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ORIGINAL ARTICLE

The effect of phosphate buffer solutions on uniconazole complexation with hydroxypropyl-β-cyclodextrin and methyl-β-cyclodextrin

Xiaofeng Zhu · Weiming Tan · Fan Zhou · Zhaohu Li · Liusheng Duan

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Abstract The inclusion complexes of uniconazole [(E)-1-(4-chlorophenyl)-4,4-dimethyl-2-(1,2,4-triazol-lyl)-1-penten-3-ol, UCZ] with two cyclodextrin derivatives, hydroxypropyl-β-cyclodextrin (HP-β-CD) and methylated-β-cyclodextrin (Me-β-CD), were prepared and characterized by ^{1}H NMR and FT-IR. The phase solubility of UCZ and HP-β-CD, UCZ and Me-β-CD, which displays the ability of CDs complexation and solubilization, was studied in aqueous solutions and phosphate buffer solutions (PBS) with different property pH values (6.2, 7.2, 8.0). The solubility results indicated that the pH of PBS showed more enhancement on the interaction of HP-β-CD and UCZ than Me-β-CD with the increasing pH value, and the optimal pH value for complexation of UCZ and HP-β-CD, UCZ and Me-β-CD was at 8.0 and at 7.2, respectively. These were also determined by UCZ release behavior and dissolution studies of the complexes in solid state.

Keywords Uniconazole · Hydroxypropyl-β-cyclodextrin · Methyl-β-cyclodextrin · Inclusion complex · PBS · Solubility · Complexation ability

Introduction

Plant growth regulators (PRGs) are playing important roles in crop production, which are increasingly being used to manipulate plant growth and yield. As a high-active member of the triazole PRGs family, uniconazole [(E)-1-(4-chlorophenyl)-4,4-dimethyl-2-(1,2,4-triazol-lyl)-1-penten-3-ol, UCZ] (Fig. 1), is increasingly applied in crops to induce a variety of morphological and biochemical responses in plants, including retarded shoot elongation, stimulated rooting, inhibited gibberellin biosynthesis, and protection from various environmental stresses, such as water deficit, salt injury, extreme temperature damage, waterlogging, sulphur dioxide and herbicide stress [1–3]. However, it has the same problems on its application as most PRGs, which are low water-solubility (8.41 mg/L), poor photo stability and low bioavailability [4, 5].

Cyclodextrins (CDs) are a cyclic oligosaccharides with hydrophilic outer surface and hydrophobic interior hollow [6, 7], which are constituted by six (α-), seven (β-) and eight (γ-) D-glucose units linked by β-1,4 glycosidic bonds [8]. Due to their special structure, CDs are able to encapsulate the hydrophobic compounds as guests to form host/guest complexes in aqueous solution. As one of the easily acquired member of CDs, β-CD has been widely used to improve stability, water-solubility and bioavailability of many drugs [9]. The native β-CD has a poor solubility in water (1.85 g/100 mL, 25 °C) and thus limiting its uses in drugs formulation [10]. In recent years, β-CD derivatives hydroxypropyl-β-cyclodextrin (HP-β-CD) and methylated-β-cyclodextrin (Me-β-CD) have been commonly used as the host to encapsulate the hydrophobic molecules since they have good water-solubility and surface activity [11]. Moreover, the host–guest forming process can be regulated by various factors, such as degree of substitution of Me-β-CD and HP-β-CD [10], substituted groups [10], and ionization degree of guest [12, 13].

So far, several studies have been performed on the reaction between β-CD derivatives and some poorly water-

The first two authors contributed equally to this work.

X. Zhu · W. Tan · F. Zhou · Z. Li · L. Duan (✉)
Engineering Research Center of Plant Growth Regulator, Ministry of Education, College of Agronomy and Biotechnology, China Agricultural University, Beijing 100193, People's Republic of China
e-mail: duanlsh@cau.edu.cn

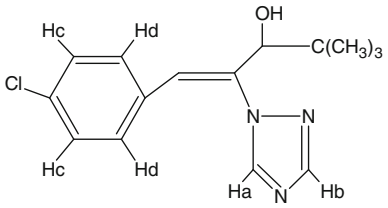

Fig. 1 Chemical structure of uniconazole

soluble or unstable pharmaceutical compounds, but the inclusion complex of PRG uniconazole has not yet been reported. In this work, the inclusion complexes of uniconazole with HP-β-CD and Me-β-CD were prepared respectively by co-precipitation method and confirmed by instrumental analysis (^{1}H NMR, FT-IR). To improve the complexation ability, different phosphate buffer solution (PBS) were chosen as the complexation environment of uniconazole and two β-CD derivatives (HP-β-CD, Me-β-CD), and the inclusion complexes manufactured in optimal PBS were also investigated.

Materials and methods

Materials

Uniconazole (purity $\geq$90%) was supplied by the Sevencontinent Green Chemicals (Zhangjiagang, Jiangsu, China). HP-β-CD (M_W = 1380–1500, degree of molecular substitution 0.8) and randomly Me-β-CD (M_W = 1310, degree of molecular substitution 1.3) were obtained commercially from Wacker Chemie AG (Germany). All the other materials and solvents were of analytical reagent grade. The PBSs (NaH_2PO_4/Na_2HPO_4) were used at pH 6.2, 7.2 and 8.0.

Solubility studies

Effects of HP-β-CD and Me-β-CD on the solubility of uniconazole were studied in water and in PBS. An excess of uniconazole (10 mg) was added into 10 mL PBS (pHs 6.2, 7.2 and 8.0) or water, containing various amounts of HP-β-CD or Me-β-CD in the 1–5 mM range, respectively. The suspensions were stirred at constant temperature 25.0 $\pm$ 0.1 °C for 72 h. Then, the suspensions were filtered through a 0.45 μm membrane filter (Millipore, USA) and analyzed by Cary 100Bio UV–Vis spectrophotometry (Varian, CARY 100) [14]. Each experiment was repeated at least three times and the results reported were the mean values. The stability constants (K_c) of the UCZ-HP-β-CD and UCZ-Me-β-CD complexes were calculated from the slope of the phase-solubility diagrams and the solubility of uniconazole in water (S_0) according to the previously reported method [15]:

$$K_c = \text{slope}/S_0(1 - \text{slope})$$

Preparation of the inclusion complexes

The accurately weighed HP-β-CD and Me-β-CD respectively dissolved in 20 mL distilled water and 20 mL pH 8.0 or pH 7.2 PBS to make 10% (w/v) CDs solutions, equimolar amount of uniconazole saturated acetone solutions were gradually added to the CDs solutions. The mixture solutions were stirred for 4 h at room temperature, and then kept at 4 °C overnight. The suspensions were filtered and washed by ethanol and distilled water successively, and then dried in vacuum drying oven at 60 °C.

Physical mixtures of uniconazole with CDs

The physical mixtures of uniconazole with β-CD derivatives were prepared by simply blending uniconazole and HP-β-CD or Me-β-CD with 1:1 molar ratio in a mortar, and noted as HP-β-CD/UCZ and Me-β-CD/UCZ, respectively.

Fourier-transform infrared spectroscopy (FT-IR)

The FT-IR spectra of uniconazole, two β-CD derivatives, two complexes, and the physical mixtures were measured as potassium bromide discs on a Nicolet 750 FT-IR Spectrophotometer.

^{1}H NMR

All ^{1}H NMR spectra were obtained in a Bruker spectrometer at 300 MHz, using dimethyl sulfoxide (DMSO-d6) as solvent, instead of other solvents such as D_2O or $CDCl_3$, to eliminate the hydrogen's disturbing to the complexes chemical shifts, and tetramethylsilane as internal standard.

Release study in water

The release behavior of uniconazole from inclusion complexes was conducted by placing suitable quantities of each inclusion complexes (containing $\sim$1.0 mg of uniconazole) in 200 mL distilled water, which was shaken gently at room temperature by a mechanical shaker. In this release behavior study, the water was not exchanged and uniconazole concentration was determined at different time intervals respectively, using the HPLC method [16]. Operating conditions of HPLC were UV-230 nm detection, ZOR-BA×SB C_{18} column (150 mm × 4.6 mm, 5 μm), methanol and water (75:25, v/v) as flow phase, the flow speed of 0.8 mL/min and 10 μL injection volume.

Dissolution studies

Dissolution studies using the USP XXIV rotating paddle apparatus (RCZ-8A, China) were carried out in diluted hydrochloric acid (0.1 M) at 37 °C for uniconazole, inclusion complexes of UCZ-HP-β-CD and UCZ-Me-β-CD. Suitable quantities of each powder containing 10 mg of uniconazole were compressed using a hydraulic press at an appropriate force to obtain discs which will not disintegrate under the test conditions. The discs were placed in 500 mL of the dissolution medium and the paddle speed was 100 rpm. The samples (1.0 mL) were withdrawn at prearranged time intervals and analyzed spectrophotometrically at 252 nm (Varian, Cary 100Bio UV–Vis spectrophotometry). Each experiment was carried out in triplicate.

Results and discussion

Phase-solubility analysis

The diagrams showed that the solubility increased in an approximately linear manner as a function of the HP-β-CD and Me-β-CD concentration (Figs. 2, 3). The obtained A_L-type curves leaded to the conclusion that soluble complexes having 1:1 stoichiometry were formed according to the present study [17]. It was observed that the apparent stability constants of HP-β-CD and uniconazole complex increased more than Me-β-CD with the increasing pH of PBS. This indicated that the pH of PBS enhanced the interaction of HP-β-CD and uniconazole more than Me-β-CD. Also, there was an optimal pH condition for both of the complexation of uniconazole with HP-β-CD and Me-β-CD, at pH 8.0 and pH 7.2 respectively (Table 1). Therefore PBS buffers at pH 8.0 and 7.2 were used for the rest of the study.

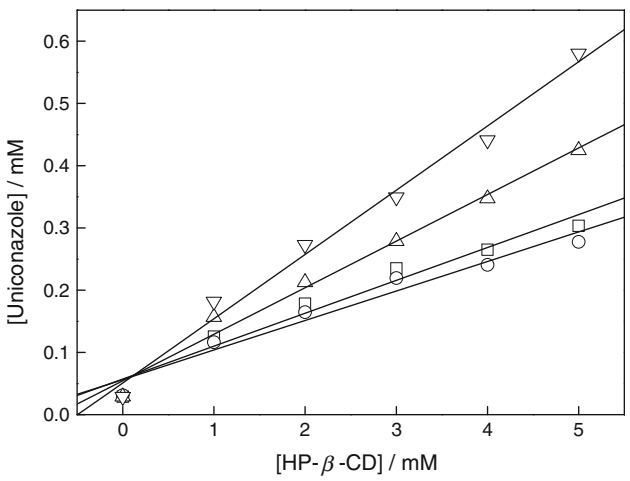

Fig. 2 Phase solubility diagram of the binary system uniconazole and HP-β-CD in water (*square*), PBS at pH 6.2 (*circle*), 7.2 (*triangle*) and 8.0 (*inverted triangle*)

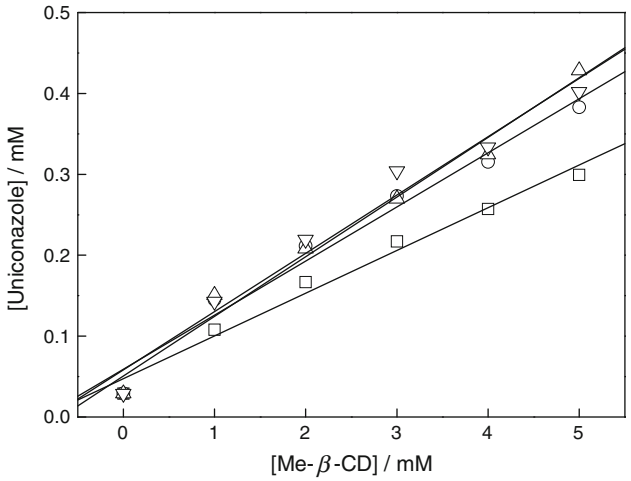

Fig. 3 Phase solubility diagram of the binary system uniconazole and Me-β-CD in water (*square*), PBS at pH 6.2 (*circle*), 7.2 (*triangle*) and 8.0 (*inverted triangle*)

In our previously work, we found the different PBS (pH 6.2, 7.2 and 8.0) all significantly reduced apparent stability constants of β-CD and uniconazole complex (data not shown), the strong interaction of the host and guest molecular was obtained in pure water. Take Consideration of the poor solubility of β-CD than HP-β-CD and Me-β-CD in water, it is difficult to get further formulations for UCZ-β-CD, even the β-CD and uniconazole complex was prepared [18].

^{1}H NMR studies

As the most powerful tool for the study of inclusion complex formation between CDs and a variety of guest molecules, NMR spectroscopy has been successfully used to confirm the conformations of inclusion complexes [19]. In the inclusion complexes, a shift in peaks can be observed for both the host and the guest [20], and almost all the CDs hydrogens presented up-field shift (Table 2). The chemical shifts of H-5 and H-3 located inside the cavity changed much more than others, probably due to some groups of uniconazole inserted into the cavity of CDs, which agreed with previously reports [21]. Because of the hydrogen signals shielded by the cavity after uniconazole complexation with CDs, the chlorophenoly and triazoly hydrogens shifted down-field (Table 3). This further proved that the complexes were formed. Most of the chlorophenoly hydrogens present changed in the chemical shift <0.010, resulting in the group inserted into the cavity probably being triazoly not chlorophenoly [22].

Table 1 Data of the phase-solubility curves of uniconazole with HP-β-CD and Me-β-CD at different pH values

Type of cyclodextrins	Solvent	Equation of regression curve	Correlation coefficient (R^2)	K_c (M^{-1})
Me-β-CD	Water	$y = 0.0528x + 0.0474$	0.982	1736.1 ± 30.9 c
	Buffer pH 6.2	$y = 0.0669x + 0.0590$	0.975	2164.6 ± 44.5 b
	Buffer pH 7.2	$y = 0.0750x + 0.0507$	0.981	2405.9 ± 28.9 a
	Buffer pH 8.0	$y = 0.0721x + 0.0583$	0.972	2321.0 ± 30.5 a
HP-β-CD	Water	$y = 0.0532x + 0.0573$	0.961	1746.5 ± 22.4 c
	Buffer pH 6.2	$y = 0.0478x + 0.0552$	0.957	1578.0 ± 39.1 c
	Buffer pH 7.2	$y = 0.0748x + 0.0544$	0.985	2401.8 ± 56.4 b
	Buffer pH 8.0	$y = 0.1032x + 0.0511$	0.988	3210.0 ± 102.7 a

Values in columns followed by the same letter are not significantly different according to Duncan's new multiple range test ($p < 0.05$)

Table 2 Change of ^{1}H NMR chemical shifts of CDs in the form of inclusion complex compared with free state

Type of cyclodextrins	$\Delta\delta$					
	External CDs protons			Internal CDs protons		
	H-1 (~4.65)	H-2 (~3.21)	H-4 (~3.33)	H-3 (~3.58)	H-5 (~3.75)	H-6,6′ (~4.23)
Me-β-CD	0.009	0.039	0.016	0.049	0.049	0.020
HP-β-CD	0.012	0.022	0.017	0.033	0.037	0.013

Table 3 Change of ^{1}H NMR chemical shifts of uniconazole in the form of inclusion complex compared with free state

Type of inclusion complexes	$\Delta\delta$			
	H_a	H_b	H_c	H_d
Me-β-CD-UCZ	−0.022	−0.021	−0.006	−0.011
HP-β-CD-UCZ	−0.027	−0.030	−0.008	−0.004

FT-IR studies

The inclusion complexes were also analyzed by FT-IR spectroscopy, which was a further supporting evidence for the formation of inclusion complex [23]. The infrared spectrum of uniconazole showed the presence of peaks at 3286 cm^{-1} (free –OH stretching vibration), 1504, 1493 cm^{-1} (stretching vibration of C=C band in chlorophenol ring) and 1273, 1192 cm^{-1} (stretching vibration of C=N band in triazole ring) (Figs. 4, 5). Spectra of all binary systems did not show new peaks indicating that no chemical bonds were created in the formed compounds. Compared with the free state of uniconazole, the characteristic absorption peaks of uniconazole from the spectra of UCZ-Me-β-CD and UCZ-HP-β-CD complexes were much broader and weaker, while the FT-IR spectra of the physical mixtures of UCZ/Me-β-CD and UCZ/HP-β-CD were no significantly different from the spectrum of CDs or uniconazole. This could be probably due to the drug complexation with CDs, which was consistent with previous reports [24, 25].

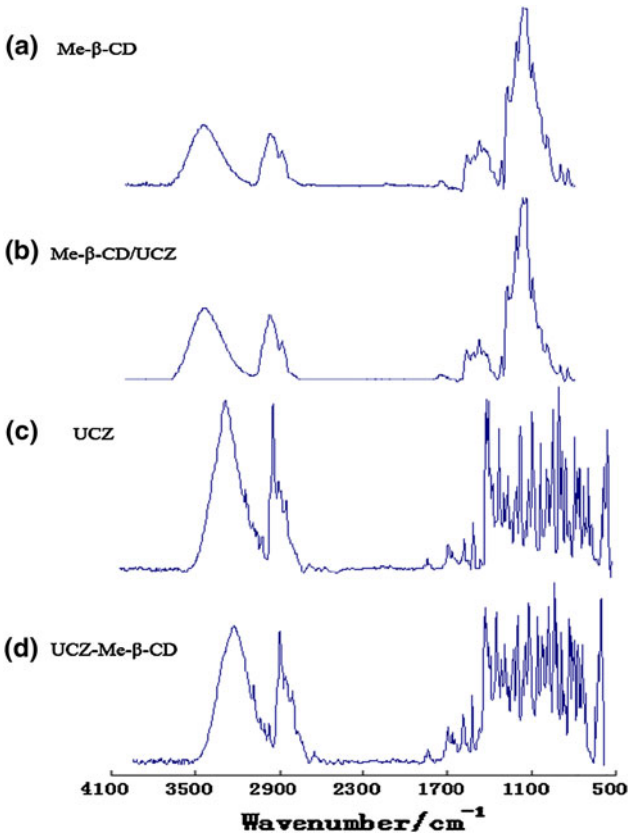

Fig. 4 FT-IR spectra of UCZ-Me-β-CD system: (*a*) Me-β-CD; (*b*) physical mixture; (*c*) uniconazole; (*d*) inclusion complex

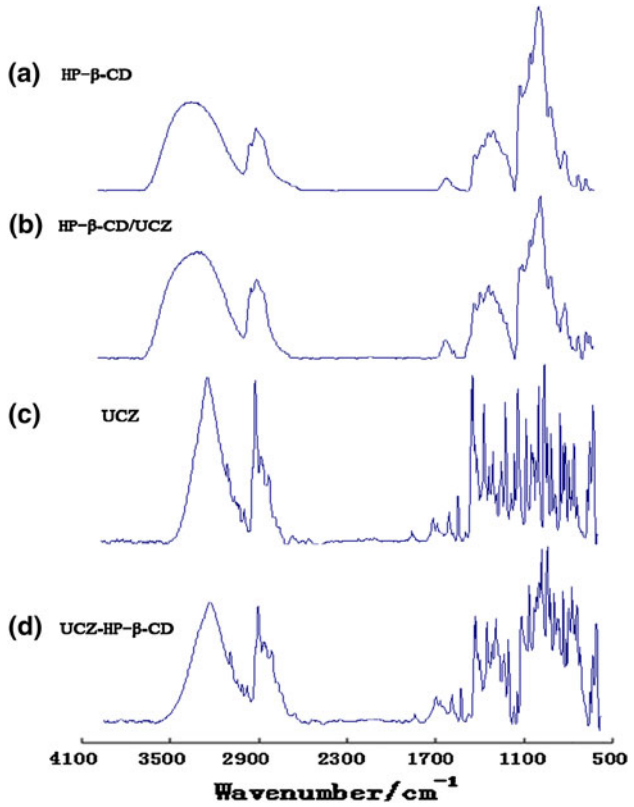

Fig. 5 FT-IR spectra of UCZ-HP-β-CD system: (*a*) HP-β-CD; (*b*) physical mixture; (*c*) uniconazole; (*d*) inclusion complex

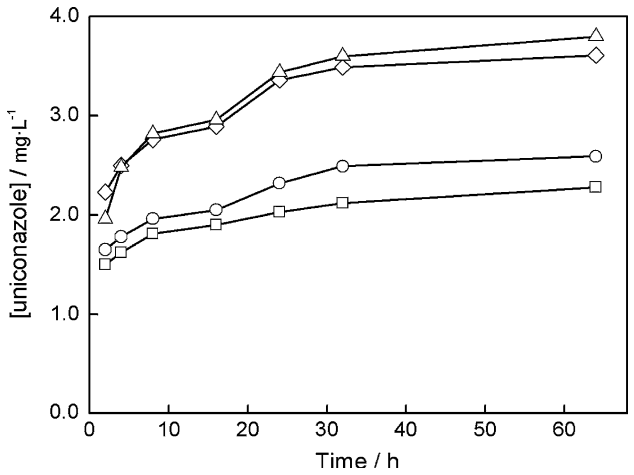

Fig. 6 Curves of uniconazole release from the inclusion complex of UCZ-Me-β-CD (*triangle* and *circle*) and UCZ-HP-β-CD (*diamond* and *square*) with inclusion conditions under the solvent of host molecular being water (*triangle* and *diamond*) and optimal PBS (*circle* pH 7.2; *square* pH 8.0)

Releasing effect of the inclusion complexes

The release profiles of uniconazole from all the inclusion complexes were similar in distilled water at room temperature. But the release values of uniconazole from the UCZ-Me-β-CD and UCZ-HP-β-CD with inclusion proceeded in water were much higher than those of in the optimal PBS (Fig. 6). This was probably attributed to the difference of complexation ability of the inclusion complexes, which was in accordance with the results of the stability constants [26]. It could also be concluded that the host–guest intermolecular force of UCZ-Me-β-CD or UCZ-HP-β-CD with inclusion proceeded in water were weaker than those of in the optimal PBS.

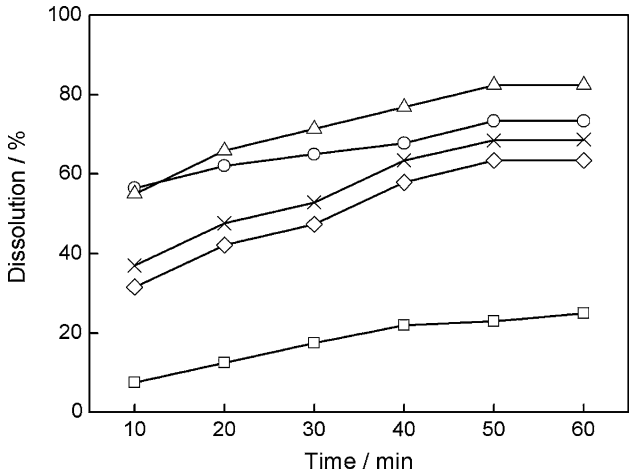

Fig. 7 The dissolution diagram of uniconazole at 25 °C uniconazole (*square*); UCZ-HP-β-CD inclusion performed in water (*circle*) and buffer pH 8.0 (*diamond*); UCZ-Me-β-CD inclusion performed in water (*triangle*) and buffer pH 7.2 (*cross*)

Dissolution studies

Significant differences were observed between the dissolved drug amounts in pure and inclusion complexes as well as in the dissolution rate (Fig. 7), and existing an certain extent increase of solubility after uniconazole complexed with CDs, as agreed with the earlier researches [27]. Among the complexes, the dissolution rates of the ones with inclusion performed in optimal PBS were much faster than in water.

Conclusion

PRG uniconazole could be complexed by both Me-β-CD and HP-β-CD, and the inclusion complexes were characterized by ^{1}H NMR and FT-IR. The pH of PBS had obvious influences on the interaction between uniconazole with Me-β-CD and HP-β-CD, and especially in the latter. Among three different property pH values, the optimal pH for inclusion between uniconazole with Me-β-CD and HP-β-CD were selected. From drug release studies and

dissolution studies, it could be observed that the inclusion complexes manufactured in optimal PBS displayed a better solubility and stability in water.

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