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Preparation and study the 1:2 inclusion complex of carvedilol with β-cyclodextrin

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Abstract

An inclusion complex of β-cyclodextrin with carvedilol was prepared by using a convenient new method of microwave irradiation. Phase-solubility studies demonstrated the ability of β-cyclodextrins to complex with carvedilol and increase drug solubility. The structure of inclusion complex was determined by fluorescence spectroscopy and ^{1}H NMR, ^{13}C NMR measurements in solution. The solid inclusion was characterised by infrared spectroscopy, differential scanning calorimetry (DSC) and element analysis. These experimental results confirmed the existence of 1:2 inclusion complex of carvedilol with β-cyclodextrin, the formation constant of complex was determined by the fluorescence method. Molecular modeling predicted the energy-minimized structure of the complex.

Keywords: Carvedilol; β-Cyclodextrin; Inclusion complex; NMR; Microwave irradiation; Molecular modeling

1. Introduction

Carvedilol ({1-[carbazolyl-(4)-oxy]-3-[2-methoxyphenoxyethylamino]propanol-(2)}) is a compound displaying antioxidant properties, used in clinical practice for the treatment of cardiovascular diseases (hypertension, congestive heart failure or myocardial infarction) [1]. However, it could not be used as a drug of oral administration due to its poor water solubility, which prevents it from being absorbed well in the body [2].

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 [3]. Owing to this ability, they have found extensive application in many fields including pharmaceutical technology (to improve the aqueous solubility, dissolution rate, bioavailability and stability of drugs) [4–6]. Now, many poorly soluble drugs have been complexed by CDs to enhance solubility, chemical stability and bioavailability of the drugs [7–11].

Currently the widely used methods to prepare inclusion compounds are: coprecipitation, kneading,

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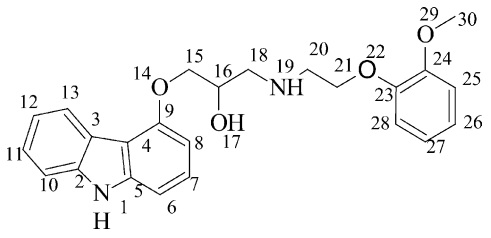

Scheme 1. Structure of carvedilol.

freeze–drying and cogrinding [12]. Recently, microwave irradiation has been developed for rapid organic synthesis [13]. 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 [14].

The complex in solution was studied by fluorescence spectrometry and NMR spectroscopy. when complexed with CDs, many guest molecules exhibit enhanced fluorescence efficiencies, since CD cavity can protect guest molecule excited states from nonradiative and quenching processes that normally readily occur in bulk aqueous solution [15], and the fuorimetric method is sensitive and selective, so it have been extensively used to determine the association constants of complexes [16–18]. NMR is the powerful tools to study the geometry of complex [19–21]. The solid inclusion complex were characterized by methods include infrared spectra (IR), differential scanning calorimetry (DSC), element analysis [22–27].

The molecular modeling using MM2 force field method have been used to study the cyclodextrin complex when the system include many atoms and have explained the experimental observation successfully [28,29].

In the present work, we prepared the inclusion compound of carvedilol-β-cyclodextrin under microwave irradiation, and determined the complex by FT-IR, DSC, element analysis, ^{1}H NMR, ^{13}C NMR, and modeled the structure of the complex using MM2 force field method (Scheme 1).

2. Experimental

2.1. Materials

β-Cyclodextrin (99.5%, Sigma) was purchased from Sigma and purified it by recrystallization from distilled water, Carvedilol was kindly provided by Zhejiang Hisun Pharmaceutical Co. Ltd. (China), and was of pharmaceutical grade, The ^{1}H NMR, ^{13}C NMR measurements were carried out in DMSO-d_6 (Aldrich) solutions, Other chemicals were of analytical reagent grade purity.

2.2. Apparatus

Bruker Avance DMX 500 MHZ superconducting NMR spectrometer, Hitachi F-4500 fluorescence. Spectrophotometer, Netzsch STA 409 thermal analyzer, Thermo Nicolet Nexus FT-470 IR spectrometry, Shunde Whirlpool T120X/T120 XS Microwave Oven (china); Elemental Analyzer EA1110, Specord 2000 UV.

2.3. Procedure

2.3.1. Phase-solubility studies

The phase-solubility diagram was recorded according to Higuchi and Connors method [30]. For that purpose, aqueous solutions of β-CD with concentrations of 0, 0.5, 1.0, 1.5, 2.0, 2.5, 3.0, 3.5 and 4.0×10^{-4} M were prepared. Excess amounts of carvedilol were added to each solution of β-CD. The solutions were sealed and shaken at ambient temperature for 48 h, then centrifuged and filtered. Their absorption was measured by UV spectrophotometry (285 nm). The phase-solubility diagram was therefore obtained. The presence of β-CD did not interfere with the spectrophotometric assay of the drug. Each experiment was performed in triplicate.

2.3.2. Fluorescence measurement

Fluorescence measurements were performed by a F-4500 spectrofluorimeter using 1 cm quartz cell, slit width was 5 nm, excitation and fluorescence emission wavelength of 250 and 360 nm, respectively. The concentration of carvedilol solution is 1×10^{-5} M, 5 ml was added into 10 ml cuvette, then, 1.0×10^{-2} mol/l β-CD solution of from 0 to 5.00 ml was added into cuvette dropwise and diluted to 10 ml with distilled water. The mixtures were oscillated 2 h before measuration.

2.3.3. NMR measurements

Carvedilol and β-cyclodextrin was dissolved into 0.5 ml DMSO-d_6, their concentration was

2×10^{-2} mol/l. Their ^{1}H NMR and ^{13}C NMR were done.

2.3.4. IR spectroscopy

IR spectra of pure carvedilol, β-CD, their physical mixture and the complex were obtained with FT-470 IR spectroscopy using KBr pelleting. The range of spectra was from 500 to 4000 cm^{-1}.

2.3.5. Differential scanning calorimetry

DSC analyses were carried out in the temperature range from 30 to 450 °C in a stream of nitrogen atmosphere on STA-409 thermal analyzer. During experiments, aluminum crucibles were used. Sample weighs were 5 mg. The heating rate was 10 °C/min and the flow rate of nitrogen atmosphere was 10 ml/min a-Al_2O_3 was used as reference.

2.3.6. Preparation of solid complex of carvedilol with β-CD

A mixture of 0.04 mmol β-CD and 0.02 mmol carvedilol was ground in a glass container, minimum amounts of solvents (ethanol:water = 1:1, v/v) were added. The mixture was reacted for 90 s at 60 °C in the microwave oven (Zhao et al. have used the same conditions to prepare the inclusion of Andrographolide with β-CD [31]). After the reaction was complete, adequate amounts of solvents (ethanol and water) were added to remove the residual β-CD and carvedilol, then the precipitate was filtered. After drying in vacuum oven at 80 °C, white powdered products was attained, This is inclusion complex of carvedilol and β-CD.

2.3.7. Molecular modeling

Molecular modeling was carried out on an IBM Pentium III 1.1 GHz personal computer. The molecular mechanics MM2 force field method implemented in Hyperchem software was used for molecular modeling calculations. The initial structures of carvedilol and β-cyclodextrin were constructed with the help of Hyperchem and optimized with PM3 from the crystal structure. Li et al. have proved that the PM3 is advantageous to AM1 in cyclodextrin (CD) structure optimization [32]. Modeling was performed by docking the optimized structure of the carvedilol molecular into the β-CD cavity and allowing for full-geometry optimization. All structures were minimized using a conjugate gradient optimization procedure until a root mean square (RMS) value of 0.01 kcal mol^{-1} $Å^{-1}$ was obtained.

3. Results and discussion

3.1. Solubility studies

The phase-solubility diagram for the complex formation between carvedilol and β-CD is presented in Fig. 1. According to the Higuchi and Connors classification, the diagrams obtained were of A_N type, where the guest solubility increases linearly with cyclodextrin concentration of deviate negatively [33]. From this curve, it can be seen that the apparent solubility of carvedilol increases due to the formation of a soluble inclusion complex between carvedilol and β-CD. The interaction mechanism for the A_N type is complicated, because of a significant contribution of solute-solvent interaction to the complexation [33].

3.2. Fluorescence study

Fig. 2 shows that adding β-CD to carvedilol solution results in a significant enhancement of the fluorescence intensity. From the figure, we can see fluorescence intensity are enhanced with increasing concentration of β-CD. These data suggest that a stable complex is formed between β-CD and carvedilol. The CD cavity provides an apolar environment for the carvedilol molecule and thus increases the quantum yield of the excited fluorophore of carvedilol. The formation

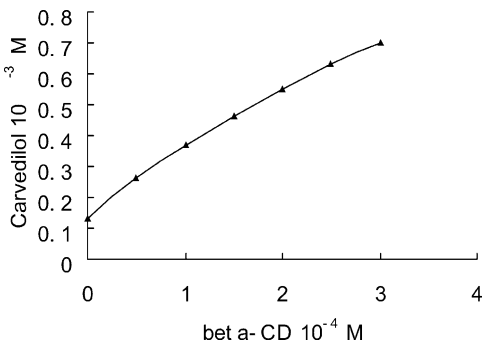

Fig. 1. Phase-solubility diagrams of carvedilol in aqueous solutions of β-CD.

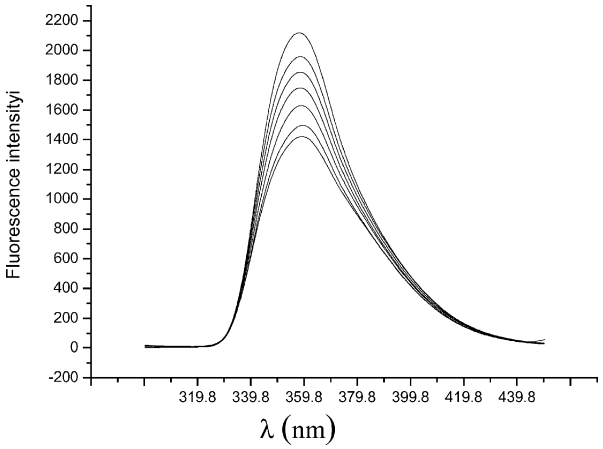

Fig. 2. The fluorescence spectrum of inclusion complex, concentration of β-CD (mol/l) are 0, 5×10^{-4}, 1×10^{-3}, 1.5×10^{-3}, 2×10^{-3}, 2.5×10^{-3}, 3×10^{-3}.

constant and the ratio of the complex were calculated from these data by use of the modified Benesi–Hildebrand equation [29]:

$$\frac{1}{F - F_0} = \frac{1}{[\mathrm{CD}]K[F_\infty - F_0] + 1/[F_\infty - F_0]}$$

or

$$\frac{1}{F - F_0} = \frac{1}{[\mathrm{CD}]^2K[F_\infty - F_0] + 1/[F_\infty - F_0]}$$

where stoichiometry of the complex is 1:1 and 1:2, respectively.

Here F is the observed fluorescence intensity of carvedilol solution at each β-CD concentration, F_0 presents fluorescence intensity of carvedilol solution in the absence of β-CD, K is forming constant. Making a plot of curve of $1/(F - F_0)$ against $1/[\beta\text{-CD}]^2$ (see Fig. 3), the calculated formation constant K is 8.5×10^5 $(\mathrm{mol/l})^{-2}$ and according to the linear fit of double reciprocal plot, the ratio of the complex is 1:2.

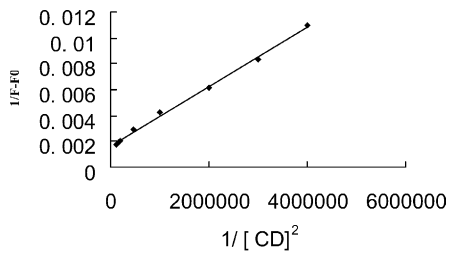

Fig. 3. Benesi–Hildebrand plots for 1:2 inclusion of β-CD with carvedilol.

3.3. ^1H NMR and ^{13}C NMR studies

To ascertain the structure of the inclusion complex, ^{1}H NMR and ^{13}C NMR spectroscopy studies of carvedilol were therefore undertaken. The difference in carbon and hydrogen chemical shift values between carvedilol in the free and complexed state are presented in Tables 1 and 2.

Table 1
^{13}C NMR chemical shifts corresponding to cavedilol in the absence and presence of β-CD.

Carve carbon	Carve (δ_0)	β-CD-carve (δ)	$\Delta\delta$
C2	141.106	141.080	−0.026
C3	112.196	112.159	−0.037
C4	111.562	111.546	−0.16
C5	138.909	138.90	−0.009
C6	103.839	103.804	−0.035
C7	122.490	122.442	−0.048
C8	100.422	100.450	0.028
C9	154.965	154.969	0.004
C10	118.586	118.542	−0.042
C11	124.531	124.509	−0.022
C12	126.508	126.408	−0.100
C13	121.744	121.603	−0.014
C15	68.322	68.391	0.069
C16	55.432	55.472	0.040
C18	52.469	52.543	0.074
C20	48.411	48.475	0.064
C21	70.414	70.512	0.098
C23	149.179	149.158	−0.021
C24	148.063	148.059	−0.004
C25	110.351	110.316	−0.035
C26	121.054	121.002	−0.048
C27	120.718	120.702	−0.016
C28	113.640	113.635	−0.005
C30	52.489	52.513	0.024

Table 2
^{1}H NMR chemical shifts corresponding to carvedil in the absence and presence of β-CD

Carve H	Carve (δ_0)	β-CD-carve (δ)	$\Delta\delta$
H6	6.9430	6.9508	0.0078
H7	7.1166	7.1193	0.0027
H21a	4.1424	4.1629	0.0205
H21b	4.1277	4.1514	0.0237
H25	6.8733	6.8881	0.0148
H26	6.9018	6.9434	0.0416
H27	6.8451	6.8568	0.0117

In the presence of β-CD the upfield shifts of the C2, C3, C10–C13, C21–C28 carbon atoms suggest that the phenyl group, indicated as A-ring, and C-ring and part B-ring, was included within the CD cavity. The downfield shifts of the C8–C9 and C21 carbon atoms confirm this hypothesis. Inoue has interpreted this phenomenon on the basis of reaction field theory [34]: a guest molecule from the free state to the CD cavity in solution cause an upfield shift of the ^{13}C NMR signals of the lead carbons included and a downfield shift of the ^{13}C NMR signals of the carbons externally close to the wider rim of the hollow CD [35,36]. The H6–H7 and H–H21, H25–H28 atom experienced downfield shift attributable to diminished freedom of rotation caused by the penetration into CD cavity [37]. It can be deduced from these information that the ring B and C probably entered the inner cavity of β-CD.

3.4. IR spectra studies

Fig. 4 shows the infrared spectra of β-CD, carvedilol, physical mixture of carvedilol and β-CD at a 1:2 molar ratios as well as the complex obtained under microwave irradiation.

Analysis of the IR spectra of the inclusion complexes and a physical mixture of the components revealed the most frequent changes to be in the range 2950–3050 cm^{-1}, which were interpreted as the valence bands of the C–H in plane vibrations of the aromatic ring. Differences were also found in the 1480–1600 cm^{-1} regions, attributed to the skeleton vibrations of the C=C bonds in the aromatic ring. The 1250 cm^{-1} region was attributed to the aromatic C–N vibration, this region of complex was disappeared. The intensity and shape of these bands changed dramatically for the inclusion compound as compared to those for pure carvedilol and physical mixture. These indicated that the vibrating and bending of the guest molecule (carvedilol) was restricted due to the formation of an inclusion complex [38], so very likely the aromatic rings in carvedilol were inserted into the cavity of β-CD.

3.5. Differential scanning calorimetry studies

Another method used to identify the inclusion complexes of drug with CD was differential scanning calorimetry. As seen from Fig. 5, DSC thermograms

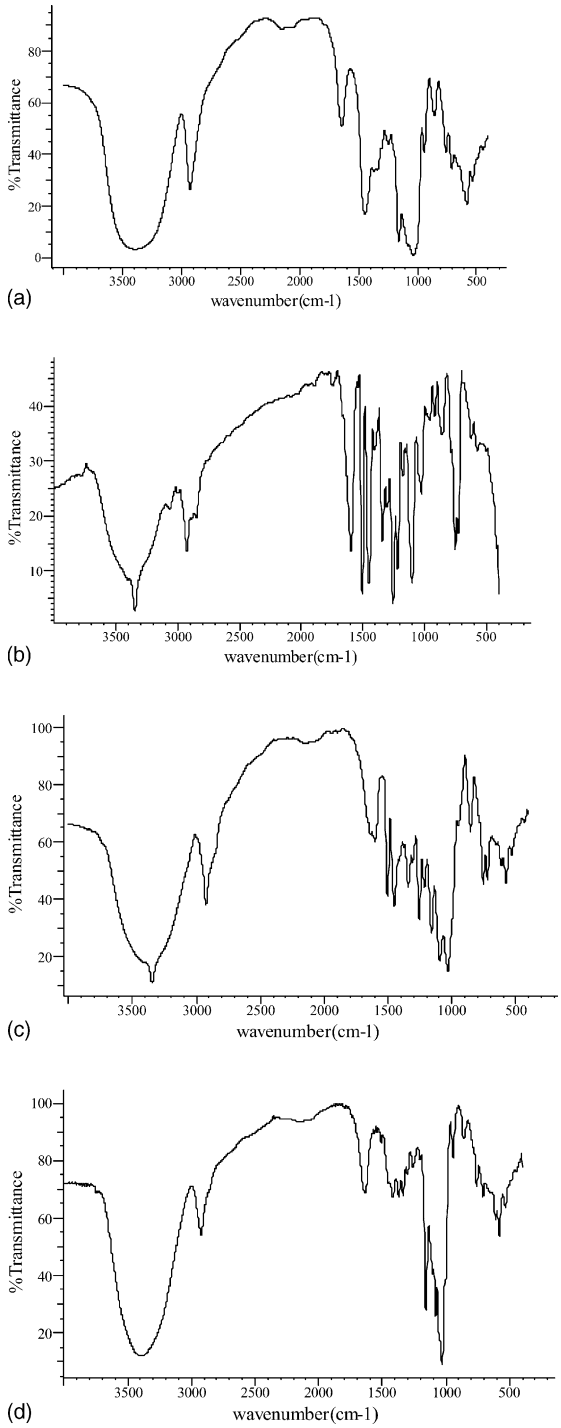

Fig. 4. FT-IR spectra of (a) β-CD; (b) carvedilol; (c) physical mixture; and (d) the inclusion complex of carvedilol/β-CD.

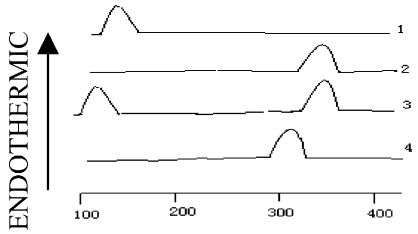

Fig. 5. DSC curves of (1) β-CD; (2) carvedilol; (3) physical mixture; and (4) inclusion complex.

of carvedilol alone (2) showed endothermic T_{max} of 324.7 °C, corresponding to the melting point of crystalline form of the drug carvedilol, and physical mixture (3) is shifted to higher, but the inclusion complex (4) is shifted towards lower temperatures 319.2 °C. The lower temperature of inclusion complex was due to melting point depression by the complex [39], the same conditions were found when felodipine and carbamazepine were complexed by β-CD, the endothermic T_{max} of inclusion complex was lowered by 3 and 5 °C, respectively [39,40].

3.6. Molecular modeling study

Computational studies on host–guest interactions were carried out, in general, to find the most probable conformation of the complex and the appropriate three-dimensional visualization of the complex. For this purpose, ΔE was calculated for the minimum energy structure according to Eq. (1) [41–43]:

$$\Delta E = E_{\text{complex}} - (E_{\text{isolated host}} + E_{\text{guest}}) \quad (1)$$

The full-geometry optimization of such complexes showed that the inclusion of the A-ring group in the wider side of the cavity was the most energetically favorable orientation. The C-ring and part B-ring groups were included into the wider side of β-CD. Molecular modeling study also showed methyoxy group was not in cavity but close to wider rim of β-CD. It is in agreement with the NMR data. The calculation energy of carvedilol and β-CD was 46.3 and 108.9 kcal/mol, respectively; E_{complex} is 224.4 kcal/mol, So ΔE is −39.71 kcal/mol, the inclusion formation is energetically favorable [44]. The energy-minimized structure of the complex was showed in Fig. 6.

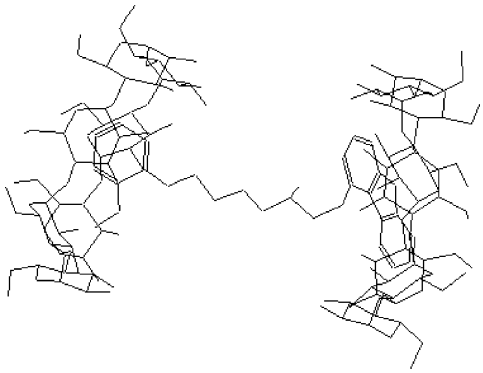

Fig. 6. Energy-minimized predicted structure of the 1:2 complex between carvedilol and β-cyclodextrin.

3.7. Element analysis experiment

The final stoichiometry is determined by elemental analysis. The result is: C%, found 48.52, calculated (1:2 complex) 48.50; H%, found 6.28, calculated (1:2 complex) 6.27; N% found 1.04 (calculated (1:2 complex) 1.04. It indicates that carvedilol formed the 1:2 inclusion complex with β-cyclodextrin.

4. Conclusion

This study shows that carvedilol interacts with β-CD and forms a complex, and the interaction increases it's solubility. The enhancement of the fluorescence intensity of carvedilol in the presence of β-CD suggests that a certain fraction of carvedilol molecules in solution are in a more hydrophobic environment, which is inside the β-CD cavity. In addition, the fluorescence spectroscopy and NMR data results suggest the formation of a stable 1:2 stoichiometric complex of carvedilol and β-CD, the energy-minimi-structure of the complex was predicted by MM2 force field.

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