}, so we can deduce that CN in sparfloxacin was included into cavity of β-CD.

3.5. Differential scanning calometry studies

The DSC curves of sparfloxacin, β-CD and inclusion complex are shown in Fig. 5. From Fig. 5 can shown that DSC curves of inclusion complex with the DSC curves of sparfloxacin and β-CD is different. This proves that the new inclusion complex is formed.

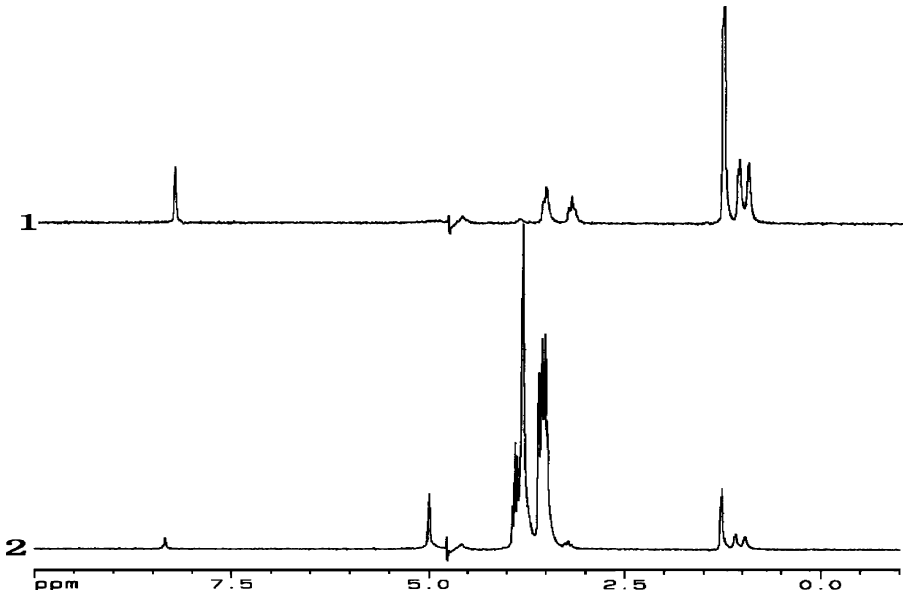

Fig. 3. ^{1}H-NMR spectra (1) sparfloxacin; (2) inclusion complex.

Table 1
Variation of ^{13}C chemical shift of sparfloxacin before and after forming inclusion complex with β-CD

		1′a-C	1′b-C	1′c-C	2′-C	3′-C	3′a-C
Sparfloxacin	δ	41.20	41.00	51.27	128.46	154.81	165.92
Inclusion	δ	41.32	41.07	51.38	128.67	154.88	166.02
Complex	$\Delta\delta$	0.12	0.07	0.11	0.21	0.07	0.10
		4′-C	4′a-C	5′-C	6′-C	7′-C	7′a-C
Sparfloxacin	δ	179.99	150.43	136.77	105.21	137.94	57.69
Inclusion	δ	117.97	150.51	136.73	105.43	137.98	57.44
Complex	$\Delta\delta$	−0.02	0.08	0.04	0.22	0.04	−0.25
		7′b-C	7′c-C	8′-C	8′a-C		
Sparfloxacin	δ	51.27	19.34	134.59	153.22		
Inclusion	δ	51.38	19.16	134.47	152.81		
Complex	$\Delta\delta$	0.11	−0.08	−0.12	−0.41		

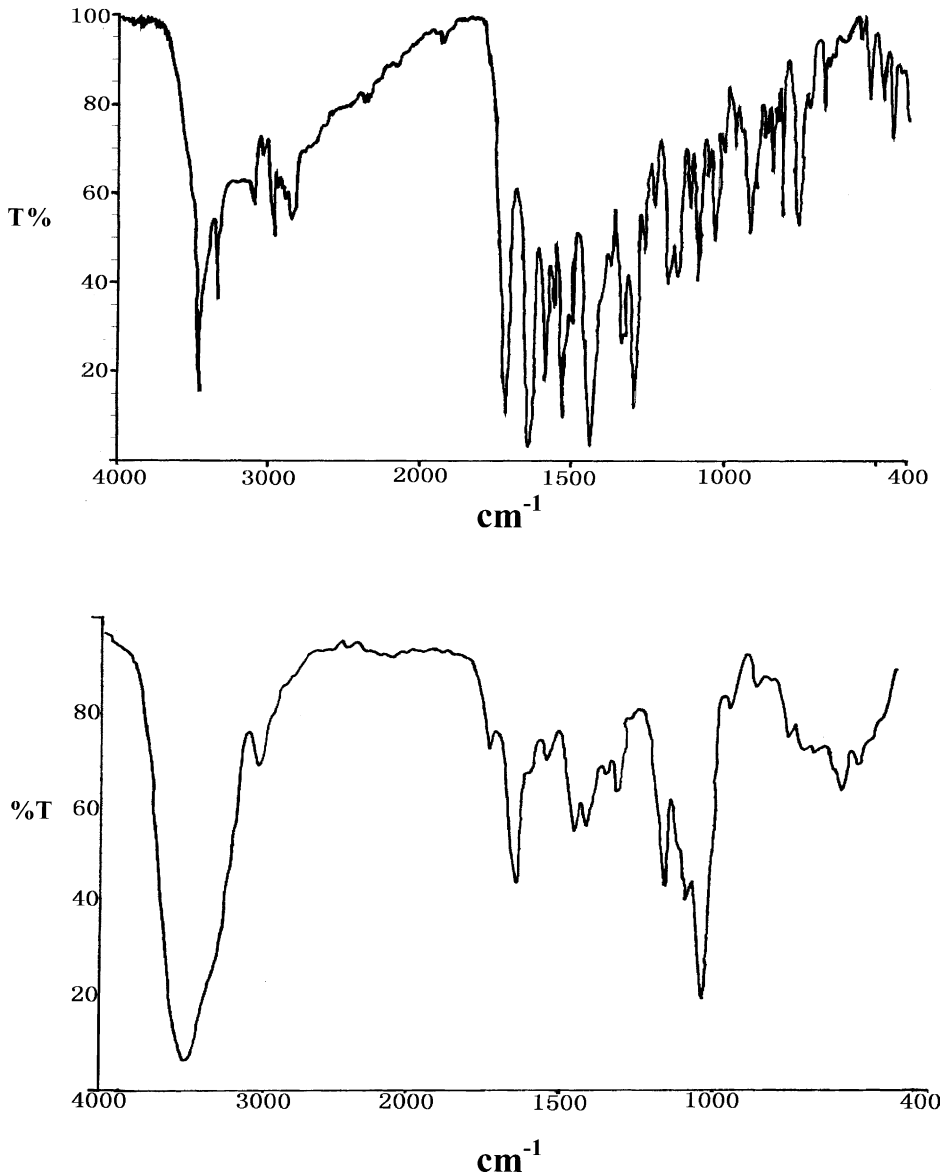

Fig. 4. IR spectra (1) sparfloxacin; (2) inclusion complex.

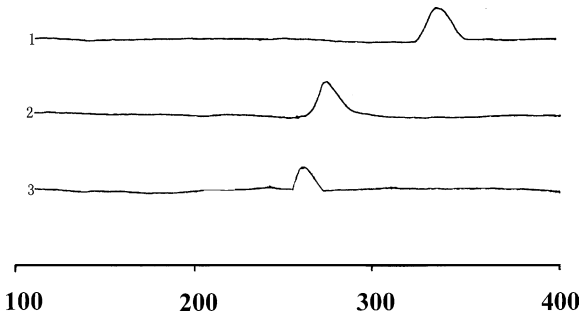

Fig. 5. Thermal spectra curves (1) β-CD; (2) sparfloxacin; (3) inclusion complex.

3.6. Microscopic morphological observation

First we observed crystal formation of sparfloxacin and β-CD by scanning electron microscope, then also we observed crystal formation of inclusion complex (Fig. 6). Pictures clearly elucidated the difference of crystal state of each other. Modification of crystallization can be assumed as a proof of the formation of new inclusion complex.

3.6.1. 2D NMR study

In the 1D proton NMR study, we discussed a possible orientation of the sparfloxacin in the cavity. However, that orientation is not definitive. In order to prove the geometry of inclusion compound of sparfloxacin with β-CD, we use a 2D ROESY NMR experiment to examine the configuration of sparfloxacin in the β-CD cavity. Fig. 7 shows a contour plot of a section (3–4 ppm) of the ROESY spectrum of sparfloxacin and β-CD complex. From Fig. 8 the cross peak is found between H-2′ on the sparfloxacin with H-5 on the β-CD, at the same time, the H-1′b relationship of the sparfloxacin with H-3 on the β-CD can also be seen.

Put information coming from ^{1}H-NMR together, we draw a conclusion: sparfloxacin interact with β-CD cavity not on the piperazine ring, but on the opposite side, and formed a super molecular system, which was suitable for interinfluence between β-CD and sparfloxacin (van der Waals force and dipole–dipole interaction). Based on the 2D ROESY NMR spectra, the proposed structure of inclusion complex of sparfloxacin with β-CD is presented in Fig. 8.

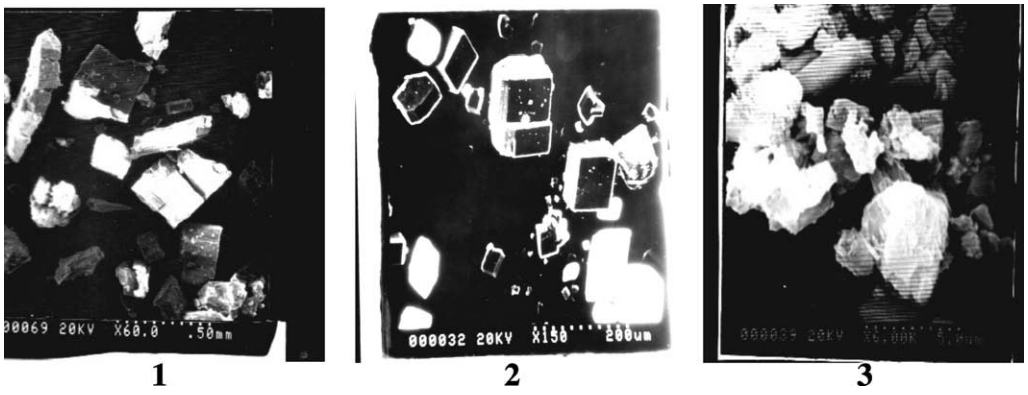

Fig. 6. Scanning electron microscope photographs (1) β-CD; (2) sparfloxacin; (3) inclusion complex.

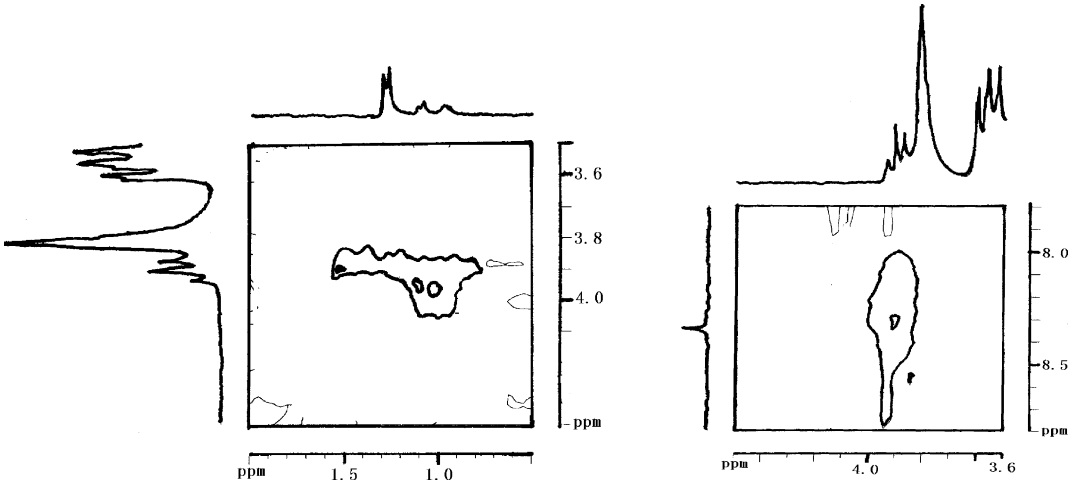

Fig. 7. ROESY spectrum inclusion complex.

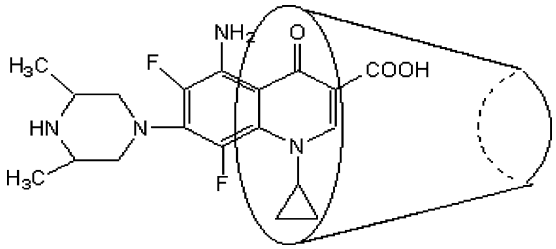

Fig. 8. Proposed structure of the β-CD and inclusion complex.

4. Conclusion

The present study shows that sparfloxacin interacts with β-CD and forms a complex. The enhancement of the fluorescence intensity of sparfloxacin in the presence of β-CD suggests that a certain fraction of sparfloxacin molecules in solution are in a more hydrophobic environment, which is inside the β-CD cavity. In addition, the fluorescence spectroscopy and ^{1}H-NMR data results suggest the formation of a stable 1:1 stoichiometric complex of β-CD:sparfloxacin in solution. Furthermore, the formation constant for the complex is estimated by using of fluorescence and proton NMR data. The structure of inclusion complex of the sparfloxacin with β-CD is suggested by the use of the 2D ROESY NMR technique. The guest/host binding is mainly due to dipole interaction, hydrophobic interaction and van der Waals forces. The sum of the interaction can be represented by the ROESY cross peaks, not only qualitatively but also quantitatively. Furthermore, the sparfloxacin: β-CD complex information obtained here may also be used to mimic the interactions of the sparfloxacin molecule with proteins.

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