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Inclusion Complex of β-CD-DZ as a nanocarrier: Preparation, Spectroscopic characterization, Thermodynamic parameters and molecular modelling Study

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ABSTRACT

The β-CD-DZ inclusion complex was prepared via freeze-drying method and characterized by using various spectroscopic techniques. The phase solubility diagram and Job plot showed the formation of a soluble complex of AL type (1:1 stoichiometric IC) with improved solubility and stability. The thermodynamic parameters for the β-CD-DZ IC formation were determined and analyzed on basis of involved driving forces. The results showed the reaction process in mainly entropy driven and endothermic that assigned the predominant role of hydrophobic interaction in the formation of β-CD-DZ complex. In conclusion, a comprehensive computational study has been done on formation process of β-CD-DZ IC using molecular docking methods. In case of β-CD-DZ IC, the results of thermodynamic parameters, molecular docking confirmed stable complex formation between β-CD-DZ and also DZ included into a narrow site of inner cavity of β-CD.

Keywords: β-Cyclodextrin (β-CD); Daidzein (DZ); 1H-NMR; inclusion complex (IC); Molecular docking.

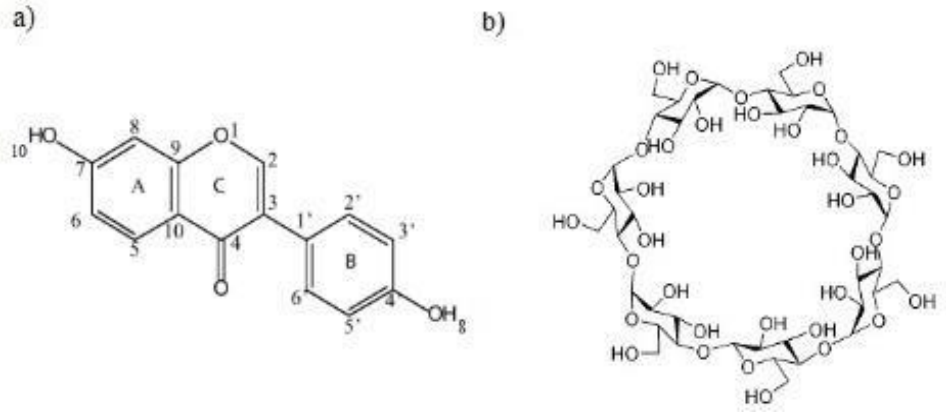
1. Introduction

Daidzein (DZ) (Scheme 1-a), one of the most important is flavones, is often found in soy foods. It has a varieties of biological activities including antioxidant, anti-inflammatory and antitumor and playing important role in female hormone. In spite of the most importance of DZ, low solubility in aqueous media, low bioavailability in oral absorption [1] and bitter taste have been limited its clinical applications. However, these limitations could be removed if DZ is encapsulated into suitable nanocarrires.

Cyclodextrins (CDs) are family of cyclic oligosaccharides that have Doughnut-shape structure. Native CDs, containing of linked 6,7 and 8 D-glucopyranose units by α-(1→4) glycosidic bonds, are named α-, β- or γ- CD, respectively [2]. The β-CD molecule (Scheme 1-b) has a hydrophilic outer surface and a relatively hydrophobic nanocavity [3] and has been used properly to improve the solubility of poor soluble guests, visible or UV light and heat, controlled release of drugs and flavors and etc. [4]. The encapsulation could be done by formation of an IC between the host CD molecule and the guest, where CD, the ‘‘host’’, includes, totally or partly, drug molecules ‘‘guest’’ by physical forces [2]. The applications of CDs in food industry, pharmaceuticals, protection and analytical chemistry [5] are based on their inclusion complaxation ability with a number of molecules.

In this research, the ability of β-CD, as a nanocarrier and protective factor that improve the solubility and bioavailability of DZ, for targeting delivery of this compound was investigated. In this regard, the IC of DZ with β-CD is prepared by the freeze-drying method and characterized by 1H-NMR spectroscopy and SEM technique. Phase solubility diagram for DZ in the IC determined. The thermodynamic parameters for the IC formation were determines and analyzed on basis of involved driving forces. An inclusive computational study has also been done on formation process of this IC using molecular docking method.

The result reveal detailed molecular features of the complex formation including the orientation of DZ in the nanocavity of β-CD.



Scheme 1. a) Structure of DZ, b) β-CD.

2. Experimental

2.1. Materials

β-CD (98 %, Sigma-Aldrich) and DZ (>99 %, Sigma Aldrich) were purchased. All solvents (acetone, DMSO and DMSO-d6) were of high purity and obtained from Merck Company (Germany). The phosphate buffer saline prepared (PBS, 10 mM, pH=7.4), as the solvent in all the studies performed.

2.2. Preparation and characterization of β-CD-DZ IC

8 mL β-CD 5 mg/mL dissolved in deionized water and 9 mg DZ were dissolved in 8 mL acetone in glass capped vials. These solutions were mixed and put on the magnetic stirrer for 1 day. After that, the mixture was centrifuged and the supernatant was separated and freezed dried (VaCo5, Zirbus, Germany; -50 $^{\circ}$C, 130×10^{-3} mBar) for preparing of the solid form of β-CD-DZ IC that was stored at 4 $^{\circ}$C. Then IC characterized by using 1H-NMR spectroscopy and SEM techniques.

2.3. Phase solubility diagram

The β-CD solution with various concentrations were prepared in PBS, Then, 1 mL portion of DZ solution (180 μM) was added to 5 mL of each these β-CD solutions in the glass capped vials. The vials were stirred gently for 3 days at 25 °C. After that the samples were filtered and DZ concentration in the filtrates was determined by measuring the absorbance at 245 nm using UV-Vis Spectrophotometer (Perkin Elmer, Lambda 265, USA).

2.4. UV-Vis absorption spectroscopy and thermodynamic parameters

All absorption spectra of β-CD-DZ complexes were recorded by UV-Vis Spectrophotometer equipped with a thermostat that control the temperature within ±0.1 °C precision. For UV- Vis titration experiments, DZ solution (2000 μL, 30 μM) was put on a quartz cuvette and titrated with fixed amounts of 20 μL solution of the β-CD (300 μM), sequentially. The UV-Vis absorption spectra were recorded in the range of 235-450 nm. The corrected absorption data for dilution was analyzed by using the Benesi-Hildebrand method Eq. (1) as follow:

$$\frac{1}{\Delta A_i} = \frac{1}{\Delta A_{max}} + \frac{1}{K_a \Delta A_{max} [\beta - CD]_i}$$

Where ΔA_i is the difference of measured absorbance at 245 nm in i^{th} step with the absorbance of free DZ, $[\beta\text{-CD}]_i$ is the total concentration of β-CD in the i^{th} step of titration, ΔA_{max} is the absorption difference between bound and free DZ and K_a is the associative binding constant for formation of β-CD-DZ complex. The spectroscopic binding experiments were carried out at 20, 25, 30 and 35 °C. The estimated binding constants were used for calculation of ΔG° using the Gibbs famous equation, $\Delta G^\circ = -RT\ln K_a$. The other thermodynamic parameters (ΔH° and ΔS°) were determined using the following Eq. (2):

$$\ln(K_a) = -\frac{\Delta H^\circ}{RT} + \frac{\Delta S^\circ}{R}$$

Where R and T are universal gas constant ($J.mol^{-1}.K^{-1}$) and absolute temperature (K), respectively.

2.5. Molecular modelling

Molecular docking calculations were performed using the software AutoDock 4.2.6. The initial structure of β-CD were extracted from the webserver:

(http://upjv.q4md forcefieldtools.org/REDDB/projects/F-85/).

The pdbqt file constructed. Macromolecules were considered rigid, while all torsional bonds of DZ were freely rotating. The grid box was set to 40×40×40 grid point cubic box for β-CD and the grid point spacing was set to 0.375Å in each dimension. The LGA was adopted to model the interactions of the β-CD-DZ IC. For calculation, set of 250 docking run for ligand was performed and for run, a maximum 25,000,000 GA operations carried out. The inclusion energy was calculated based on the ranking functions, and their configurations were ranked into the clusters from the lowest to highest binding energy.

3. Results and discussion

3.1. Characterization of β-CD-DZ IC

All 1H-NMR experiments were carried out in DMSO-d6 at 298 K in the range of 0 to 10 ppm using NMR (Bruker, Ultra shield 400 MHz, Germany). All 1H-NMR analysis were recorded for three samples β-CD, DZ and the β-CD-DZ IC (1:1). Chemical shift changes (Δδ) were calculated according to Eq. (3):

$$\Delta\delta = \delta_{complex} - \delta_{guest}$$

Where δc_{omplex} and δ_{guest} are the chemical shifts of IC and β-CD, respectively.

As shown in Table (1), for studying the inclusion behavior of β-CD-DZ, the 1H-NMR spectra of β-CD, DZ and IC β-CD-DZ were compared. According to Table 1, the most chemical shift change is observed for H-5 (Δδ=0.089 ppm) and 6-OH (Δδ=0.0782 ppm) groups which could be an indicative that DZ is more located on the narrow side of the cavity of β-CD. Accordingly, the NMR study confirms the formation of IC and the location of DZ in the narrow side of β-CD cavity.

Table 1. 1H-chemical shift (δ [ppm]) corresponding to pure β-CD and DZ and β-CD-DZ IC.

DZ	δ [ppm]	β-CD-DZ	$\Delta\delta=\delta_{complex}-\delta_{DZ}$
7-OH	9.5783	9.6096	0.0313
4'-OH	10.8295		
H-5	8.0324	8.1646	0.1322
H-2'/6'	7.4479	7.4423	-0.0056
H-8	6.8741	6.8662	-0.0079
H-3'/5'	6.7986		
H-6	6.978	6.8883	-0.0897
H-3	8.3428	8.3595	0.0167
β-CD	δ [ppm]	β-CD-DZ	$\Delta\delta=\delta_{complex}-\delta_{\beta\text{-}CD}$
2-OH	5.7398	5.7593	0.0195
H-1	4.8362	4.9069	0.0707
6-OH	4.4606	4.5388	0.0782
H-3	3.6578	3.7281	0.0703
H-2	3.5542	3.5713	0.0171
H-5	3.3324	3.4214	0.089

The FE-SEM obtained for DZ, β-CD, and β-CD-DZ IC are illustrated in Fig. 1. DZ arranged in columns (Fig. 1-a), for β-CD (Fig. 1-b) has a rhombus shape, and also for freeze dried DZ: β-CD IC the form of amorphous powder circular could be observed (Fig. 1-c).

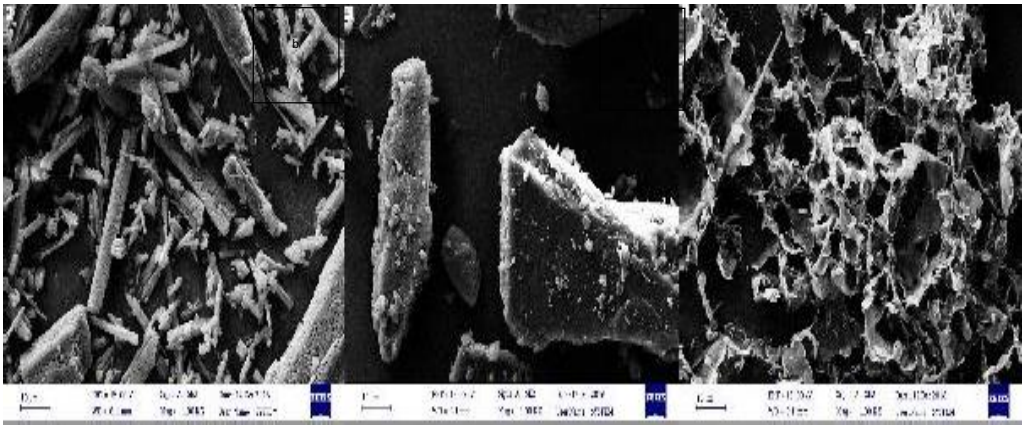


Fig.1. The FE-SEM images for a) DZ, b) β-CD and c) IC of DZ: β- CD.

3.2. Phase solubility diagram

The apparent stability constant (K_s) of the complexes was calculated from the phase-solubility diagrams according to the following Eq. (4):

$$K_S = \frac{slope}{S_0(1-slope)}$$

Where S_0 is y-intercept, (the intrinsic solubility of DZ in water in the absence of CD). Fig. 2 shows the phase-solubility diagram for DZ with β-CD. According to the phase solubility diagram results, the solubility of DZ increased as a linear function of the β-CD concentration. Based on Higuchi and Connors's theory [6], the diagram shown in this figure belongs to AL-type group; and therefore represents the 1:1 stoichiometric between DZ and β-CD in the complex. The value of 1091.87 M-1 for the apparent stability constant (Ks) of β-CD-DZ

complex at 25 °C was obtained with respect to Eq. (4). From these results, we found that solubility of DZ improved via complexation with β-CD.

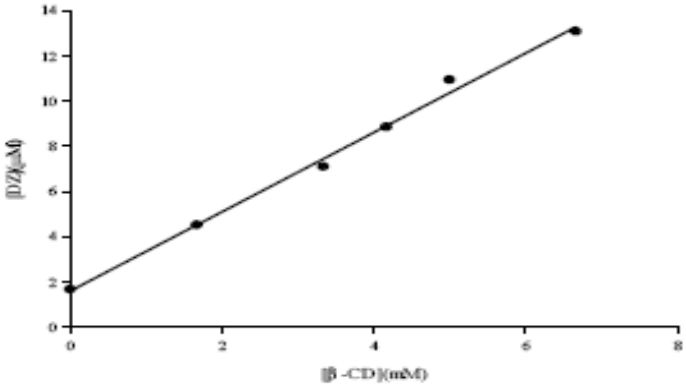


Fig. 2. Phase solubility diagram for β-CD and DZ at 25 °C.

3.3. Thermodynamics of DZ-β-CD complex formation

As shown in Fig. 3-a, the absorbance increased in all spectral region due to the addition of β-CD at 25 °C. The same behavior was observed at all other studied temperatures. The spectral titration data were analyzed on basis of Eq. 1 and the corresponding plots were shown in Fig. 3-b. The high linearity of these plots represents the formation of 1:1 complex that is in agreement with the results of phase diagram task.

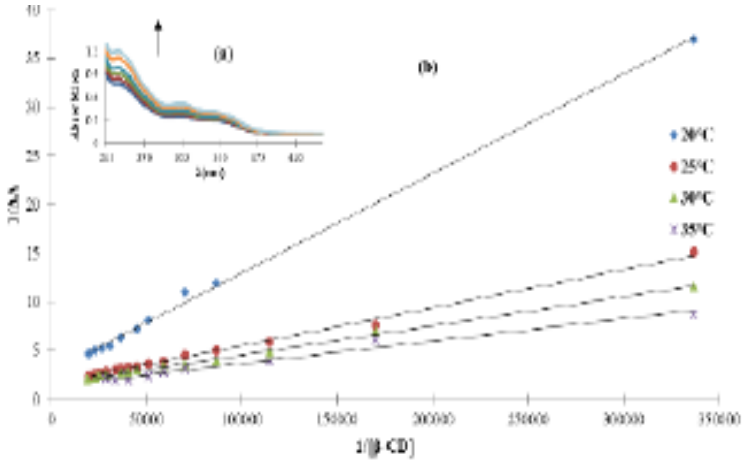


Fig. 3. a) Increase of the UV-Vis absorption spectra for DZ (30 μM) with different concentration of β-CD in the PBS (pH=7.4) at 25 °C. b) Benesi-Hildebrand plot (1/ΔA vs. 1/ [β-CD]) for absorption titration data of DZ in the presence of different concentration of β-CD at 20, 25, 30 and 35°C.

The values of associative binding constant for formation of β-CD-DZ IC, Ka, at various temperatures have been calculated and the results are shown in Table 2.

Table 2. Thermodynamic parameters for the formation of IC between DZ and β-CD.

T (K)	K_a (M^{-1})	ΔG (kJ/mol)	ΔH (kJ/mol)	ΔS (J/mol)
293.15	27091.20±964.62	-99.90±9.21	37.43±6.59	213.12±21.94
298.15	40500.00±1950.91	-100.97±9.29		
303.15	51366.70±14342.44	-102.04±9.36		
308.15	57250.00±2575.72	-03.10±9.44		

The results represents the reaction process in mainly entropy driven and endothermic that assigned the predominant role of hydrophobic interaction in the formation of DZ: β-CD complex.

3.4. Molecular Docking

Molecular docking was employed to investigate the interaction of β-CD with DZ in the IC. IC between β-CD:DZ showed two hydrogen bonds between H of –OH (B ring) of DZ with O of – OH (C-6) and O of –OH (B ring) of DZ with H of –OH (C-6) of glucopyranose units (No. 4 and 2) of the narrower truncated cone. (Fig. 4) ΔG° obtained from molecular docking for interaction of β-CD with DZ is -5.97 kcal/mol. Comparison of the molecular docking results of β-CD to the titration absorption spectroscopy data showed that ΔG° and binding constant (K_a) results were in good agreement with each other. The results of molecular docking for β-CD were compared to the experimental data of 1H- NMR and showed that the most chemical shift in δcomplex belongs to the primary hydroxyl (C6) that located in the narrower truncated cone.

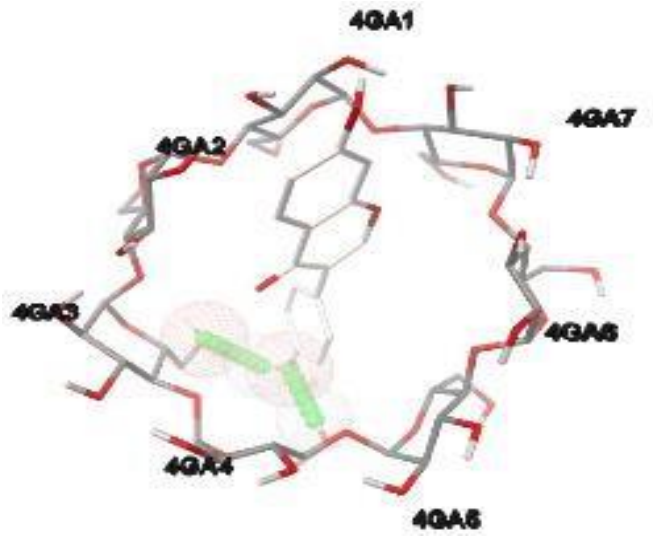


Fig. 4. Molecular docking for β-CD with DZ.

4. Conclusions

The results of this work show that DZ was complexed with β-CD to form an inclusion complex by the freeze drying method in molar ratios of 1:1. The β-CD-DZ IC was studied using UV-Vis spectroscopy, 1H-NMR, SEM. Phase solubility diagram by using UV-Vis spectroscopy analysis showed that DZ could form 1:1 stoichiometry inclusion complex with β-CD. The thermodynamic parameters for the β-CD-DZ IC formation showed the reaction process in predominantly entropy driven and endothermic and thus the main role of hydrophobic interaction in the formation of β-CD-DZ complex and this result in agreement with the computational study. 1H-NMR spectroscopy showed that the formation of inclusion complex between β-CD-DZ and the location of DZ (Bring) in the narrow side of β-CD cavity and this result was confirmed by molecular docking.

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