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RESEARCH ARTICLE

Daidzein/cyclodextrin/hydrophilic polymer ternary systems

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Abstract

Objective: To evaluate the effect of different cyclodextrins (β-cyclodextrin [β-CD], methyl-β-cyclodextrin [Mβ-CD], or hydroxypropyl-β-cyclodextrin [HPβ-CD]) and/or hydrophilic polymers (carboxymethylcellulose, hydroxypropylmethylcellulose [HPMC], polyethyleneglycol, or polyvinylpyrrolidone [PVP]) on daidzein solubility in water.

Materials and methods: The corresponding associations were characterized in aqueous media using phase-solubility studies. The morphology of daidzein/cyclodextrin freeze-dried complexes was characterized using scanning electron microscopy, and their spatial configuration was proposed by means of nuclear magnetic resonance spectroscopy.

Results and discussion: In the presence of 6 mM of cyclodextrins, the solubility of daidzein in water was significantly enhanced: 5.7-fold (β-CD), 7.2-fold (Mβ-CD), and 9.4-fold (HPβ-CD). The analysis of the three solid complexes proved that the formation of inclusion complexes occurred through the insertion of the B and C rings of daidzein molecule into the cyclodextrins cavity. The association of daidzein/cyclodextrin complexes to the hydrophilic polymers HPMC or PVP (1%, w/w) was able to improve the solubility of daidzein even further.

Conclusion: The highest solubilizing effect was obtained for daidzein/HPβ-CD/PVP ternary system (12.7-fold).

Keywords: Complexation, cyclodextrins, daidzein, hydrophilic polymers, solubility

Introduction

Daidzein (4′,7-dihydroxyisoflavone), presented in Figure 1, belongs to the isoflavone class and is often found in soy foods[1]. Daidzein is structurally similar to the estrogens, whose receptors (ERα and ERβ) are present in different tissues of the human body, such as the skin (epidermis and dermis). Daidzein has been shown to bind to estrogen receptors and, for this reason, there has been growing interest in its effects on hormone-deficient diseases[2,3]. In the same way, daidzein has been shown to stimulate the hyaluronic acid production[4] and to protect the skin from oxidative damages induced by ultraviolet radiation[5] following topical application. Taken together, daidzein seems to be a promising agent for skin aging prevention, especially for postmenopausal women. However, its estrogenic and antioxidant activities are conditioned to the aglicone form, which presents reduced solubility in aqueous media[6–8]. This fact also impairs its incorporation into a hydrophilic vehicle for topical use, as well as its delivery to the skin[4,5,8–10].

The complexation of daidzein with cyclodextrins has been reported to improve the solubility of the aglicone form in water. It was firstly investigated by Ge et al.[11], who characterized daidzein/hydroxypropyl-β-cyclodextrin (HPβ-CD) solid complexes using thermal analysis, infrared spectroscopy, and X-ray diffractometry. More recently, Stancanelli et al.[7] characterized daidzein/HPβ-CD complexes in liquid media using phase-solubility studies by means of UV–Vis spectrophotometry and circular dichroism. However, for a variety of reasons such as high molecular weight, cost, toxicology, and drug bioavailability, the amount of cyclodextrin that can be incorporated into a pharmaceutical dosage form is limited[12].

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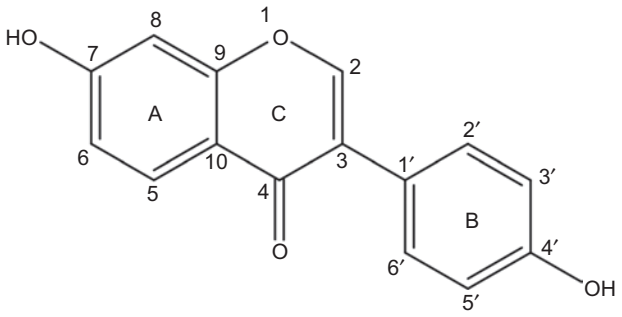

Figure 1. Chemical structure of daidzein.

Hydrophilic polymers have been also reported to present solubilizing effect because of the increased viscosity of the media and/or due to drug/polymer interactions, allowing the reduction of the cyclodextrin amount required for drug solubilization[12]. Previous studies realized in our laboratory[13] have shown that the efficiency of complexation between the flavonol quercetin and cyclodextrins (β-cyclodextrin [β-CD] or HPβ-CD) was enhanced by the use of hydroxypropylmethylcellulose (HPMC) 0.1% (w/w). In another study, Phillip Lee et al.[14] reported that the solubility in water of the anticancer drug gefitinib was significantly increased by complexation with HPβ-CD in the presence of polymers such as polyvinylpyrrolidone (PVP) or HPMC. According to Cappello et al.[15], hydrophilic polymers may interact with drug/cyclodextrin complexes forming drug/cyclodextrin/polymer aggregates or a co-complex, that is, a complex between several drug/cyclodextrin complexes and a polymer chain.

In the present study, daidzein/cyclodextrin (β-CD, methyl-β-cyclodextrin (Mβ-CD), or HPβ-CD) complexes were prepared in aqueous media followed by freeze-drying. For the first time, the solid complexes were characterized using scanning electron microscopy and their spatial configuration was proposed by means of nuclear magnetic resonance (NMR) spectroscopy. The possibility of achieving increased solubilizing effect of cyclodextrins through the formation of daidzein/cyclodextrin/hydrophilic polymer ternary systems was also investigated.

Materials and methods

Materials

Daidzein (purity 98%) was purchased from Sigma-Aldrich (St. Louis, MO). β-CD, Mβ-CD, and HPβ-CD were kindly supplied by Roquette Frères (Lestrem, France). Sodium carboxymethylcellulose (CMC; Akucell AF0305) and HPMC (Methocel E15LV) were kindly supplied by Akzo Nobel (New York, NY) and Colorcon (São Paulo, Brazil), respectively. Polyethyleneglycol 1500 (PEG) and PVP K-30 were purchased from Cromato (São Paulo, Brazil) and Henrifarma (São Paulo, Brazil), respectively. Water was purified using a Milli-Q system (Millipore, Bedford, MA). Analytical grade methanol was purchased from Vetec (Rio de Janeiro, Brazil). Deuterated water (D_2O) and dimethyl sulfoxide (DMSO-d_6) were purchased from Cambridge Isotope Laboratories (Andover, MA).

Phase-solubility study

Phase-solubility diagrams were obtained according to Higuchi and Connors[16]. Excess amount of daidzein (1.5 mM) was added to 5.0 mL of water or aqueous solutions containing increasing concentrations of β-CD, Mβ-CD, or HPβ-CD (0.75–6.0 mM). Daidzein:cyclodextrin molar ratios were 1:0, 1:0.5, 1:1, 1:2, and 1:4. The resulting dispersions were stirred using a water bath at 37°C for 48 h and then filtered at room temperature through a 0.45-μm membrane (Millipore HAWP). Aliquots of the supernatant were diluted with methanol when necessary and daidzein content was measured in triplicate by spectrophotometry at 250 nm (Hewlett-Packard 8452A UV–Vis Spectrophotometer). The method was validated over the concentration range of 1.0–8.5 μg/mL according to ICH guidelines[17].

The apparent stability constant (K_s) of a drug/cyclodextrin complex represents the binding strength between the drug and the cyclodextrin. The K_s of daidzein/cyclodextrin complexes was calculated based on the phase-solubility diagrams according to the following equation:

$$K_s(\mathrm{M}^{-1}) = \frac{\text{slope}}{S_o \times (1\text{-slope})}$$

where S_o is the intrinsic solubility of daidzein in water (solubility of daidzein in the absence of cyclodextrin) (M).

A more reliable method for evaluating the solubilizing effect of cyclodextrins is to determine their complexation efficiency (CE), that is, the complex to free cyclodextrin concentration ratio. For 1:1 drug/cyclodextrin complexes, CE is calculated from the slope of the phase-solubility diagram according to the following equation:

$$\mathrm{CE} = \frac{\text{slope}}{(1\text{-slope})}$$

Preparation of daidzein/cyclodextrin solid complexes

Aqueous solutions containing daidzein/cyclodextrin (β-CD, Mβ-CD, or HPβ-CD) complexes were prepared at a molar ratio of 1:2 (1.5 mM of daidzein and 3.0 mM of cyclodextrin) following the above-described phase-solubility study procedure, and freeze-dried (Edwards Modulyo 4 K, –60°C). Solid complexes samples were dissolved in methanol:water (1:1, v/v) (0.4 mg/mL), filtered through a 0.45-μm membrane (Millipore HVLP), and daidzein content was measured in triplicate by spectrophotometry at 250 nm.

Scanning electron microscopy

Photomicrographs of daidzein, cyclodextrins (β-CD, Mβ-CD, or HPβ-CD), and the corresponding solid complexes were taken at a voltage of 10 kV and a magnification of 1000× using a JSM 6060 microscope. Samples

were mounted on brass stubs using double-sided tape and vacuum-coated with a thin layer of gold.

NMR spectroscopy

NMR spectra of daidzein and daidzein/cyclodextrin solid complexes were recorded in a Bruker DRX400 AVANCE spectrometer operating at 27°C (300 K), 400 MHz to the hydrogen nucleus or 100 MHz to the carbon nucleus, equipped with direct detection ϕ5 mm $^{1}H/^{13}C$ dual probe and inverse detection ϕ5 mm probe with *z*-gradient coil, using D_2O or DMSO-d_6 as solvent and tetramethylsilane as internal standard (δ 0.0 ppm). One-dimensional ^{1}H and ^{13}C NMR spectra were acquired under standard conditions. Two-dimensional inverse hydrogen-detected heteronuclear shift correlation spectra were obtained by HSQC pulse sequence [^{1}J (C, H)] and HMBC pulse sequence [^{n}J(C, H); n = 2,3,4]. ^{1}H homonuclear correlation spectroscopy (COSY) and ^{1}H homonuclear 2D-ROESY (spinlock pulse= 600 msec) experiments were used to confirm the assignments of all carbon and hydrogen atoms of the samples. Nuclear Overhauser effect (NOE) correlations were used in order to confirm daidzein/cyclodextrin interactions and to get information about the geometry of the complexes, mainly by means of ^{1}H homonuclear 2D-ROESY experiment.

Association of daidzein or daidzein/cyclodextrin complexes to hydrophilic polymers

Daidzein/hydrophilic polymer binary systems

Excess amount of daidzein (1.5 mM) was added to 5.0 mL of water or aqueous solutions containing different concentrations of CMC, PEG, HPMC, or PVP (0.1–1.0%, w/w).

Daidzein/cyclodextrin/hydrophilic polymer ternary systems

Excess amount of daidzein (1.5 mM) was added to 5.0 mL of water or aqueous solutions containing increasing concentrations of β-CD, Mβ-CD, or HPβ-CD (0.75–6.0 mM), in the presence of HPMC or PVP (1.0%, w/w).

The resulting dispersions were stirred using a water bath at 37°C for 48 h and then filtered at room temperature through a 0.45-μm membrane (Millipore HAWP). Aliquots of the supernatant were diluted with methanol when necessary and daidzein content was measured in triplicate by spectrophotometry at 250 nm. The apparent stability constant and the complexation efficiency of daidzein/cyclodextrin/hydrophilic polymer systems were calculated according to the above-described equations.

Statistical analysis

A one-way analysis of variance was employed to evaluate the significance of the results obtained in the evaluation of the isolated or combined effect of cyclodextrins (6 mM) and hydrophilic polymers (1%, w/w) on the solubility of daidzein. Post-hoc multiple comparisons were performed by Tukey's test for significance at *P*-values <0.05.

Results and discussion

Phase-solubility study

As illustrated in Figure 2, the phase-solubility diagrams obtained for all daidzein/cyclodextrin complexes showed a linear relationship between increases in the solubility of daidzein and cyclodextrin concentration (R^2 > 0.99). According to Higuchi and Connors[16], these curves can be classified as A_L type.

The effect of different cyclodextrins on the solubility of daidzein in water and on the apparent stability constant and complexation efficiency of the corresponding complexes is presented in Table 1.

The intrinsic solubility of daidzein in water (S_o) was 0.0104 mM. In the presence of 6 mM of β-, Mβ-, or HPβ-CD, the solubility of daidzein enhanced significantly (P < 0.05): 5.7-, 7.2-, and 9.4-folds, respectively.

The apparent stability constants determined for daidzein/cyclodextrin complexes were 781 M^{-1} (β-CD), 1055 M^{-1} (Mβ-CD), and 1410 M^{-1} (HPβ-CD), suggesting a relatively strong interaction between the isoflavone and the cyclodextrins. According to previous studies reported by our group[18], the drug/cyclodextrin complexation efficiency can be influenced by the operating conditions employed in the phase-solubility studies. Thus, the excess amount of daidzein (1.5 mM) employed in our study may have contributed to the high apparent stability constant found for daidzein/HPβ-CD complex (K_s = 1410 M^{-1}) in comparison with the apparent stability constant obtained by Stancanelli et al.[7] when daidzein (0.018 mM) was complexed with the same cyclodextrin (K_s = 210 M^{-1}).

For poorly soluble drugs (aqueous solubility <0.1 mM), such as daidzein, the intrinsic solubility is in general much larger than the intercept of the phase-solubility diagram, resulting in an erroneous K_s value. Therefore, it can be more convenient to compare the CE than the K_s values since CE is less sensitive to errors related to estimation of intrinsic drug solubility[19]. The CE values obtained revealed that the solubilizing power of the cyclodextrins

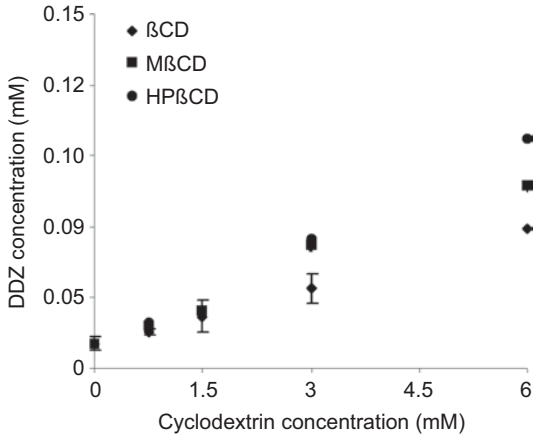

Figure 2. Phase-solubility diagrams of daidzein/cyclodextrin complexes (DDZ = daidzein, β-CD = β-cyclodextrin, Mβ-CD = methyl-β-cyclodextrin, HPβ-CD = hydroxypropyl-β-cyclodextrin); n = 3.

studied toward the daidzein follows the order HPβ-CD (0.0151) > Mβ-CD (0.0112) > β-CD (0.0083). Based on the CE values, the complex to free cyclodextrin concentration ratios were determined: only one out of every 67 (HPβ-CD), 90 (Mβ-CD), and 121 (β-CD) cyclodextrin molecules is able to form a water-soluble complex with daidzein in the medium. Among the three cyclodextrins studied, HPβ-CD presented the highest solubilizing effect since lower amounts of this cyclodextrin were required to solubilize the isoflavone.

Table 1. Isolated or combined effect of cyclodextrins (6 mM) and hydrophilic polymers (1.0%, w/w) on the solubility of daidzein in water and on the apparent stability constant and complexation efficiency of the corresponding associations ($n = 3$).

Sample[a]	Apparent solubility of DDZ (mM)	Solubility increase of DDZ (fold)	K_s[b] ($M^{(-1)}$)	EC[c]
DDZ + CMC	0.0117	1.1	—	—
DDZ + PEG	0.0125	1.2	—	—
DDZ + HPMC	0.0230	2.2	—	—
DDZ + PVP	0.0443	4.3	—	—
DDZ + β-CD	0.0593	5.7	781	0.0083
DDZ + β-CD + HPMC	0.0744	7.1	942	0.0100
DDZ + β-CD + PVP	0.0833	8.0	979	0.0104
DDZ + Mβ-CD	0.0745	7.2	1055	0.0112
DDZ + Mβ-CD + HPMC	0.0845	8.1	1102	0.0117
DDZ + Mβ-CD + PVP	0.1051	10.1	1336	0.0143
DDZ + HPβ-CD	0.0975	9.4	1410	0.0151
DDZ + HPβ-CD + HPMC	0.1080	10.4	1485	0.0159
DDZ + HPβ-CD + PVP	0.1319	12.7	1745	0.0188

[a]DDZ = daidzein, CMC = carboxymethylcellulose, PEG = polyethyleneglycol, HPMC = hydroxypropylmethylcellulose, PVP = polyvinylpyrrolidone, β-CD = β-cyclodextrin, Mβ-CD = methyl-β-cyclodextrin, HPβ-CD = hydroxypropyl-β-cyclodextrin.
[b]K_s = apparent stability constant.
[c]EC = efficiency complexation.

Scanning electron microscopy

The photomicrographs obtained for daidzein, cyclodextrins, and the corresponding solid complexes[20,21] are presented in Figure 3. Daidzein presented columnar particles (Figure 3A). β-CD (Figure 3B) and HPβ-CD (Figure 3D) particles had a parallelogram shape, whereas Mβ-CD (Figure 3C) is composed of spherical particles. Finally, all daidzein/cyclodextrin solid complexes were found to be lesser crystalline structures with soft and fluffy appearance (Figure 3E–3G). These observations are in agreement with the study reported by Figueiras et al.[20], which revealed by means of X-ray diffractometry analysis that host/guest solid complexes occur in the amorphous state. However, we cannot suggest an interaction between the two components (daidzein and cyclodextrin) because it was not possible to differentiate the morphology of the isolated compounds and that of the solid complexes.

NMR spectroscopy

The chemical shifts of hydrogens and carbons of daidzein and the chemical shifts of hydrogens of daidzein/cyclodextrin solid complexes are summarized in Table 2. The attributions were compared with the NMR spectral data previously reported in the literature[22,23]. The comparison between the one-dimensional ^{1}H NMR spectra obtained for daidzein (Figure 4A) and the three daidzein/cyclodextrin solid complexes (Figure 4B–4D) reveals changes in the signals intensity, probably due to the low concentration of daidzein in the solid complexes (~0.51%, w/w). Chemical displacements (~0.2 ppm downfield shift) were also observed in the hydrogen signals H6/H8 (A ring), H3′/H5′ (B ring), and H2 (C ring) of the isoflavone and suggested an interaction between daidzein and cyclodextrins due to perturbations in molecular electron density.

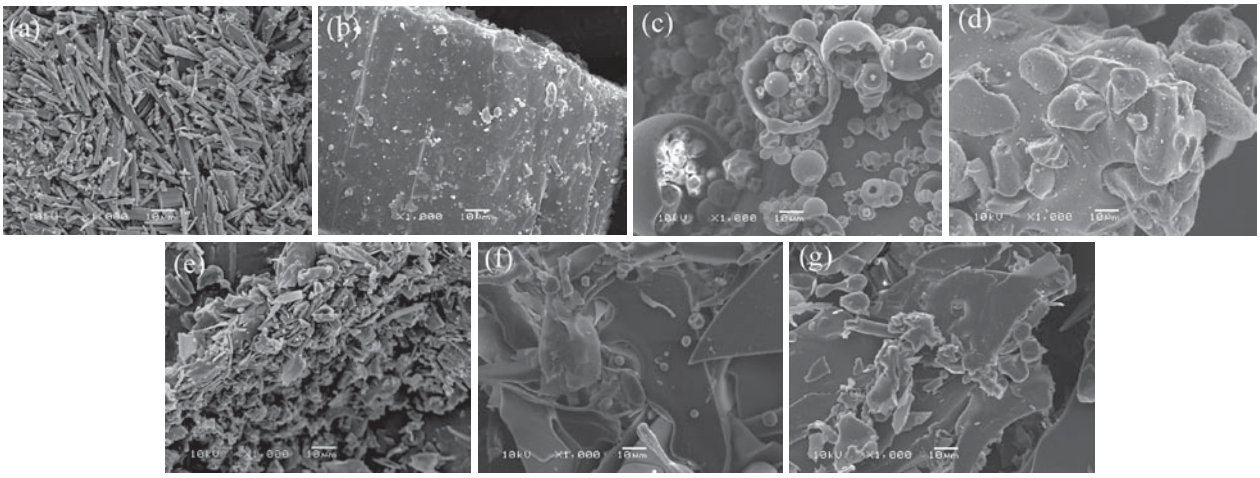

Figure 3. Photomicrographs obtained by scanning electron microscopy of (A) daidzein, (B) β-cyclodextrin (β-CD), (C) methyl-β-cyclodextrin (Mβ-CD), (D) hydroxypropyl-β-cyclodextrin (HPβ-CD), (E) daidzein/β-CD solid complex, (F) daidzein/Mβ-CD solid complex, and (G) daidzein/HPβ-CD solid complex.

Table 2. ^{1}H (400 MHz, DMSO-d_6) and ^{13}C (100 MHz, DMSO-d_6) spectral data of daidzein and ^{1}H (400 MHz, D_2O) spectral data of daidzein/cyclodextrin solid complexes obtained by nuclear magnetic resonance.

				δ_H[a] (ppm) daidzein/cyclodextrin complexes		
Atoms	δ_H[a] (ppm)	*J* (Hz) Daidzein	δ_C (ppm)	β-CD[b]	HPβ-CD[c]	Mβ-CD[d]
1	—		—	—	—	—
2	8.29 (s)		152.70	7.94 (b)	8.05 (s)	8.03 (s)
3	—		123.50	—	—	—
4	—		174.74	—	—	—
5	7.98 (d)	8.75	127.30	7.91 (b)	8.02 (b)	8.07 (b)
6	6.94 (dd)	8.75/2.33	115.10	6.77 (b)	6.90 (b)	6.98 (b)
7	—		162.63			
8	6.86 (d)	2.33	102.02	6.66 (b)	6.78 (b)	6.90 (b)
9	—		157.47	—	—	—
10	—		116.60	—	—	—
1′	—		122.56	—	—	—
2′/6′	7.39 (m)		129.96	7.31 (m)	7.37 (m)	7.39 (m)
3′/5′	6.82 (m)		114.89	6.97 (m)	6.96 (m)	6.94 (m)
4′	—		157.16	—	—	—
7-OH	9.63 (b)		—	—	—	—
4′-OH	9.63 (b)		—	—	—	—

[a](s) simplet, (d) duplet, (dd) double duplet, (m) multiplet, (b) broad.
[b]β-CD = β-cyclodextrin.
[c]HPβ-CD = hydroxypropyl-β-cyclodextrin.
[d]Mβ-CD = methyl-β-cyclodextrin.

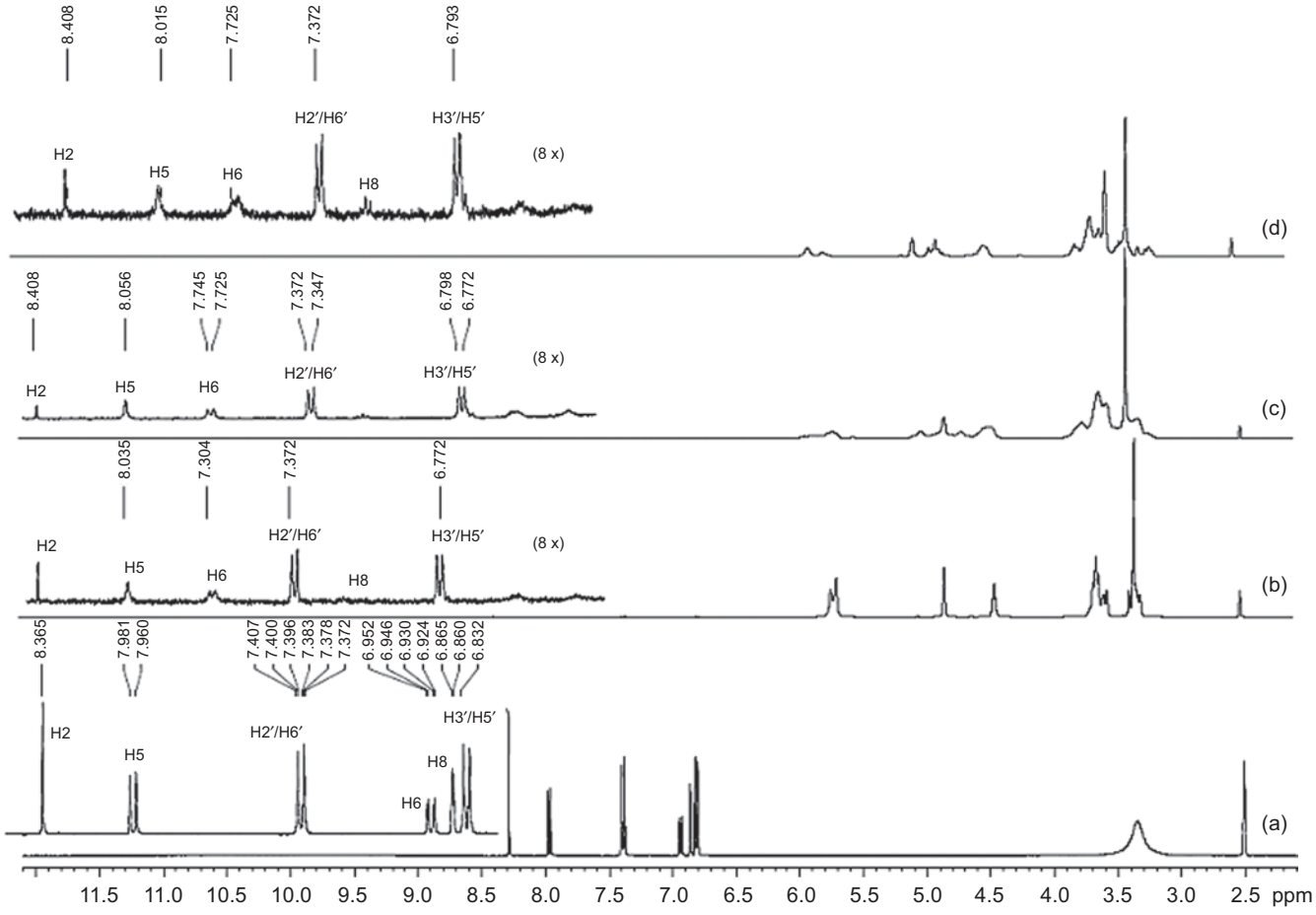

Figure 4. One-dimensional ^{1}H nuclear magnetic resonance spectra (400 MHz, DMSO-d_6) of (a) daidzein, (b) daidzein/β-cyclodextrin solid complex, (c) daidzein/hydroxypropyl-β-cyclodextrin solid complex, and (d) daidzein/methyl-β-cyclodextrin solid complex.

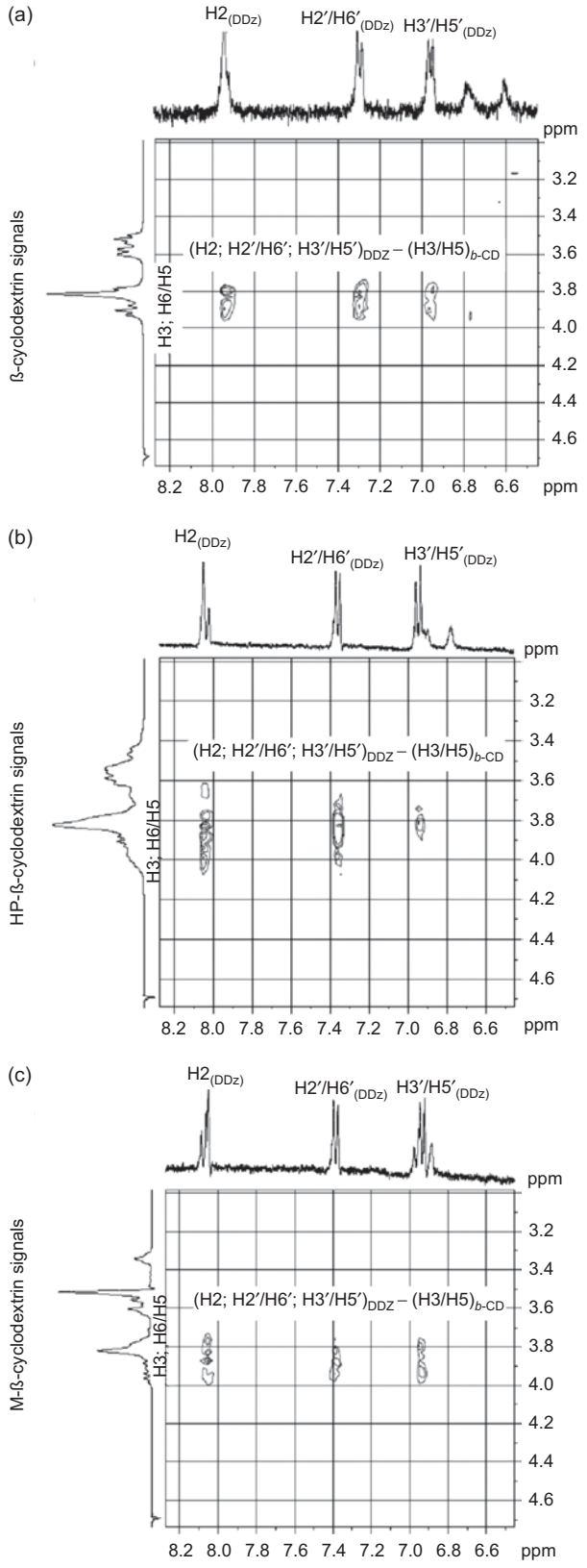

Figure 5. Expanded region of ^{1}H homonuclear 2D-ROESY contour map of solid complexes (400 MHz, D_2O) (A) daidzein/β-cyclodextrin complex, (B) daidzein/hydroxypropyl-β-cyclodextrin complex, and (C) daidzein/methyl-β-cyclodextrin complex (DDZ = daidzein).

Figure 5 presents expanded regions of ^{1}H homonuclear 2D-ROESY contour maps of daidzein/cyclodextrin solid complexes. The NOEs observed between the hydrogens H2, H2′/H6′, and H3′/H5′ of daidzein and the hydrogens H3 and H5 of β-CD (Figure 5A), HPβ-CD (Figure 5B), and Mβ-CD (Figure 5C) could only arise if daidzein/cyclodextrin inclusion complexes have been formed, since these hydrogens are located inside the cyclodextrins cavity. These data suggest, for the three solid complexes, that daidzein molecule (B and C rings) is situated in the inner face of the cyclodextrins. A recent study conducted by Daruházi et al.[24] reported that genistein, another isoflavone whose structure contains an additional hydroxyl group in the A ring (C5) in comparison with daidzein molecule, has been formed an inclusion complex with β-CD and that the mode of insertion into the cyclodextrin cavity would occur from the B ring, similar to our findings.

Association of daidzein or daidzein/cyclodextrin complexes to hydrophilic polymers

The isolated effect of different hydrophilic polymers and their combinations with cyclodextrins on the solubility of daidzein in water and on the apparent stability constant and complexation efficiency of the corresponding associations is presented in Table 1.

As illustrated in Figure 6, CMC and PEG did not present solubilizing effect over the concentration range of 0.1–1.0% (w/w). On the other hand, in the presence of HPMC or PVP (1.0%, w/w), the solubility of daidzein was significantly ($P < 0.05$) increased: 2.2- and 4.3-folds, respectively. Differences in the conformational structure, polymerization degree, charge density, and type of functional groups could explain the distinct effects of the hydrophilic polymers tested on the solubility of daidzein[25]. Therefore, HPMC and PVP were selected for investigating the effect of daidzein/cyclodextrin/hydrophilic polymer ternary systems on the solubility of the isoflavone.

The phase-solubility diagrams obtained for all daidzein/cyclodextrin/hydrophilic polymer ternary

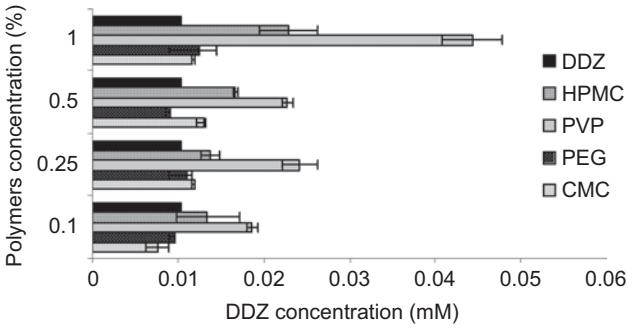

Figure 6. Solubility of DDZ (daidzein) in water in the presence of hydrophilic polymers (HPMC = hydroxypropylmethylcellulose, PVP = polyvinylpyrrolidone, PEG = polyethyleneglycol, CMC = carboxymethylcellulose) at different concentrations (0.1–1.0%, w/w); $n = 3$.

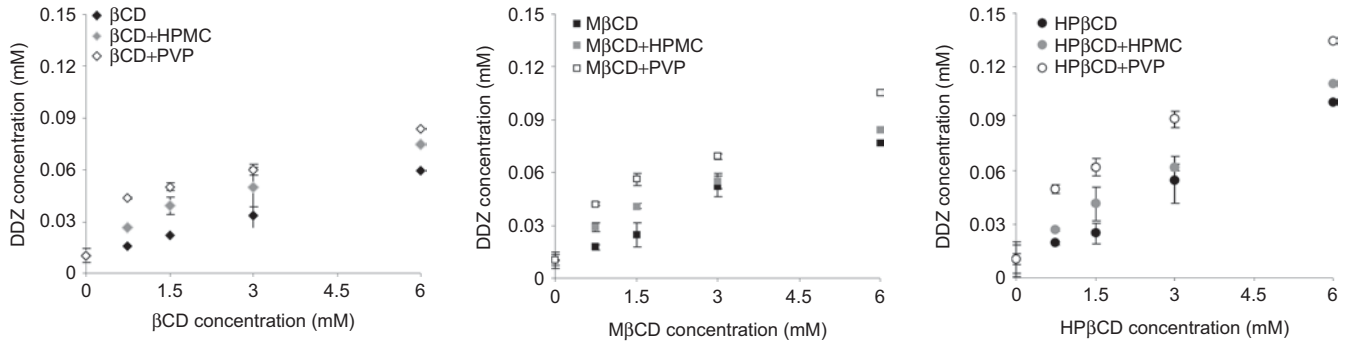

Figure 7. Phase-solubility diagrams of daidzein/cyclodextrin complexes (DDZ = daidzein, β-CD = β-cyclodextrin, Mβ-CD = methyl-β-cyclodextrin, HPβ-CD = hydroxypropyl-β-cyclodextrin) in the absence or presence of hydrophilic polymers 1.0%, w/w (HPMC = hydroxypropylmethylcellulose, PVP = polyvinylpyrrolidone); $n = 3$.

systems (Figure 7) showed similar behaviors (A_L-type curves), independent on the type of cyclodextrin and hydrophilic polymer, and revealed that the combined effect of daidzein complexation with cyclodextrins and its association with hydrophilic polymers (1%, w/w) was able to improve the solubility of daidzein and the apparent stability constant and the complexation efficiency of the associations even further. PVP and HPβ-CD, which presented the highest solubilizing effects in daidzein/hydrophilic polymer and daidzein/cyclodextrin binary systems, respectively, likewise revealed the highest solubility enhancement in daidzein/HPβ-CD/PVP ternary system (12.7-fold; $K_s = 1745\ M^{-1}$; CE = 0.0188; complex to free cyclodextrin concentration ratio = 1:54).

Conclusions

The present study revealed, for the first time, using SEM and NMR techniques, that the formation of inclusion complexes between the isoflavone daidzein and the cyclodextrins β-CD, Mβ-CD, or HPβ-CD is possible in aqueous media. These complexes, when associated to the hydrophilic polymers HPMC or PVP, allow high solubilization of daidzein. Such novelties are very relevant, since biological properties of daidzein are conditioned to its aglicone form, which presents reduced solubility in water.

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Declaration of interest

The authors report no declarations of interest.

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