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Studies on inclusion effect of andrographolide /β-cyclodextrin

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ABSTRACT

Objective: Studies on inclusion effect of andrographolide and β-cyclodextrin. **Method:** The inclusion compound of andrographolide-β-cyclodextrin was prepared by saturated solution method assist with microwave, The inclusion compound was confirmed by the DTA ▪ AFT-IR and 1H-NMR. **Result:** The result indicated that andrographolide and β-cyclodextrin formed inclusion compound. **Conclusion:** The andrographolide could be inclused by β-cyclodextrin and the inclusion effect was better.

KEYWORDS

Andrographolide;
β-cyclodextrin;
Inclusion complex;
DTA;
^{1}H-NMR;
IR.

INTRODUCTION

Andrographis Paniculata (Burm.f.) Nees belong to Acanthaceae, and use the dried aerial parts as medicine in China, It is used extensively as an antipyretic and antivirus medicine for a long time[1,2], it's newly pharmacological activities were found, such as immune activity, antitumor effect, hepatoprotective activity, inhibitory effect on platelet aggregation, antihypotensive effect, antifertility activity and antihyperglycemic effect. It's main component is diterpene lactams which is represented by andrographolide (A).

However, andrographolide could not be used as a drug due to its poor water solubility, which prevents it from being absorbed well in the body. In addition, it is unstable towards oxygen. These disadvantages limit the application of andrographolide.

In pharmaceutics, *β*-cyclodextrin (*β*-CD) is used to increase solubility and stability and to reduce the toxicity of drugs[3]. Currently the widely used methods to prepare inclusion compounds are coprecipitation, kneading, freeze-drying and cogrinding[4]. According to a very recent report, carrying out the inclusion reactions using microwave heating, as opposed to conventional methods, has the major advantages of shorter reaction times and higher yield of products In the present work, we prepared the inclusion compound of andrographolide /*β*-CD under microwave irradiation.

The product was characterized by FTIR spectroscopy. Furthermore, since the structural characterization is of particular significance for supramolecular host-guest complexes, we also investigated the structure of andrographolide /*β*-CD by ^{1}H-NMR. In addition, the thermal stability of the inclusion complex was studied by DTA.

MATERIALS AND METHODS

Materials

β-CD (99.5%, Mengzhou Huaxing Co., Ltd, China) was purified by recrystalliz- ation from distilled water. andrographolide was extracted from Andrographis paniculata Nees in our laboratory. (Its structure was identified by ^{1}H-NMR, ^{13}C-NMR, FT-IR, MS, its purity is 99.8% detected by HPLC) The ^{1}H-NMR measurements were carried out in DMSO-d6 (Aldrich Co.) solutions. Other chemicals were of analytical reagent grade purity.

Instrumental methods

Differential thermal analysis (DTA) measurements were obtained using a EXSTAR 6000 (Seiko Instruments Inc). The experiments were performed at a linear heating rate of 10°C/min in the temperature range from 25°C to 400°C. Infrared spectra were recorded with a Nicolet AVATAR 360, FT-IR using the KBr disc method. ^{1}H-NMR, were recorded on a Bruker Advance 400 NMR spectrometer in DMSO-d6. Microwave irradiation was carried out with a Sanle WP 750-1 Microwave Oven (Nanjing, China) under atmospheric pressure.

Preparation of the inclusion complex of β-CD with andrographolide

A mixture of 0.2237g (0. 2 mmol) β-CD and 0.0352g (0. 1 mmol) andrographolide was ground in a glass container. Minimum amounts of solvents (ethanol/ water = 1:1 v/v) were added. The mixture was reacted for 90s at middle step in the microwave oven. After the reaction was complete, adequate amounts of solvents were added to remove the residual β-CD and andrographolide, then the precipitate was filtered and the inclusion complex was thus obtained.

RESULTS AND DISCUSSION

DTA measurements

Differential thermal analysis (DTA) were performed to investigate the thermal stability of andrographolide / β-CD. Figure 1 (2) shows the DTA curves of andrographolide. In the DTA curve, an endothermic behavior was observed at 236°C. Coupled with the melting point of andrographolide (236°C), we can conclude that this point represents a phase change of andrographolide from the solid state to the liquid state.

The DTA curves of β-CD are shown in Figure 1 (1). Undoubtedly, water was evolved beginning at about 87.7°C, An endothermic behavior was observed at 307°C, And the melting point of β-CD is this.

The DTA curves of physics mixture are shown in Figure 1 (3). From this Figure we can see two endothermic behavior were observed at 236°C, A307°C. These were the melting point of andrographolide and β-CD.

The DTA curves of andrographolide -β-CD inclusion compound are shown in Figure 1 (4). From this Figure we can see an endothermic behavior was observed at 301°C. These were not the melting point of andrographolide and β-CD. This chang prove the reality of the inclusion.

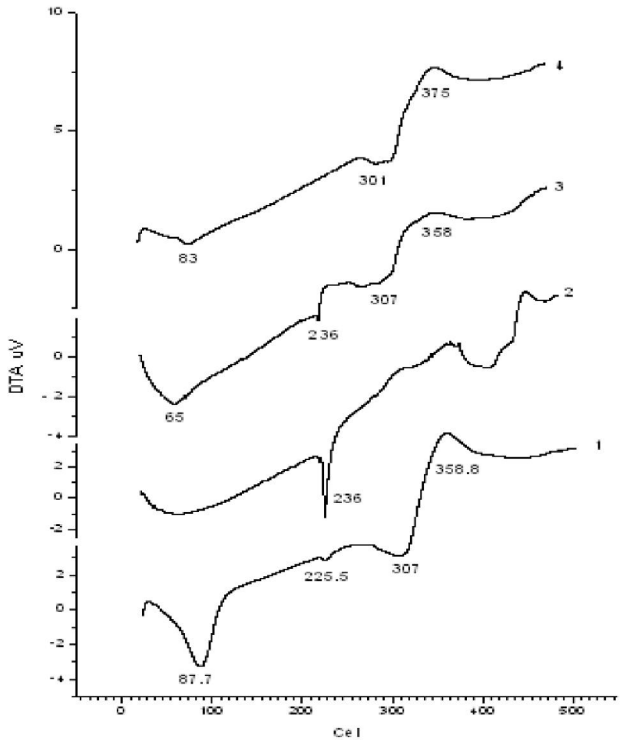

Figure 1 : DTA (1) β-CD; (2) Andrographolide; (3) Physics mixture; (4) Inclusion compound

FT-IR study

Figure 2 shows the infrared spectra of β-CD, andrographolide, a physical mixture of andrographolide and β-CD at a 1:2 molar ratio as well as the complex obtained under microwave irradiation. Characteristic bands arising from –C=O (1727.83 cm^{-1}) and –C=C (1634.08 cm^{-1}) observed for pure andrographolide and the physical mixture, shift to 1741.47 cm^{-1} and 1669.99 cm^{-1} respectively in the case of the complex. These in-

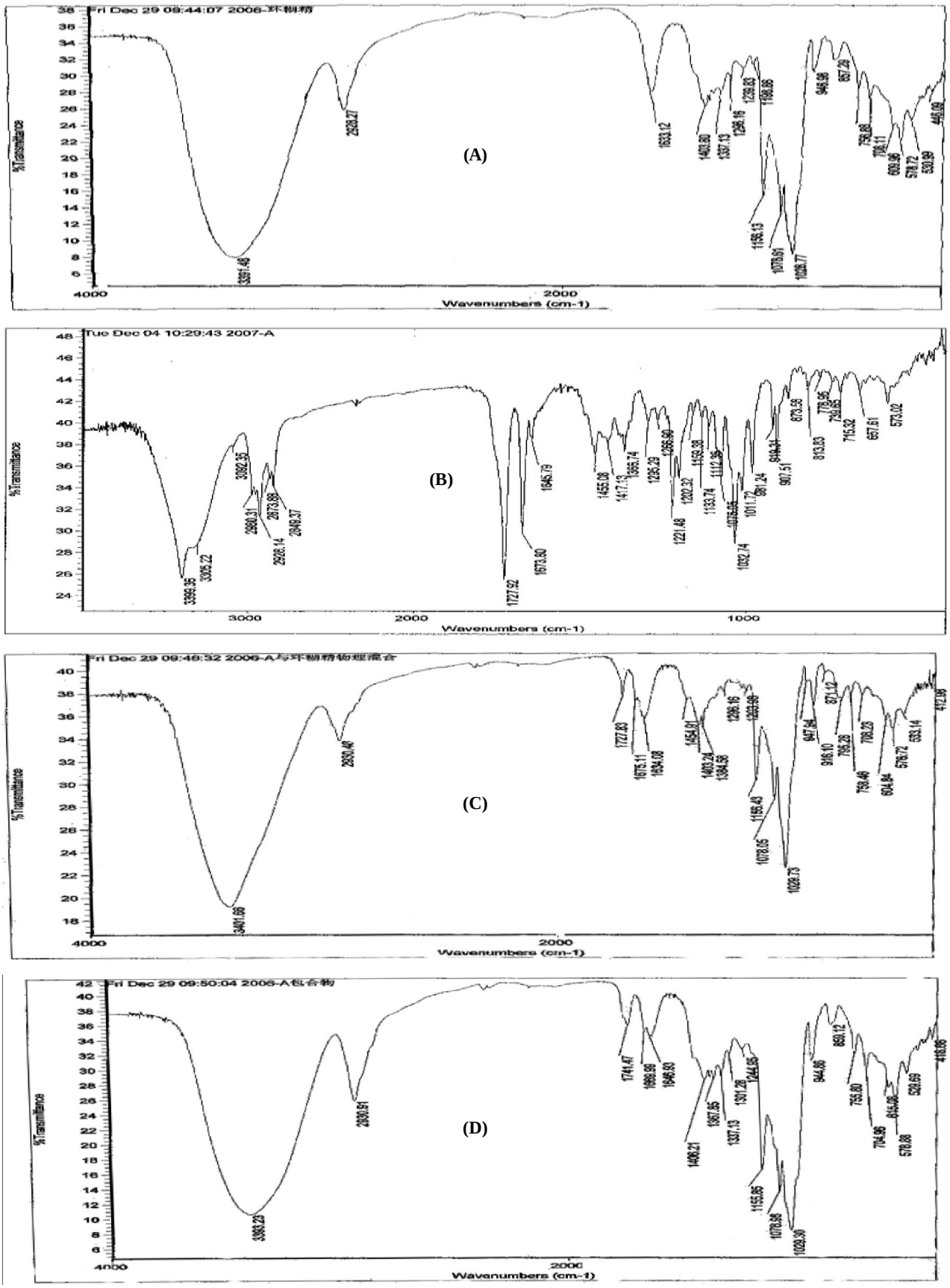

Figure 2 : Infrared spectrogram (A) β-CD; (B) Andrographolide; (C) Physics mixture; (D) Inclusion compound

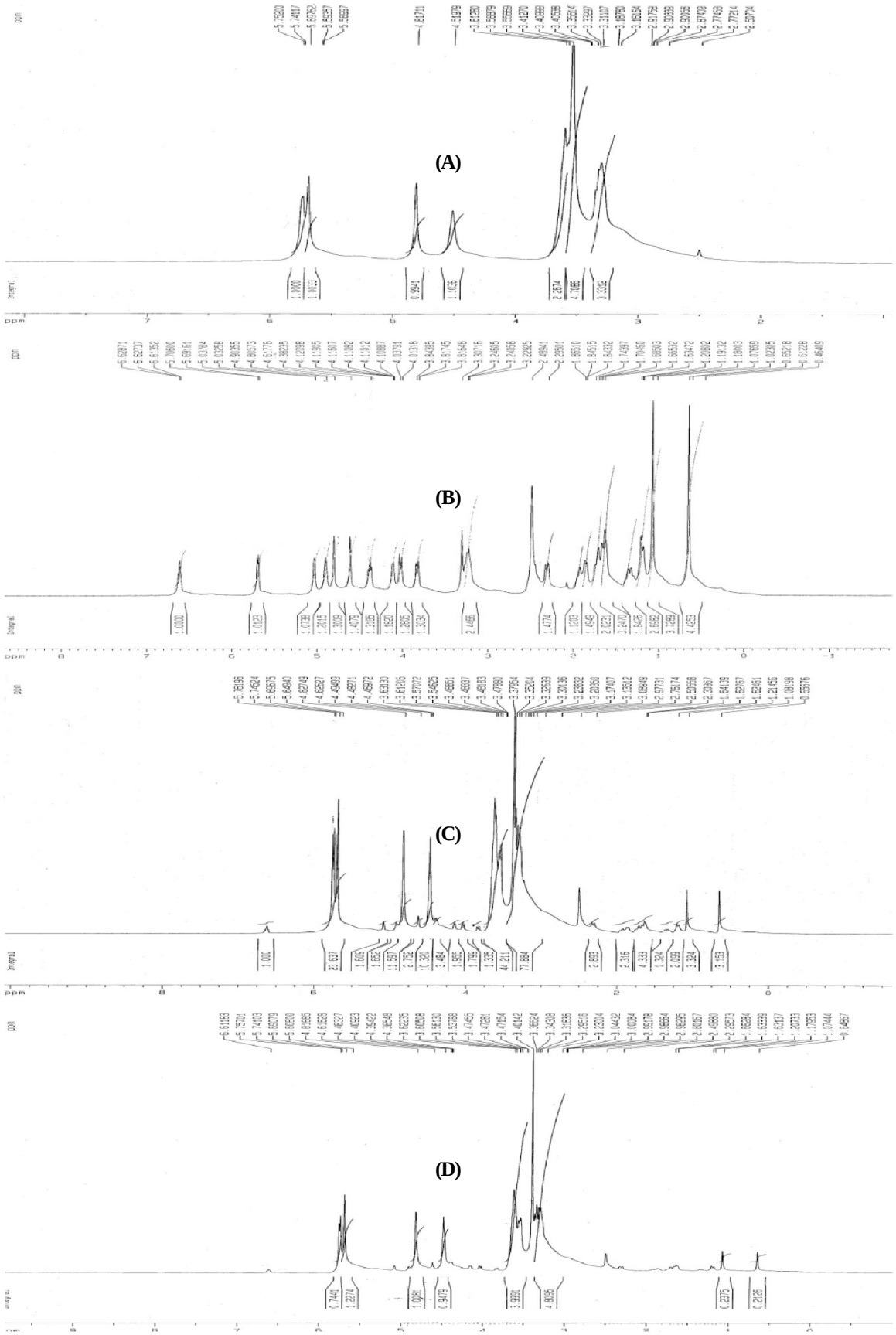

Figure 3 : ^{1}H NMR spectrogram (A) β-CD; (B) Andrographolide; (C) Physics mixture; (D) Inclusion compound

dicated that the vibrating and bending of the guest molecule (andrographolide) was restricted due to the formation of an inclusion complex[5], and very likely the lactone ring in andrographolide was inserted into the cavity of β-CD. The inclusion compound was also confirmed by this change.

NMR studies

NMR spectroscopy has been previously used to establish inclusion modes and stoichiometries. In the present work, ^{1}H-NMR, measurements were performed to elucidate the structure of andrographolide / β-CD. The ^{1}H-NMR chemical shift values of β-CD in the free and complexed state are shown in TABLE 1. 2-H,3-H,5-H, 6-H is up-field shifts,1-H, 4-H, is down-field shifts. These observations prove the reality of the inclusion. Since the andrographolide molecule contains two parts, ring A and the connected rings B&C, this may lead to two isomeric 1:1 complexes and a 1:2 complex. To ascertain the structure of the inclusion complex, ^{1}H-NMR spectroscopy studies of andrographolide were therefore undertaken. The difference in chemical shift values between andrographolide in the free and complexed state are presented in TABLE 2. All this change proved the reality of the inclusion.

TABLE 1 : $\delta_{\beta\text{-CD}}$ change of inclusion compound and physics mixture

β-CD (Proton)	β-CD (δ_0)	β-CD-A (δ)	$\Delta\delta$ (δ-δ_0)
1-H	4.8171	4.8188	0.0017
2-H	3.3551	3.3431	-0.0120
3-H	3.6128	3.6051	-0.0077
4-H	3.3110	3.3662	0.0552
5-H	3.5567	3.5379	-0.0188
6-H	3.5888	3.5613	-0.0275

TABLE 2 : δ_A change of inclusion compound and physics mixture

A (Proton)	A (δ_0)	β-CD-A (δ)	$\Delta\delta$ (δ-δ_0)
9-H	2.306	2.297	-0.009
12-H	6.634	6.613	-0.021
14-H	4.914	4.911	-0.003
15-Ha	4.393	4.398	0.005
15-Hb	4.036	4.026	-0.010
17-Ha	4.816	4.820	0.004
17-Hb	4.628	4.617	-0.011
18-H	1.087	1.076	-0.012
19-Ha	3.837	3.826	-0.010
20-H	0.663	0.650	-0.013

CONCLUSIONS

The inclusion complex of andrographolide with β-CD prepared under saturated solution method assist with microwave was studied in this work. The inclusion compound was confirmed by the DTA, AFT-IR and ^{1}H-NMR. The structure of the inclusion complex was inferred from the NMR data. However, since the X-ray crystal diffraction has not been carried out, the structure interpretation based on the NMR experiments is tentative and awaits confirmation.

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