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ORIGINAL ARTICLE

A thermodynamic study of the cyclodextrin-UC781 inclusion complex using a HPLC method

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Abstract UC781, a very potent HIV-1 non-nucleoside reverse transcriptase inhibitor with extreme hydrophobicity and poor water solubility, is under development as a topical vaginal microbicide product to prevent HIV transmission. In this study, the thermodynamic behavior of the interaction between UC781 with three cyclodextrins (CDs): β-cyclodextrin (βCD), hydroxypropyl-β-cyclodextrin (HPβCD) and methyl-β-cyclodextrin (MβCD), was investigated using a reversed-phase HPLC method. A mobile phase consisting of acetonitrile: H_2O (30:70) solution containing various CD concentrations was used. The retention time at different temperatures was determined to evaluate the inclusion process. The influence of βCDs on the solubility and hydrophobicity of UC781 was characterized by retention time values. The results showed that the inclusion capacity of cyclodextrins follows the order MβCD > βCD > HPβCD. An enthalpy–entropy compensation effect was also observed. In addition, the results revealed that the change of ΔH is greater than that of ΔS. These results suggested that the complexation of UC781 with βCDs is an enthalpy driven process. The modification on β-cyclodextrin will influence the inclusion process.

Keywords UC781 · β-Cyclodextrin inclusion complex · Non-nucleoside reverse transcriptase inhibitor (NNRTI) · Thermodynamics · HPLC · Enthalpy

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Introduction

Cyclodextrins (CDs) are cyclic (α-1,4)-linked oligosaccharides with different numbers of α-D-glucopyranose units [1]. Cyclodextrins are produced from the enzymatic degradation of starch by bacteria [2, 3]. α-Cyclodextrin (αCD) comprises six glucopyranose units, β-cyclodextrin (βCD) seven such units, and γ-cyclodextrin (γCD) eight such units. The inner diameter of the relative hydrophobic cavity is approximately 4.7–5.3, 6.0–6.5, and 7.5–8.3 Å for αCD, βCD, and γCD, respectively [4, 5]. Thus, each cyclodextrin has its own ability to form host–guest inclusion complexes by partially or fully incorporating specific guest molecules with appropriate dimensions into the hydrophobic cavities of cyclodextrins. The chemically modified βCDs, particularly hydroxypropyl-β-cyclodextrin, have received considerable attention due to their pharmaceutical properties and safety profile as compared to the other βCDs [6]. Their application in pharmaceutical research is known to bring about an enhancement of the solubility [4, 6], chemical stability [1, 4], permeability [7], and bioavailability [8, 9] as novel drug carriers.

The thiocarboxanilide UC781, (*N*-[4-chloro-3-(3-methyl-2-butenyloxy) phenyl]-2-methyl-3-furancarbothioamide) (Fig. 1), is a tight-binding HIV-1 non-nucleoside reverse transcriptase inhibitor (NNRTI) that is an extremely potent inhibitor of HIV-1 in cell culture [10–12]. It belongs to the class of (thio)carboxanilide derivatives, the prototype of which (UC84) was originally reported as an antiviral agent by Bader et al. [13]. UC781 can neutralize several laboratory and clinical strains of HIV-1, including both syncytium- and non syncytium-inducing phenotypes [10]. It has also been shown to inhibit HIV-1 strains which are resistant to nucleoside RT inhibitors with potency similar to that for inhibition of wild-type virus [14]. Furthermore, UC781 targets HIV-1 RT and is effective against a variety of NNRTI resistant HIV-1 strains. Importantly, high resistance to UC781 is only obtained when more than one mutation occurs in the NNRTI binding pocket [12]. However, the extremely high hydrophobicity and low solubility (<30 ng/mL) of UC781 poses a huge challenge in formulation development of UC781.

A β-cyclodextrin based drug delivery system has been developed in our laboratory to enhance the aqueous solubility of UC781 [15]. The thermodynamic properties are important for the understanding and processing method optimization of βCD complex development. Several physical–chemical methods have been used to characterize the thermodynamic properties of inclusion complexes including UV spectroscopy [16, 17], NMR spectroscopy [17], as well as liquid chromatographic methods, such as thin layer chromatography [18] and high performance liquid chromatography (or High Pressure Liquid Chromatography HPLC) system [19, 20]. The difference on retention characteristics between complexed drug and neat drug [21, 22] can be used to evaluate their thermodynamic properties.

The aim of this paper is to examine the inclusion and thermodynamic properties of UC781 with natural and modified βCDs using HPLC method and to compare the complexation ability of UC781 with these different βCDs.

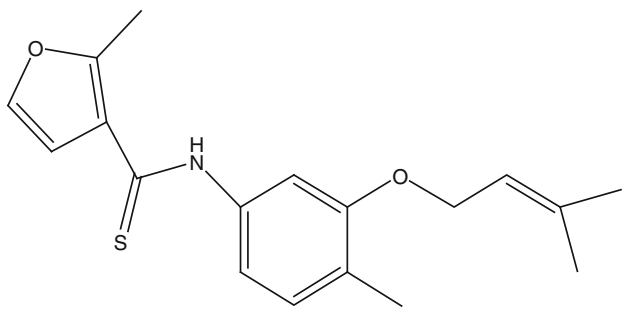

Fig. 1 Structure of UC781 *N*-[4-chloro-3-(3-methyl-2-butenyloxy) phenyl]-2-methyl-3-furancarbothioamide (MW:335.48)

This HPLC method is advantageous in that it requires only a limited amount of material and full evaluation can be completed quickly.

Materials and methods

Materials

UC781 for these studies was initially provided by Biosyn Co. Ltd. (Huntington, PA). However the licensing rights to this drug were transferred to CONRAD who provided subsequent supply as needed. βCD (MW 1134), MβCD (MW: ~1320; mean degree of substitution: 1.7–1.9), and HPβCD (MW: ~1380 and mean degree of substitution: 0.8) were purchased from Sigma-Aldrich (St Louis, MO). All other reagents used were of reagent grade and all solvents were of HPLC grade. Milli-Q water was used to prepare buffer solutions and other aqueous solutions.

Experimental

HPLC analysis for UC781 was briefly described as follows: a Waters HPLC system equipped with an auto injector model Waters 717, and a Waters 2487 dual wavelength (λ) absorbance detector at 300 nm were used (Waters Corporation, Milford, MA). Separation of compound in the experiment was achieved by using an Alltech ODS-C8 column (4.6 mm i.d. × 7.5 mm, 5 μm, Columbia, MD). A mobile phase of acetonitrile (ACN)/milliQ water (30:70 v/v) containing different CD concentrations at a flow rate of 1.0 mL/min was used. Temperature (from 25 to 50 °C) was controlled using a water bath within a range of ±0.1 °C. UC781 was dissolved in ACN at a concentration of 3 μg/mL. 10 μL of the UC781 solution was injected for the thermodynamic study. The retention time of UC781 was recorded for thermodynamic parameters calculations.

Retention time of UC781 was determined using HPLC method. $K_{1:1}$ (complexation constant between drug and cyclodextrin) is calculated based on the capacity factor change described in the results section. Thermodynamic properties, such as ΔG, ΔH, and ΔS were calculated according to Van't Hoff equation.

HPLC theoretical treatment of UC781-cyclodextrins inclusion process

HPLC is a separation technique using column chromatography to separate, identify, and quantify compounds. Retention time (t_R) of drugs in HPLC varies depending on the interactions between the stationary phase, the molecules being analyzed, and the solvent(s) used. In a given HPLC system, t_R can be thought of as the character of the

drug itself, reflecting the interaction between drug and the stationary phase.

In addition to t_R, another important parameter used in this study is "capacity factor" (K_c). Historically, a slightly different retention parameter, K_c was introduced by the analogy based on the liquid partitioning theory and is widely accepted in chromatographic practice. K_c is dimensionless and independent of any geometrical parameters of the column or HPLC system. It can be considered a thermodynamic characteristic of the solid phase-compound-mobile phase system [23]. K_c is defined by Eq. 1 [24]

$$K_c = \frac{M_s}{M_m} = \frac{t_{drug} - t_m}{t_m} \quad (1)$$

where *s* is the solid phase and *m* is the mobile phase; M_s is the mass of analyte in the solid phase, M_m is the mass of analyte in the mobile phase; t is the retention time of drug (t_{drug}) and mobile phase (t_m).

In a given mobile phase, the following equilibrium can be reached between drug and solid phase. The mass balance can be expressed as Eq. 2

$$[\text{Drug}] + [\text{S}] \Leftrightarrow [\text{Drug} - \text{S}] \quad K_1 = \frac{[S - \text{Drug}]}{[\text{Drug}][S]} \quad (2)$$

where S is the solid phase of column; K_1 is the binding constant for drug and solid phase;[Drug] is free drug in mobile phase; and [Drug−S] is drug binding to solid phase. When CD is added into the mobile phase, equilibrium can occur between the drug and CD. This equilibrium can be expressed as Eq. 3

$$[\text{Drug}] + [\text{CD}] \Leftrightarrow [\text{Drug} - \text{CD}] \quad K_{1:1} = \frac{[\text{CD} - \text{Drug}]}{[\text{Drug}][\text{CD}]} \quad (3)$$

$K_{1:1}$ is the complexation constant of the drug and cyclodextrin; the total drug in the HPLC system can be given by Eq. 4

$$[\text{Drug}]_{\text{Total}} = [\text{Drug} - \text{S}] + [\text{Drug}]_m + [\text{Drug} - \text{CD}] \quad (4)$$

here $[\text{Drug}]_{\text{Total}}$ is total drug in HPLC; [Drug−S] is drug in solid phase; $[\text{Drug}]_m$ is free drug in mobile phase; and [Drug−CD] is drug in complexed for with cyclodextrin in mobile phase. Combining Eqs. 4 and 1 obtain Eqs. 5 and 6

$$\frac{M_s}{M_m} = \frac{[\text{Drug} - \text{S}]}{[\text{Drug}]_m + [\text{Drug} - \text{CD}]} = K_c = \frac{t_{drug} - t_m}{t_m} \quad (5)$$

$$\frac{[\text{Drug}] \cdot [\text{S}] \cdot K_1}{[\text{Drug}]_m + [\text{Drug}] \cdot [\text{CD}] \cdot K_{1:1}} = K_c \quad (6)$$

the drug concentration and retention time are now correlated with each other through K_c. Here, [Drug] equals to free drug in mobile phase $[\text{Drug}]_m$. Re-arranging the expression gives an Eqs. 7 and 8

$$\frac{1}{K_c} = \frac{t_m}{t_{drug} - t_m} = \frac{1 + K_{1:1} \cdot [\text{CD}]}{[S] \cdot K_1} \quad (7)$$

$$\frac{t_m}{t_{drug} - t_m} = \frac{K_{1:1}}{[S] \cdot K_1} \cdot [\text{CD}] + \frac{1}{[S] \cdot K_1} \quad (8)$$

Thus, plotting $\frac{t_m}{t_{drug} - t_m}$ versus [CD] will results in a linear relationship between the t_{drug} $\left(\frac{t_m}{t_{drug} - t_m}\right)$ and βCD concentration ([CD]) where the slope $= \frac{K_{1:1}}{[S] \cdot K_1}$ and intercept $= \frac{1}{[S] \cdot K_1}$. $K_{1:1}$ under the experimental conditions can be calculated using Eq. 9

$$K_{1:1} = \text{slope/intercept} = \frac{K_{1:1}/([S] \cdot K_1)}{1/([S] \cdot K_1)} \quad (9)$$

Results and discussion

Inclusion behavior of βCDs with UC781

The effect of CD concentration on UC781 retention time is shown in Table 1. A significant decrease in UC781 t_R was observed with increasing βCDs concentration in the mobile phase for all types of cyclodextrin. Both HPβCD and MβCD exhibit better solubility properties than native βCD in mobile phase which greatly enhance the complexation ability with UC781. These results indicate that the hydrophobicity of UC781 controls its retention on column and complexation with βCDs in mobile phase. In addition, amount by which the UC781 retention time was reduced was observed to be greater with changes in MβCD concentration as compared to those with observed with changes in HPβCD concentration. This result demonstrates a stronger complexation ability of MβCD with UC781 than that of HPβCD. The effect of βCD on retention time was not compared with that of MβCD and HPβCD due to its limited solubility in mobile phase.

The effect of βCDs on the lipophilicity change of UC781

The partition coefficient is a parameter that is used to describe the degree of lipophilicity of a compound typically expressed as logP specifically; it is experimentally determined by evaluating the distribution of a compound between two immiscible phases (such as octanol and water). Drug molecules with larger logP values are more lipophilic and tend to be less able to be dissolved into water. This parameter is useful in estimating distribution and permeability of the drug within the body [25–27]. However, the traditional shake-flask method [28, 29], does not provide a rapid determination of lipophilicity. In addition, this method requires a large amount of compound

Table 1 Effect of βCDs on the retention time of UC781 in HPLC Studies

	Concentration %	0.5	1	2	4	5
MβCD	t_R (min)	34.18	31.36	26.47	19.81	17.65
HPβCD		32.89	31.49	30.62	28.52	27.25
	Concentration %	0.1	0.2	0.3	0.4	0.5
βCD	t_R (min)	36.99	34.67	33.68	31.84	32.20

when the compound is very lipophilic. A HPLC method was used to rapidly determine logP from K_w [30, 31]. K_w is the capacity factor when the mobile phase is pure water. It has been demonstrated that the extrapolated logarithm of the capacity factor for a pure aqueous eluent (log k_w) gives the best correlation with logP [32]. Moreover, the relationship between the logarithm of the capacity factor (log K_c) and log K_w can be expressed through Eq. 10

$$\log K_c = A \cdot [C] + \log K_w \quad (10)$$

where A is the slope, C is the volume fraction of organic solvent in mobile phase and log K_w the intercept of the regression curve. In these studies, the CD concentration change was used instead of the change in organic solvent for the calculation of log K_w since it was desired to determine the impact of CD on lipophilicity. The log K_w is a fixed value obtained from Eq. 10 in these studies. Therefore, the slope values obtained from linear regression can be compared to evaluate the complexation ability of the three βCDs with UC781.

Figure 2 represents the logarithm of the K_c of UC781 as a function of CD concentration in the mobile phase. In the presence of βCDs in the mobile phase, log K_c of UC781 decreased with increasing CD concentration. Linear relationships between log K_c and CD concentration in the mobile phase were observed, indicating a classical reverse phase elution mechanism [20, 33]. The absolute value of slopes (Table 2) obtained in the linear regression treatment

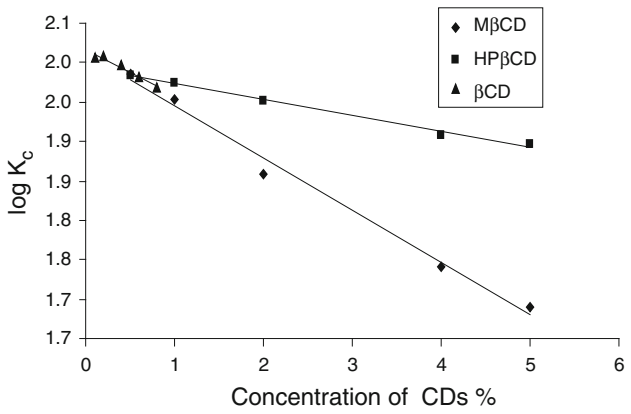

Fig. 2 Influence of βCDs on the lipophilicity of UC781 at 30 °C

Table 2 Data from linear regression of Eq. 10 (slopes, log K_w, and correlation coefficients (r^2))

	Slope	Log K_w	r^2
βCD	−0.057	2.01	0.96
MβCD	−0.066	2.01	0.99
HPβCD	−0.020	1.99	0.99

followed a rank order of MβCD > βCD > HPβCD, which also corresponds with the retention time changes shown in Table 1. This phenomenon can be explained by the alteration of hydrophobic interaction between UC781 and the solid phase in the HPLC column, which is induced by the complexation of UC781 with CD in the mobile phase. Furthermore, the substitution of different groups on βCD can cause steric interactions between gust molecules and βCD. The 2-hydroxypropyl groups may occlude the entrance for the guest molecules which hinder the complexation ability [34, 35]. The cyclodextrin cavity is lengthened upon methylation of the OH(2) and OH(6) groups of the cyclodextrin rim without significant distortion of the ring, and this would enhance the complexation ability of MβCD [36, 37]. The results also indicate that MβCD has a better ability to form a complex with UC781 under these experimental conditions.

The effect of temperature on the inclusion process of UC781 with βCDs

The effect of temperature on the inclusion constants of UC781 with different βCDs is given in Fig. 3. ΔG values were calculated from the inclusion constants and are listed in Fig. 4. $K_{1:1}$ and absolute value of ΔG change decreased with increasing temperature. The results reveal that higher temperature is unfavorable for the inclusion process, which can be explained by extensive movement of molecules at high temperature leading to the disassociation of the cyclodextrin complex.

Inclusion process thermodynamics

All ΔG values shown in Fig. 4 are negative, which suggests that the inclusion process can proceed spontaneously.

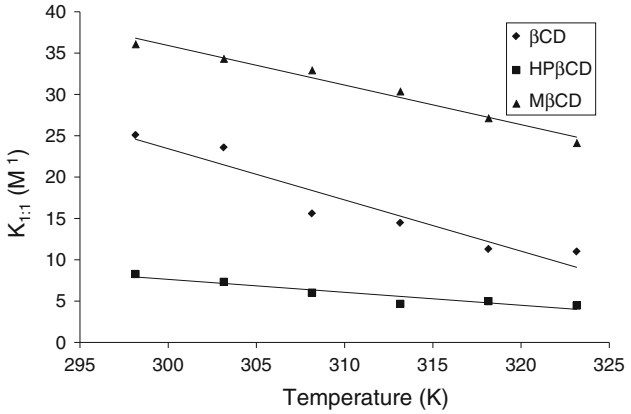

Fig. 3 $K_{1:1}$ values obtained for UC781-βCD complexes at different temperatures

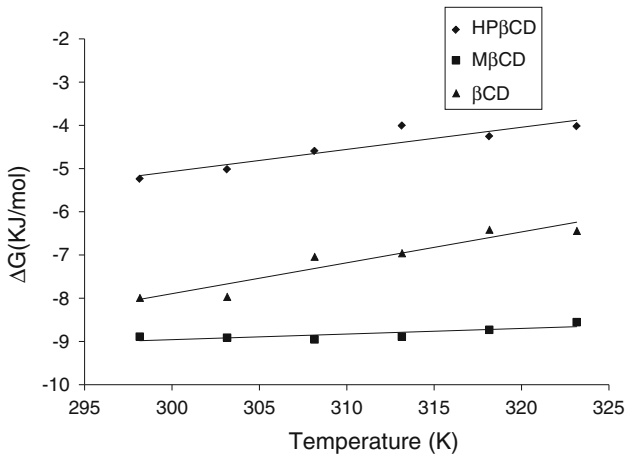

Fig. 4 Temperature effect on Gibbs free energy change for UC781 complexation with βCDs

Given the ΔG values, other thermodynamic parameters can be obtained using the Van't Hoff equation. Enthalpy (ΔH) and entropy (ΔS) changes for complexes were calculated as following:

$$\frac{\Delta G}{T} = \Delta H \frac{1}{\mathrm{T}} - \Delta S. \tag{11}$$

The slope and the intercept of the plot of ΔG/T versus 1/T are ΔH and –ΔS, respectively. Figure 5 represents the plot of ΔG/T versus 1/T for the three βCDs. The ΔH and ΔS values are shown in Table 3. Negative values for ΔH mean that the inclusion process is exothermal and low temperature is suitable for the inclusion process. A relatively large negative ΔH and small negative ΔS suggests that the inclusion process of UC781 with βCDs is primarily an enthalpy driven process indicating that Van der Waals force is the major driving force in this complex formation [4, 38].

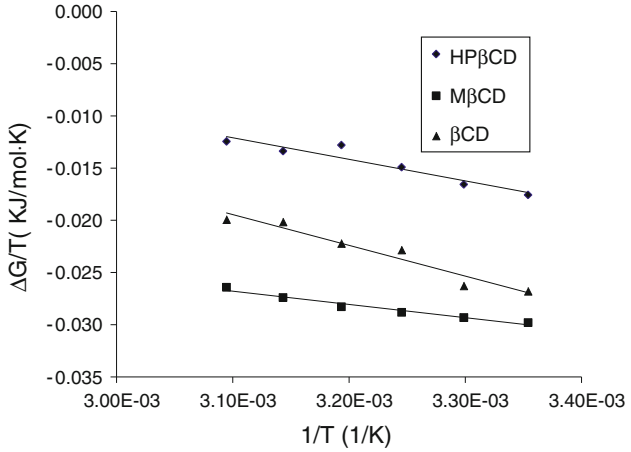

Fig. 5 The relationship between ΔG/T and 1/T for UC781 complexation with cyclodextrins

Table 3 Enthalpy–entropy values for UC781 cyclodextrin complexes

	ΔH KJ/mol	ΔS J/mol
βCD	−29.42	−0.072
MβCD	−12.69	−0.013
HPβCD	−20.64	−0.052

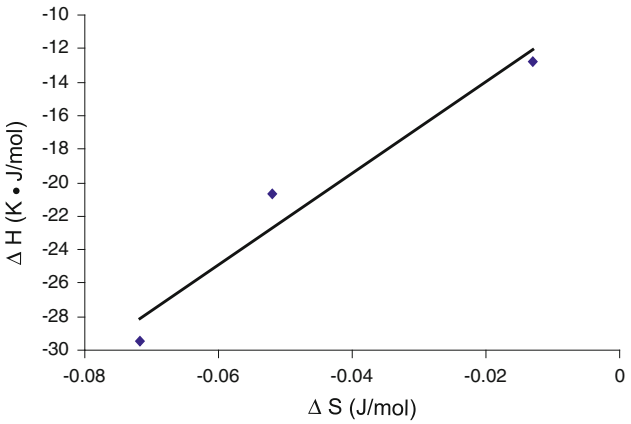

Fig. 6 Enthalpy–entropy plot for UC781-βCDs inclusion complexes

Enthalpy–entropy compensation is the phenomenon in which the change in enthalpy is offset by a corresponding change in entropy resulting in a smaller net free energy change. It is believed to play an important role in reactions in solution [39, 40]. The enthalpy-entropy compensation relationship has been observed in these βCDs complex [41]. The enthalpy–entropy compensation is also attributed to the interactions with water molecules in the βCD cavity [42]. In this study, the plot of ΔH versus ΔS shows a straight line (Fig. 6). The slope is the compensation temperature, T = 304 K. It demonstrates that the inclusion

process also displays an enthalpy–entropy compensation effect [43].

Conclusions

Different mechanisms of complex formation were widely investigated for decades. The formation of cyclodextrins complex may involve several driving forces: Van der Waals interactions, hydrophobic interactions, exclusion of cavity-bound high-energy water, hydrogen bonding, electrostatic interactions, etc. [4, 44]. The thermodynamic properties of complexation are considered important for governing the complex formation between cyclodextrin and guest molecule. The enthalpy and entropy changes of the complex formation can be utilized to analysis the major driving force for the formation of cyclodextrin complex in spite of the anthalpy–entropy compensation. Although, the extent of complex formation mainly depends on the chemical properties of the guest molecule itself. Many auxiliary methods can be used to further enhance the formation of complex, such as change the species and concentration of cyclodextrins, temperature, procedures of complex formation, pH, co-solvent, etc. which can reflect the changes on the thermodynamic properties [4].

The thermodynamic behavior of the complexation between UC781 and three different βCDs was investigated systematically using a HPLC method utilizing retention difference between UC781 and its complex. The inclusion ability and hydrophilicity enhancement effects of βCDs on UC781 were found to follow the rank order of MβCD > βCD > HPβCD which can be explained by sterically influence caused by different substitution groups. The thermodynamic behavior of UC781 and all the βCDs studied were shown to be an enthalpy-driven processes suggesting that Van der Waals forces plays an important role in the complexation. Enthalpy–entropy compensation was also observed for complexation of UC781 with βCDs indicating a replacement of water molecules in the cyclodextrin cavity with UC781.

The HPLC method provides rapid evaluation of the thermodynamics of inclusion and requires minimal amount of material. The results from this study increase our understanding of the mechanism of complexation of UC781 with βCD and its derivatives and offer useful information which can be utilized in the formulation development of a cyclodextrin based drug delivery system for UC781. Additionally, the HPLC method developed in these studies provides a novel means for evaluating other hydrophobic drug candidates and their complexation with βCDs.

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