

Determination of binding constants between cyclodextrins and sulconazole antifungal drug by Hildebrand-Benesty approximation

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Determinarea constantelor de legare între ciclodextrine și sulconazol prin aproximare Hildebrand-Benesty

Abstract:

Cyclodextrins are well known host molecules able to form inclusion complexes with a wide variety of guest molecules, including drugs (such as sulconazole nitrate - a very efficient, but also toxic antifungal drug). The effects of the addition of different cyclodextrins on the light absorption of sulconazole have been investigated in aqueous media. The formation of host-guest inclusion complexes was indicated by UV spectroscopy. The binding constants sulconazole to α -, β -, γ - and hydroxypropyl- β -cyclodextrin in bidistilled water have been determined Hildebrand-Benesi and Scatchard approximation. This method offers the possibility to investigate the association probability between cyclodextrins and Sulc, the strength of the binding forces and the future changes of drug behavior in aqueous medium induced by complexation process.

Keywords: *cyclodextrins; sulconazole; inclusion complex, binding constants.*

Rezumat:

Ciclodextrinele sunt bine cunoscute și caracterizate molecule gazda, capabile de a forma compusi de incluziune cu o gama variată de molecule oaspete, inclusiv medicamente (cum este sulconazolul nitrat- un antifungic eficient, dar cu o toxicitate ridicată). Efectul adăugării de ciclodextrine asupra absorbției UV-vis a sulconazolului a fost analizată în mediu apos, prin spectroscopie UV-vis, valoarea constantei de legare a SULC de α -, β -, γ - și hidroxipropil β - ciclodextrina a fost realizată prin aproximarea Hildebrand-Benesi. Aceasta metodă permite analiza interacției posibile între SULC și ciclodextrine, taria forțelor de legare și posibilele modificări ale comportamentului medicamentului în mediu apos, induse de procesul de complexare.

Cuvinte cheie: *ciclodextrine, sulconazol, complex de incluziune, constante de legare*

Introduction

Cyclodextrins (CDs) act as host molecules to form inclusion complexes rather nonspecifically with a wide variety of guest molecules. The relatively hydrophobic cavity of native cyclodextrins and their derivatives induces the ability to complex guest molecules of appropriate size and shape. Complexation of guest compounds with cyclodextrins can modify guest solubility and reduce its volatility, increase its stability against the effects of light, heat, and oxidation, and mask unwanted physiological effects [1].

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The most common application of CDs in pharmaceutical industry is to enhance drug solubility in aqueous solutions. Generally, the lower the aqueous solubility of pure drug, the greater is the relative solubility enhancement gained by cyclodextrin complexation.

Due to its molecular structure, sulconazole is a suitable guest for the macrocycles of different cyclodextrins [2]. Sulconazole nitrate salt, (1-(2-[p-chlorobenzylthio]-2-[2,4-dichlorophenyl]ethyl)-1H-imidazole) mononitrate, is a broad-spectrum antifungal agent intended for topical application. It is an imidazole derivative with *in vitro* antifungal and antiyeast activity. SULC is freely soluble in pyridine, slightly soluble in ethanol, acetone, and chloroform, and very slightly soluble in water. It has a melting point of about 130°C [3].

The effectiveness of solubilization of a sparingly soluble hydrophobic compound in aqueous medium by means of its guest-host inclusion complexation with cyclodextrins depends mainly on the structure and dimensional complementarity of cyclodextrin host and of guest molecule. Both the complexation stoichiometries and the equilibrium binding constants are depending on these molecular parameters [4]. Generally speaking, the formation of a complex between a host and a guest is a basic and important process in supramolecular chemistry. That is why the binding constant has to be determined in order to evaluate the efficiency of inclusion process and to quantitatively determine the complex formation [5].

The non covalent interactions between mutually interacting entities were characterized by different parameters such as the binding constant, association constant, equilibrium constant or stability constant. A large part of already existing knowledge is based on the measurement of equilibrium constants. Equilibrium constants offer information about the mechanism of the involved process. The basic process in complexing systems can be described as reversible association or binding of one or more ligands (guest molecules) to one or more host molecules. The process reversibility allows for very efficient use and re-use

of guest entities. Given a set of initial conditions, the efficiency of the inclusion complexation can be quantified using association constants, directly or indirectly (by estimating the dissociation constant) [6].

This work deals with the dissolution of solid SULC as a function of cyclodextrins concentration in order to determine reliable values of the corresponding equilibrium concentrations and the binding constants. To characterize and compare the complexation ability of the most known commercially available CDs and CD derivatives, besides the native α -, β -, γ -cyclodextrins, hydroxypropyl- β -cyclodextrin was also included in our study.

Material and Method

α -, β -, γ -cyclodextrins (α -CD, β -CD, γ -CD) (Aldrich) and hydroxypropyl- β -cyclodextrin (HP β -CD) (average molecular weight, 1500 Da) (Cyclolab) were dried at 105°C in a vacuum oven for 48 h. Sulconazole nitrate salt (SULC) (Fluka) was used as received. Double distilled water was used throughout the study.

UV visible spectroscopy studies were performed on a Specord 200 Analytik Jena 200 spectrophotometer. To reach the thermal equilibrium the samples were maintained at 23°C for 1 h under stirring before the experiment.

The binding constants were obtained by using two stock aqueous solutions. One solution containing SULC ($[SULC] = 0.52 \times 10^{-4} M$) and CD ($[CD] = 52 \times 10^{-4} M$) was obtained by dissolution of SULC powder in a CD water solution. The other stock solution contains $0.52 \times 10^{-4} M$ SULC. Aliquots of the first stock solution were diluted with SULC solution in different ratios to give solutions of the same concentration of guest and various concentrations of host (from 0.26 to $7.8 \times 10^{-4} M$). The UV spectra of the obtained solutions were registered (Figure no. 1) and the concentration of bind SULC was determined at 196 nm (Figure no. 1) with reference to the standard curve (Figure no. 2).

HP β-CD/SULC

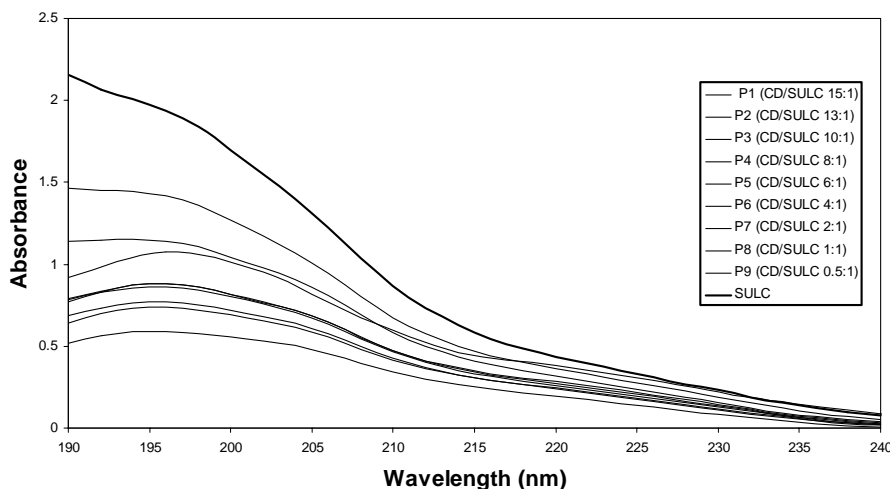


Figure no. 1. UV spectra of HP β-CD/SULC solutions as a function of SULC/CD molar ratio

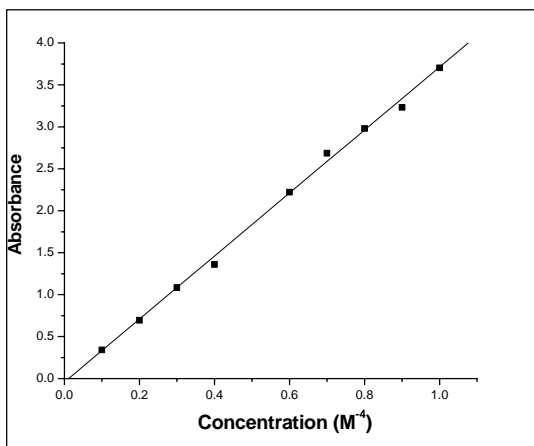


Figure no. 2. Sulc etalon curve in distilled water

Results and Discussion

The host-guest complexation can be described by the Equations (2)–(5).



$$K = \frac{[C]}{[H]^a \cdot [G]^b} \quad (3)$$

$$[H]_t = [H] + a \cdot [C] \quad (4)$$

$$[G]_t = [G] + b \cdot [C] \quad (5)$$

where: H, host; G, guest; C, complex; *a*, *b*, stoichiometric values (*a* and *b* are integers larger than or equal to 1); $[H]_t$ and $[G]_t$ total host and guest concentration at initial state, respectively; total guest concentration at initial stage; $[H]$, $[G]$, $[C]$, concentrations of host, guest, and complex at final stage, namely, at equilibrium, respectively; *K* is the host-guest binding constant.

Equation (6) is derived from Equations (2)–(5).

$$K = \frac{[C]}{([H]_t - a \cdot [C])^a \cdot ([G]_t - b \cdot [C])^b} \quad (6)$$

Assuming that *a*=*b*=1 in Equation (6) its reverse is:

$$\frac{1}{K} = [C] - ([H]_t + [G]_t) + \frac{[H]_t \cdot [G]_t}{[C]} \quad (7)$$

To obtain the binding constants (*K*) between α-CD and SULC, UV spectroscopy data were used. After collecting all UV parameters, the next step is how to treat the collected data to obtain the *K* value. Different approximation and regression methods were already suggested [5]. Typical examples are Benesi and Hildebrand [7], Ketelaar, Nagakura and Baba, Scott, Scatchard and

Hammond [5], Rose and Drago [8], Nakano and Creswell, Allred [5] approximations.

The association between cyclodextrin molecules and SULC can be treated from ligand-host molecule interaction point of view. As this interaction is reversible, given a set of initial conditions, the binding efficiency can be quantified using aforementioned association constants. One such constant frequently reported by biochemists in multi-site binding systems is called the dissociation constant K_d . Much as the name suggests, dissociation constants directly measure how well host molecules bind ligands. The derivation of K_d is straightforward and is in fact the inverse of the equilibrium association constant K_{ass} , $K_{ass} = 1 / K_d$. At equilibrium, ligand molecules can either be found in the free state, G , or in the bound state, HG (Equation 17).

$$K_d = \frac{[H] \cdot [G]^n}{[HG_n]} \quad (17),$$

where $[H]$, $[G]$ represents guest and host concentration; $[HG_n]$ represents complex concentration; n represent the number of bonding sites.

In our case $n = 1$

The fraction of total occupied binding sites, τ , could be considered as below:

$$\tau = \frac{[G]_{bound}}{[H]_{total}} = \frac{[HG]}{[H] + [HG]} \quad (18)$$

From Equations (17) and (18) results:

$$\tau = \frac{[G]}{K_d + [G]} \quad (19)$$

where: $[G]$ represents free guest concentration; $[G]$ values are obtained from UV measurements, by difference between the complexed SULC (determined from the reference curve, Figure 1) and the total concentration of the guest.

Plotting $1/\tau$ versus $1/[G]$ at constant $[H]$ concentration will yield a so called Benesi-Hildebrand binding curve and its slope will surrender K_d (Benesi, 1949). The major advantage of the Benesi-Hildebrand plot is that the variables τ and $[G]$ remain independent on the abscissa and ordinate, whereas other graphical representations allow the variables to become mixed.

K_d and K_{ass} values obtained for the inclusion complexes of SULC using Benesi-Hildebrand plot approximation (Figure no. 3) are presented in the Table 1.

Table 1

K_d and K_{ass} values for the inclusion complexes

Complex	α -CD-Sulc	β -CD-Sulc	γ -CD-Sulc	HP β -CD-Sulc
K_d	0,41	0,38	0,98	0,19
K_{ass}	2,43	2,57	1,03	5,04

Plots of $\tau/[G]$ to $[G]$ yield straight lines (called Scatchard plots) having slopes equals to $-1/K_d$ [9, 10].

From Table 1 one can conclude that the association constant is maxim in HP β -CD-SULC binding, being followed by β -CD-SULC association, α -CD-SULC and γ -CD-SULC.

The association probability is bigger for HP β -CD and β -CD as initially expected and demonstrated using two different methods. These results are in accordance with the initial hypothesis that the complexation process is more probable to take place with β -CD and HP β -CD considering the

size of the inner cavity of the four analyzed cyclodextrins and SULC molecules dimensions.

Different methods offer the possibility to investigate the association probability between cyclodextrins and Sulc, the strength of the binding forces and the future changes of drug behavior in aqueous medium induced by complexation process. Because of different aspects of complex guest-host interactions, evaluated with each method described here, the results obtained are not intercomparable, but they allow us to see various particular aspects of inclusion processes.

As initially hypothesized the most stable inclusion complexes are those obtained with HP β -

CD and β -CD, results confirmed also by other experimental data described in literature.

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