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Investigation of inclusion complex of miconazole nitrate with β-cyclodextrin

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Abstract

Inclusion complex of miconazole nitrate with β-cyclodextrin (β-CD) was prepared by the coprecipitation method. The stability constant K_f and ratio of inclusion complex, as well as the thermodynamic parameters of inclusion reaction were determined by phase solubility method. The inclusion complex was characterized by IR spectra, DSC and X-ray diffraction. It was testified that the inclusion complex was formed between β-CD and miconazole nitrate and found that the benzene ring of miconazole nitrate entered into the cavity of β-CD, meanwhile leaving the *N*-ring outside.

Keywords: Miconazole nitrate; β-Cyclodextrin; Phase solubility; Inclusion complex; DSC

1. Introduction

Recently, much interest has been concentrated on cyclodextrins (CDs), which are cyclic glucose oligomers having six, seven or eight glucose units, linked by 1,4-α-glucosidic bonds and called, respectively, α-, β- and γ-cyclodextrin. They have the ability to form inclusion complexes with a wide variety of organic compounds, which enter partly or entirely into the relatively hydrophobic cavity of CDs, simultaneously expelling the few high-energy water molecules from inside (Vaios, Karathanos, & Mourtzinos, 2007). The size of the cavity of the cyclodextrin allows selectively for the complexation of guest molecules, therefore the CDs can act as molecular encapsulants (Brewster, Simphins, & Hora, 1989; Higuchi & Connors, 1965; Jimenez Sanchez, 1997; Nakajuma & Hirobasbi, 1984; Shuang, Guo, & Pan, 1998). The physical, chemical and biological properties of molecules, which are encapsulated by CDs, may be drastically modified, such as masking or reducing undesired taste, increasing the aqueous solubility and improving the shelf life of encapsulated substrate, and protecting sensitive substrates from oxidation, light-induced decompositions and heat-induced changes, and so on (Szente & Szejtli, 2004). Due to these advantages, CDs have been used extensively during the last decades in the cosmetics, food and pharmaceutical industry. In recent years, some researchers in textile industry have also taken advantage of CDs to prepare the fragrant fabrics and have gained the desirable results (Buschmann & Knittel, 2001; Hara, Mikuni, Hara, & Hashimoto, 2002; Martel, Morcellet, & Ruffin, 2002; Yong & Zhu, 2006).

Miconazole nitrate is a kind of safety, high-efficiency and broad-spectrum antibiotic external drug, which performs the functions such as killing bacteria, diminishing inflammation and relieving tickle, etc (National pharmacopeia committee, 2006) (Fig. 1). In this paper, the inclusion complex of miconazole nitrate with β-CD was prepared. The stability constant and ratio of inclusion complex of miconazole nitrate with β-CD were determined by phase solubility method. In addition, the characteristics of the inclusion complex were tested by means of IR spectra, X-ray diffraction and DSC.

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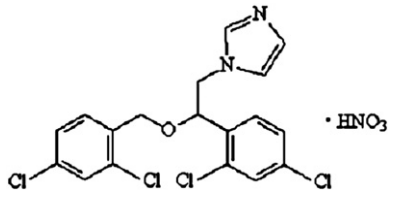

Fig. 1. The structure of miconazole nitrate.

2. Experiment

2.1. Materials

β-CD was purchased from Shanghai chemical reagent Co. Ltd (Fig. 2). miconazole nitrate was kindly provided by Zhejiang Shengda Pharmaceutical Co. Ltd. All other reagents were of analytical grade. The water used was double-distilled and deionized.

2.2. Apparatus

NETZSCH 204F1 Differential scanning calorimetry (DSC) (NETZSCH Corporation, Germany); NICOLET-380 Fourier Transform infrared spectrophotometer (FTIR) (Thermo Nicolet Corporation, USA); X-ray Diffractometer (Rigaku Corporation, Japan); Shimadzu-3000 ultraviolet spectrophotometer (UV-3000) (Shimadzu Corporation, Japan); DZF-6020 vacuum dryer and sonicator (precision apparatus factory, Shanghai, China).

2.3. Procedures

2.3.1. Phase solubility studies

Phase solubility studies were carried out according to the method described by Higuchi and Connors. An excess amount of miconazole nitrate was mixed in a series of phosphate sodium buffer solutions (pH = 6, 7, 8, 9) containing increasing amounts of β-CD, dispersing by sonicator for 30 min, and then oscillating by a laboratory stirrer for 24 h at room temperature. The degree of absorption in solution was measured by UV/visible spectrophotometer at 258 nm after equilibrating, and converted into the concentration of miconazole nitrate by using a calibration absorption curve. Before the measurement the samples were filtered with syringe through a 0.45um PVDF filter. The experiments were carried out in triplicate for each buffer. The phase solubility diagram was plotted by using β-CD concentration as x-coordinate and miconazole nitrate concentration as y-axis. The calibration curve of miconazole nitrate is shown in Fig. 3.

The stability constants, K_f, were calculated from the straight-line portion of the phase solubility diagram according to the Higuchi-Connors equation (Eq. (1)):

$$K_f = A/[B(1-A)] \quad (1)$$

A is slope and B is intercept, which could be obtained from the straight-line of the phase solubility diagram.

2.3.2. Preparation of the physical mixture

A physical mixture consisting of miconazole nitrate and β-CD in the same weight ratio as the inclusion complex was prepared. miconazole nitrate and β-CD were admixed together in a mortar and pestle for 5 min to obtain a homogeneous blend.

2.3.3. Preparation of the inclusion complex

Miconazole nitrate dissolved by ethanol was dispersed in phosphate sodium buffer with β-CD (molecular ratio of miconazole nitrate/β-CD was 1:1) and mixed in a laboratory stirrer for 24 h at room temperature and then inclusion complex solution was prepared. The suspension was filtered through a 0.45 μm PVDF filter and the solid inclusion complexes were obtained by vacuum drying the filter.

2.3.4. Characterization

Four different types of samples were used: β-CD alone, miconazole nitrate alone, physical mixture and inclusion complex, to identity difference in IR spectra, DSC curves and X-ray diffraction. DSC studies were performed to confirm the inclusion formation in the solid state. Samples of about 5 mg, placed in a DSC pan, were heated from 20 °C to 215 °C at a scanning rate of 10 °C/min, under a constant flow of dry nitrogen. The X-ray diffraction patterns of the four different samples were measured with a D/max-2550PC X-ray Diffractometer, which used Cu–K target at 40 kv, 300 Ma, $\lambda = 1.542$ Å. Infrared absorption spectra of four types of samples were obtained using

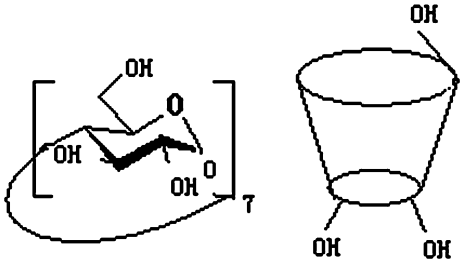

Fig. 2. The structure and sketch of β-CD.

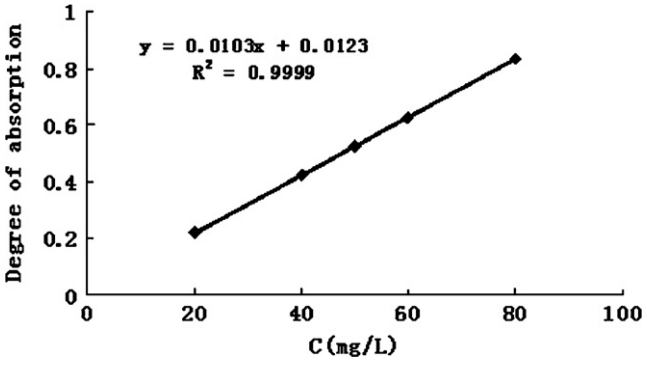

Fig. 3. The calibration absorption curve of miconazole nitrate.

NICOLET-380 FTIR spectrophotometer. The samples were pressed with KBr into a pellet before measuring the infrared absorption spectra.

3. Results and discussion

3.1. Phase solubility studies

3.1.1. Phase solubility graph and the stability constant

Phase solubility studies were carried out at room temperature in different four buffers to calculate the solubility constants K_f, The phase solubility diagrams of miconazole nitrate with β-CD are shown in Fig. 4.

In Fig 4, the plot at each of the buffers shows a linear trend, therefore, all of them can be considered as AL-type diagrams (Higuchi and Connors, 1965), which indicates to form 1:1 inclusion complex in systems.

The stability constants, K_f, of the complexes of miconazole nitrate with β-CD at different four buffers as calculated using Eq. (1) are given in Table 1.

From Table 1, K_f decrease with increasing pH is observed, indicating that the ability of inclusion between miconazole nitrate and β-CD is larger at lower pH. However, there is a conflict phenomena between Fig. 4 and Table 1, i.e., the solubility of miconazole nitrate improves with increasing pH, whereas the K_f decrease, which may be the hydrolysis of miconazole nitrate at a higher pH, and need further to study.

3.1.2. Effect of temperature on inclusion reaction

Temperature is an important parameter in thermodynamics study. Fig. 5 shows the effect of temperature on inclusion reaction.

It is obvious that the K_f decreases with temperature up, revealing the inclusion reaction between miconazole nitrate and β-CD was an exothermic process. Therefore, the inclusion complex was suitable to be prepared at lower temperature.

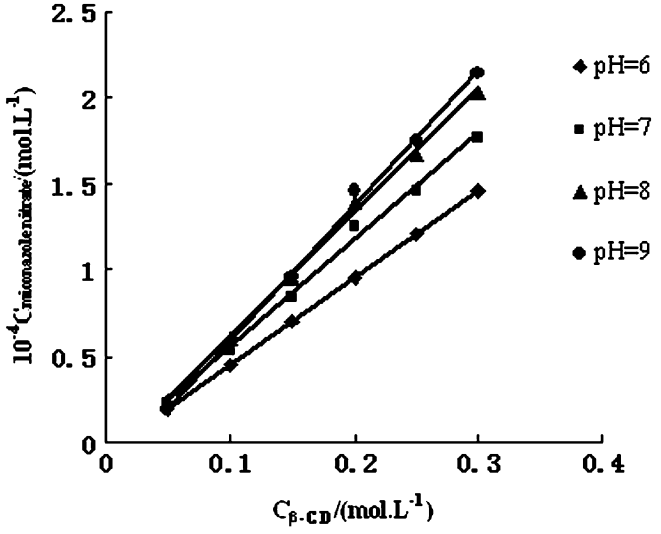

Fig. 4. Phase solubility study of miconazole nitrate with β-CD in different pH buffers (25 °C).

Table 1
The stability constant K_f, of miconazole nitrate at different pH (25 °C)

pH	Linear equation	R^2	K_f (L mol^{-1})
6.0	$Y = 5.04 \times 10^{-4}$c-5.20×10^{-6}	0.9999	97
7.0	$Y = 6.21 \times 10^{-4}$c-6.87×10^{-6}	0.9946	82
8.0	$Y = 7.21 \times 10^{-4}$c-1.11×10^{-5}	0.9975	65
9.0	$Y = 7.88 \times 10^{-4}$c-2.00×10^{-5}	0.9969	39

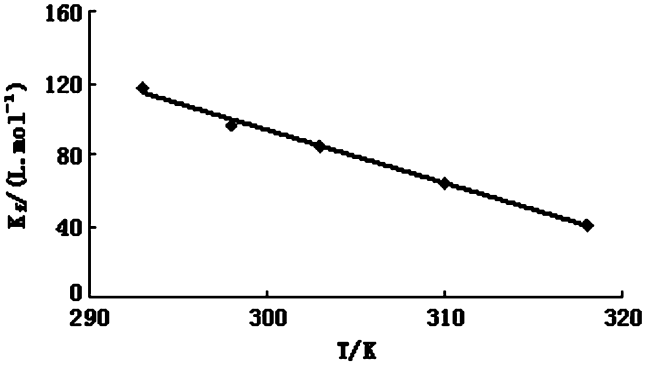

Fig. 5. Effect of temperature on the stability constant of inclusion reaction (pH = 6).

3.1.3. Change of entropy and free energy of the reaction

The phase solubility data can also give additional information, such as the thermodynamic parameters involved in the complex formation. The integrated form of the Van't Hoff equation (Eq. (2)) permits the calculation of the values of enthalpy and entropy changes, depending on the variations of the stability constants with temperature.

$$\ln K_f = -\Delta H/RT + \Delta S/R \quad (2)$$

The Van't Hoff plot for the complex of miconazole nitrate/β-CD is a linear function between $\ln K_f$ and the inverse of the absolute temperature ($1/T$), as shown in Fig. 6.

From the intercept of Fig. 6, the entropy changes (ΔS) could be calculated directly using Eq. (2). According to

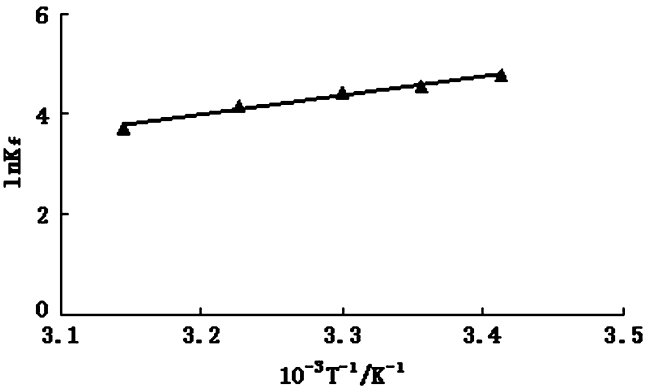

Fig. 6. The relationship between ln K_f and $1/T$.

Table 2
The effect of temperature on K_f, ΔH, ΔS and ΔG of the inclusion complex of miconazole nitrate with β-CD

T (K)	K_f (L mol^{-1})	ΔS (J mol^{-1} K^{-1})	ΔH (kJ mol^{-1})	ΔG (kJ mol^{-1})
293	117	−69.60	−31.99	−11.60
298	96	−69.60	−32.34	−11.31
303	85	−69.60	−32.69	−11.20
310	63	−69.60	−33.18	−10.71
318	41	−69.60	−33.73	−9.8

Eqs. (3) and (4), the enthalpy changes (ΔH) and the Gibbs free energy changes (ΔG) were calculated and given in Table 2.

$$\Delta G = -RT \ln K_f \quad (3)$$

$$\Delta H = \Delta G + T \cdot \Delta S \quad (4)$$

The negative values of ΔH indicate that the interaction processes between miconazole nitrate and β-CD are exothermic. ΔH is relatively small and in relation to some typical low energy interactions including: hydrophobic interactions, due to the displacement of water molecules from the cavity of the β-CD; increase of van der Waals interactions between the molecules; formation of hydrogen bonds, etc. ΔS are also negative in these processes. The reason may be that inclusion reaction causes a decrease in translational and rotational degrees of freedom of the encapsulated molecules, giving a more ordered system. These results confirm further that the inclusion of miconazole nitrate with β-CD has occurred. The negative value of ΔG displays that the inclusion process is a spontaneous one.

3.2. Characterization of inclusion complex

3.2.1. DSC analysis

Fig. 7 shows the DSC results of four different types of samples: β-CD alone, miconazole nitrate alone, physical mixture and inclusion complex.

The DSC thermogram of β-CD shows an endothermic peak at 186 °C (Fig. 7a), possibly due to elimination of the high-energy water in the cavity of β-CD. The DSC curve of miconazole nitrate (Fig. 7b) shows an endothermic peak at 142 °C, which corresponds to the melting point. The DSC scan of the physical mixture (Fig. 7c) appears two endothermic peaks, one of which is nearly identical to that of pure miconazole nitrate at approximately 142 °C, the other corresponds to that of β-CD, showing that no inclusion reaction has occurred by physical blends. However, the thermogram of the inclusion complex does not show the endothermic peak assigned to miconazole nitrate at 142 °C (Fig. 7d), which indicates that an inclusion complex between miconazole nitrate and β-CD was produced.

3.2.2. X-ray diffraction

The X-ray diffraction spectra of four different samples are shown in Fig 8. There is no obvious peak in the

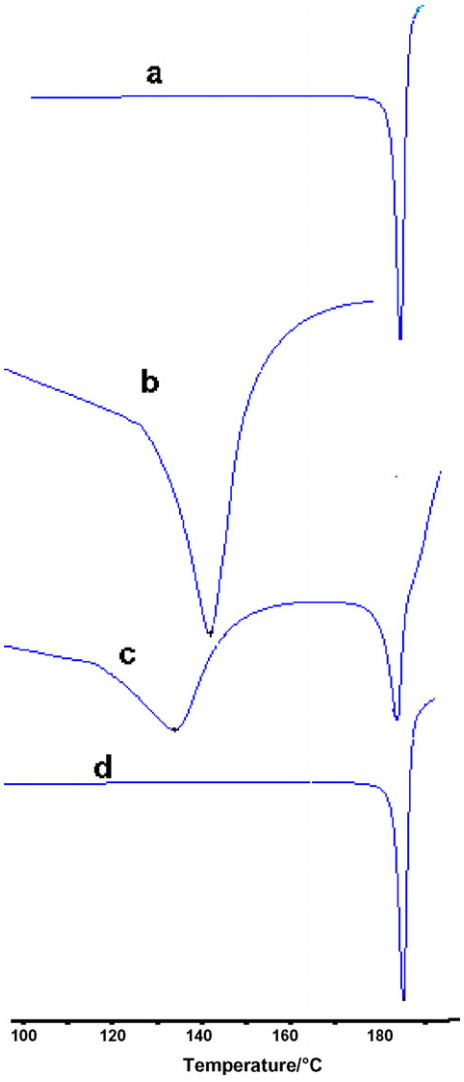

Fig. 7. DSC curve of: (a) β-CD, (b) miconazole nitrate, (c) physical mixture, (d) inclusion complex.

X-ray diffraction spectrum of the pure β-CD (Fig. 8a). A sharp diffraction peak appears in spectra of both miconazole nitrate (Fig. 8b) and physical mixture (Fig. 8c), whereas the peak disappears in the spectrum of the inclusion complex (Fig. 8d). This phenomenon once again verifies the inclusion formation between β-CD and miconazole nitrate.

3.2.3. IR spectra

Fig. 9 presents the IR spectra of four different types of samples. As seen in Fig. 9, the IR absorption peak of the physical mixture is simple superposition of the peak of CD and that of miconazole nitrate, whereas the spectra of the inclusion complex and that of the CD are pretty alike. The result is the same as that of X-ray diffraction mentioned above. This also testifies that the miconazole nitrate have entered into the cavity of CD and formed inclusion complex.

It is well known, the absorption bands of benzene ring and hybrid ring are located at 1600–1500 cm^{-1} and 1300–1500 cm^{-1}, respectively. There are absorption peaks at

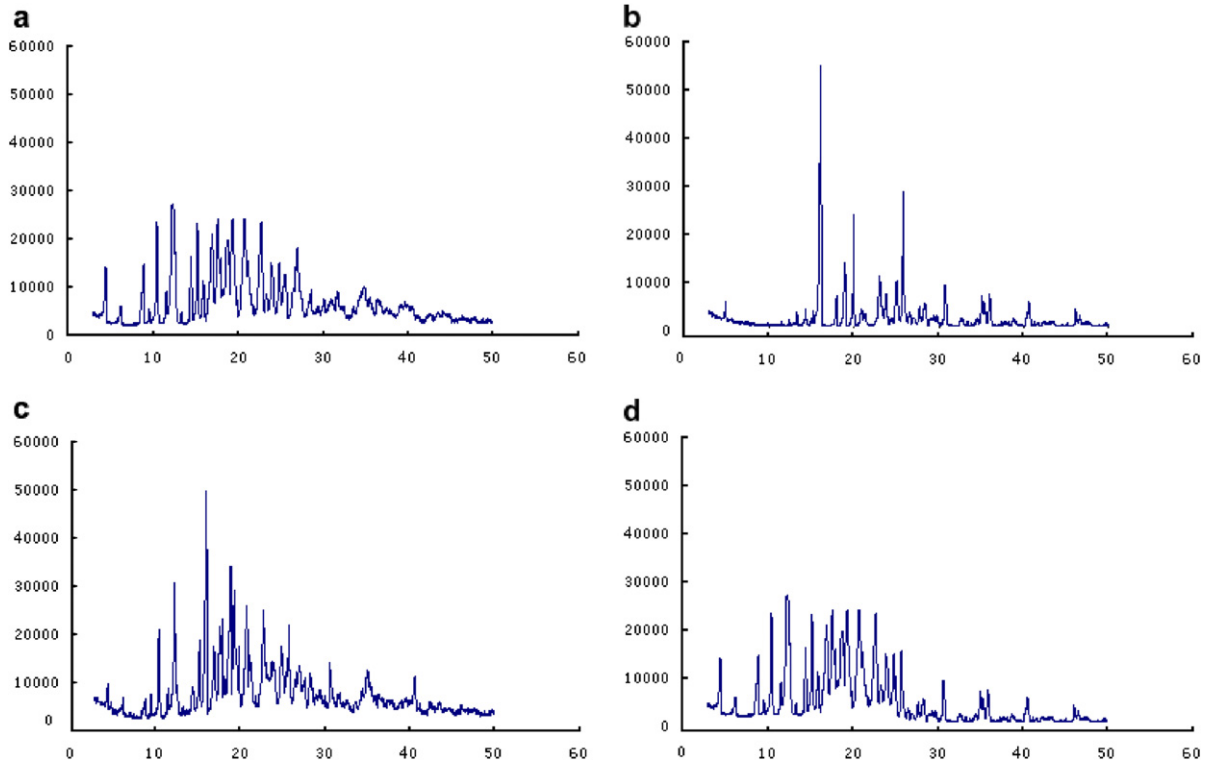

Fig. 8. X-ray diffraction spectra of: (a) β-CD, (b) miconazole nitrate, (c) physical mixture, (d) inclusion complex.

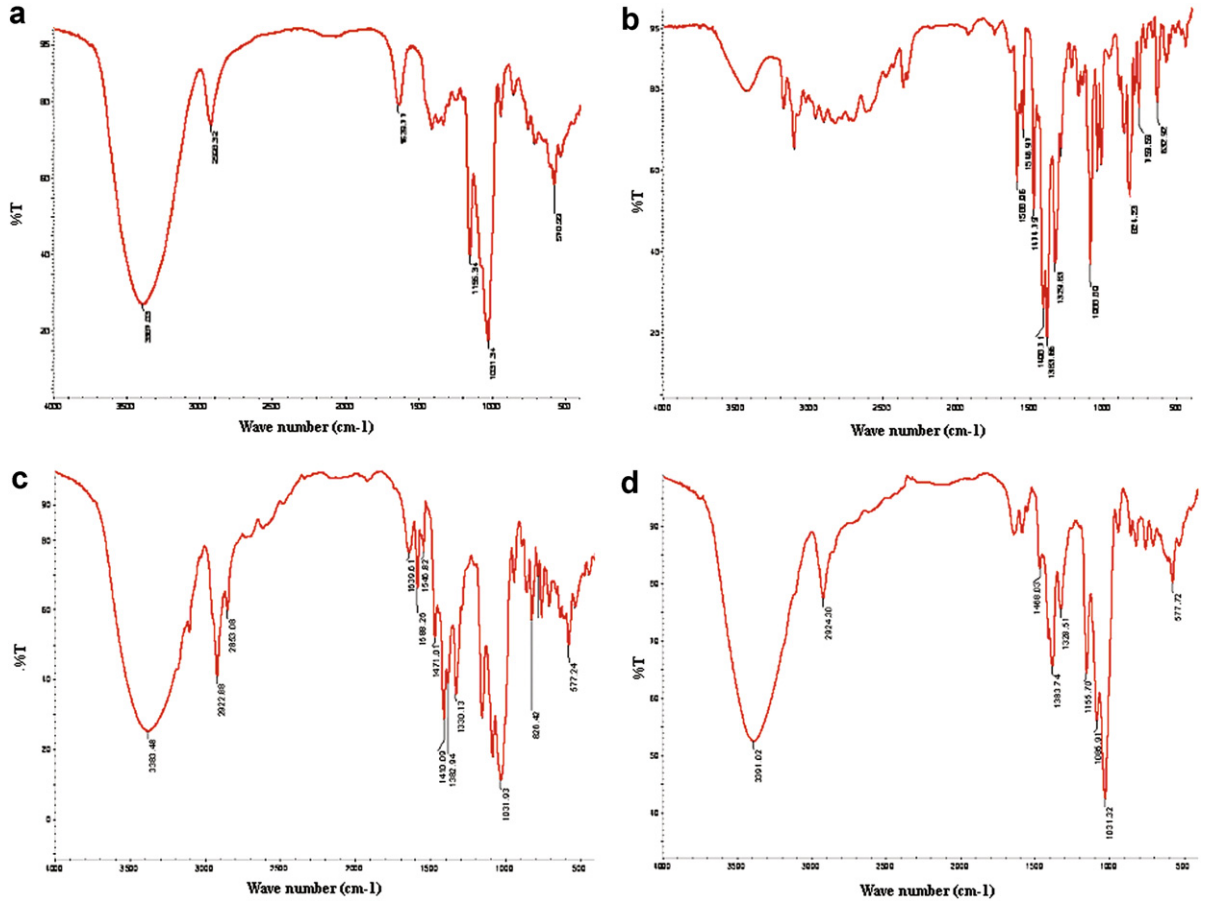

Fig. 9. IR spectra of: (a) β-CD alone, (b) miconazole nitrate alone, (c) physical mixture, (d) inclusion complex.

1474 cm^{-1}, 1410 cm^{-1}, 1383 cm^{-1} and 1329 cm^{-1} in the IR spectra of miconazole nitrate (Fig. 9b), the physical mixture (Fig. 9c) and the inclusion complex (Fig. 9d), while the peaks at 1588 cm^{-1} and 1546 cm^{-1} disappear in the inclusion complex, which certifies that the benzene ring of miconazole nitrate enters into the cavity of CD, meanwhile leaving the *N*-ring outside.

4. Conclusion

Miconazole nitrate was encapsulated by β-CD, forming an inclusion complex. The ratio of inclusion complex was valued by phase solubility method as 1:1, and the thermodynamic parameters of inclusion reaction were also determined. The result showed that the inclusion process was a spontaneous one. Its structure was characterized by IR spectra, DSC and X-ray diffraction, which all verified the inclusion complex formation between β-CD and miconazole nitrate. These data will offer us the first-hand information to develop the skin-care textiles in our subsequent research.

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References

Brewster, M. E., Simphins, J. M., & Hora, M. S. (1989). *Journal of Parenteral Science and Technology, 43*, 231.

Buschmann, H. J., & Knittel, D. (2001). New textile applications of cyclodextrins. *Journal of Inclusion Phenomena and Macrocyclic Chemistry, 40*, 169–172.

Higuchi, T., & Connors, K. A. (1965). *Advances in Analytical Chemistry and Instrumentation, 4*, 117.

Jimenez Sanchez, J. C. (1997). A new method for determining the composition and stability constant of complex of the form AmBn. *Analytical Chimica Acta, 90*, 223–231.

Hara, K., Mikuni, K., Hara, K., & Hashimoto, H. (2002). Effects of cyclodextrins on deodoration of "aging odor". *Journal of Inclusion Phenomena and Macrocyclic Chemistry, 44*, 241–245.

Martel, B., Morcellet, M., & Ruffin, D. (2002). Capture and controlled release of fragrances by CD finished textiles. *Journal of Inclusion Phenomenon and Macrocyclic Chemistry, 44*, 439–442.

Nakajuma, T., & Hirobasbi, M. (1984). Complex between cyclodextrins and bencylane in aqueous solution. *Chemical & Pharmaceutical Bulletin, 32*(2), 383.

National pharmacopeia committee. (2006). The pharmacopeia for the People's Republic of China (p. 3984). Beijing, China. Chemical Industry Publishing Company.

Shuang, S. M., Guo, J. Y., & Pan, J. H. (1998). *Chinese Journal of Analytical Chemistry, 26*, 564.

Szente, L., & Szejtli, J. (2004). Cyclodextrins as food ingredients. *Trends in Food Science and Technology, 4*(15), 137–142.

Vaios, T., Karathanos & Mourtzinos, Ioammis (2007). Study of the solubility, antioxidant activity and structure of inclusion complex of vanillin with b-cyclodextrin. *Food Chemistry, 101*, 652–653.

Yong, G. L., & Zhu, J. F. (2006). The preparation of inclusion complex of ipriflavone and b-cyclodextrin. *Chinese additional machine leader, 8*, 817–818.