

Spectroscopic characterization of α -cyclodextrin-sulconazole inclusion complexes. Determination of binding constants by Rose-Drago approximation

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Caracterizarea spectroscopică a complexelor de incluziune α -ciclodextrină-sulconazol. Determinarea constantelor de legare prin aproximare Rose-Drago

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). *Aim.* To investigate the effects of the addition of α -cyclodextrin (α -CD) on the light absorption by sulconazole nitrate (SULC) in aqueous media. *Material and method.* To obtain the binding constants between α -CD and SULC, the UV spectra of solutions containing the same total concentration of CD and SULC and different molar ratios between CD and SULC were analyzed. The formation of host-guest inclusion complexes was indicated by UV spectroscopy. The binding constants sulconazole to α -cyclodextrin in bidistilled water have been determined using Rose-Drago method. *Results.* After calculation, we found that the stoichiometry for the inclusion complexation is 1:1, indicating a good interaction between the two components. *Discussions.* The α -CD-SULC inclusion complex exhibits a high value of the inclusion complex binding constants, reflecting the good stability of the obtained compound, and indicating this structure as a promising drug carrier useful in therapy.

Keywords: *cyclodextrins, sulconazole nitrate, inclusion complexes, binding constants*

Rezumat: Ciclodextrinele sunt molecule gazdă capabile de a forma complecși de incluziune cu o gamă variată de molecule oaspete, incluzând diferite medicamente (de exemplu sulconazol nitratul - un antifungic eficient, dar cu o toxicitate ridicată). *Scop:* Efectul adăției α -ciclodextrinei asupra absorbției luminii de către sulconazol (SULC) a fost studiată în mediu apos. *Material și metodă:* Pentru obținerea constantelor de legare între α -CD și SULC, au fost analizate spectrele UV ale soluțiilor conținând aceeași concentrație totală de CD și SULC, ca și ale celor cu diferite raporturi molare între cei doi reactanți. Formarea complecșilor de incluziune a fost evidențiată prin spectroscopie UV-Vis. Valoarea constantei de legare a SULC de ciclodextrină a fost studiată în apă bidistilată, utilizând metoda Rose-Drago. *Rezultate:* După efectuarea calculelor matematice, s-a determinat că stoechiometria complexării este de 1:1, indicând o bună interacțiune între cele două componente. *Discuții:* Complexul de incluziune α -CD-SULC prezintă o valoare crescută a constantelor de legare, indicând o bună stabilitate a compusului final și sugerând utilizarea viitoare în terapie a acestui *carrier* de substanțe antifungice.

Cuvinte cheie: *ciclodextrine, sulconazol nitrat, complecși 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

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effects of light, heat, and oxidation, and mask unwanted physiological effects [1]. 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 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 cyclodextrin concentration in order to determine reliable values of the corresponding equilibrium concentrations and the binding constants.

Materials and Methods

Materials

α -cyclodextrin (α -CD) (Aldrich, Germany) was dried at 105°C in a vacuum oven for 48 h. Sulconazole nitrate salt (SULC) (Fluka, Germany) was used as received. Double distilled water was used throughout the study.

Methods

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 determined by using two solutions of the same concentration (0.33×10^{-4} M) of guest (SULC) and host (α -CD) which were mixed in different proportions in order to obtain various solutions with the same total concentration and different ratio between host and guest. In the mixed solutions, the host concentration varied from 4×10^{-6} to 2.88×10^{-5} M. The molar absorptivity of the complex (ϵ_c) was measured on solutions of the inclusions complexes obtained by coprecipitation.

Results

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).

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

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

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

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.

To obtain the binding constants between α -CD and SULC, the UV spectra of solutions containing the same total concentration of CD and SULC and different molar ratios between CD and SULC were analyzed.

Originally the Rose–Drago method [8] was used to evaluate by UV visible spectroscopy the acid-base equilibrium, the molecular addition compound of iodine. The only assumption for this original method is that there are at most two observing species which obey Beer's law in the employed concentration range [7].

Because

$$A_H = \varepsilon_H \cdot [H] = \varepsilon_H \cdot ([H]_t - a \cdot [C]),$$

$$A_G = \varepsilon_G \cdot [G] = \varepsilon_G \cdot ([G]_t - b \cdot [C]) \quad A_C = \varepsilon_C \cdot [C],$$

$$A_{obs} = A_H + A_G + A_C,$$

where: ε_H , ε_G , ε_C represents the molar absorptivities of the host, guest and complex; A_H , A_G , A_C , A_{obs} are absorption experimental values of host, guest and complex, and experimental observed absorption of the complex, respectively, the Equation (7) could be rewritten:

$$A_{obs} = \varepsilon_H \cdot ([H]_t - a \cdot [C]) + \varepsilon_G \cdot ([G]_t - b \cdot [C]) + \varepsilon_C \cdot [C]$$

$$A_{obs} - \varepsilon_H \cdot [H]_t - \varepsilon_G \cdot [G]_t = (\varepsilon_C - a \cdot \varepsilon_H - b \cdot \varepsilon_G) \cdot [C] \quad (8)$$

Equation (8) shows that $A_{obs} - \varepsilon_H[H]_t - \varepsilon_G[G]_t$ is proportional to $[C]$ because $(\varepsilon_C - a\varepsilon_H - b\varepsilon_G)$ is constant. The molar absorptivities ε_H , ε_G , ε_C are determined from other measurements using the pure host, pure guest and the complex obtained by coprecipitation, respectively.

Plotting the values of $A_{obs} - A_{guest}$ as a function of $[H]_t/[H]_t + [G]_t$ complex concentrations

(modified Job's plot) [5] the stoichiometry of the complexation process was estimated.

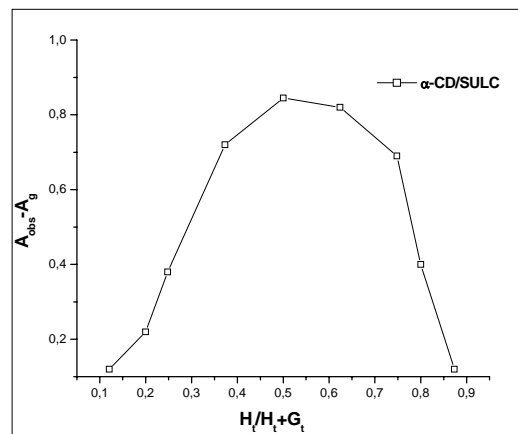


Figure 4. Modified Job's plot of SULC in the presence of CD

As one can see from Figure 1, the maximum X is around 0.50 - 0.55. That value indicates that the stoichiometry for the inclusion complexation is 1:1, as described elsewhere [5].

From Equation (8), the Equation (9) can be obtained:

$$[C] = \frac{A_{obs} - \varepsilon_H \cdot [H]_t - \varepsilon_G \cdot [G]_t}{\varepsilon_C - a \cdot \varepsilon_H - b \cdot \varepsilon_G} \quad (9)$$

Combining Equation (10) with Equation (8) gives:

$$\frac{1}{K} = \frac{A_{obs} - \varepsilon_H [H]_t - \varepsilon_G [G]_t}{\varepsilon_C - \varepsilon_H - \varepsilon_G} - ([H]_t + [G]_t) + \frac{\varepsilon_C - \varepsilon_H - \varepsilon_G}{A_{obs} - \varepsilon_H \cdot [H]_t - \varepsilon_G \cdot [G]_t} \cdot [H]_t \cdot [G]_t \quad (10)$$

As for our systems $\varepsilon_H = 0$ and considering that: $Y = \frac{1}{K}$, $X = \varepsilon_C - \varepsilon_G$, $a_n = A_{obs} - \varepsilon_g \cdot [G]_t$,

$$b_n = [H]_t + [G]_t, \quad c_n = \frac{[H]_t \cdot [G]_t}{A_{obs} - \varepsilon_G [G]_t}, \quad \text{Equation 10 becomes:}$$

$$Y = \frac{a_n}{X} - b_n + c_n \cdot X \quad (11)$$

Discussions

Each {X; Y} pair is obtained under the premise, anterior demonstrated, of 1:1 complexation and no approximation is introduced into this solution. The reciprocal of the obtained Y is the binding constant K [5]. Using the values calculated for a_n , b_n , c_n , from the experimental values the K values corresponding to each set of a_n , b_n , c_n values were determined (Table 1). The final K value of the considered system is an average of those calculated K values, considering the confidence interval 95 %. The degree of liberty and standard deviation were determined, using T-student test [5] (Table 2).

The α -CD-SULC inclusion complex exhibits a high value of the inclusion complex binding constants, reflecting the good stability of the obtained compound, indicating this structure as a promising drug carrier useful in therapy. The stoichiometry for the inclusion complexation is 1:1, indicating a good interaction between the two components.

Table 1
Binding constants values for the α -CD-SULC inclusion complexes

a_n	b_n	c_n	Y	K
4.51E-01	-3.30E-05	2.63E-10	7.38E-05	1.36E+04
5.14E-01	-3.30E-05	3.39E-10	7.99E-05	1.25E+04
4.90E-01	-3.30E-05	4.13E-10	7.88E-05	1.27E+04
4.51E-01	-3.30E-05	5.62E-10	7.73E-05	1.29E+04
3.18E-01	-3.30E-05	8.55E-10	6.98E-05	1.43E+04
1.79E-01	-3.30E-05	1.41E-09	6.49E-05	1.54E+04
3.03E-01	-3.30E-05	6.69E-10	6.62E-05	1.51E+04
5.21E-01	-3.30E-05	3.34E-10	8.05E-05	1.24E+04
9.98E-01	-3.30E-05	1.15E-10	1.18E-04	8.51E+03

Table 2
Binding constants values for the inclusion complexes

Complex	α -CD/SULC
Mean K	5050±245
Confidence interval	95%
Degree of liberty	2.306

References

- Hedges A R - Industrial applications of cyclodextrins; *Chemistry Reviews* 1998; 98:2035-2044.
- Loftsson T, Brewster M E - Pharmaceutical applications of cyclodextrins: 1. Drug solubilization and stabilization; *Journal of Pharmaceutical Sciences* 1996; 85:1017-1025.
- Stroescu V, (eds). *Bazele farmacologice ale practicii medicale*; 7th edn; București: Editura Medicală; 2001.
- Kopecky F, Kopecka B, Kaclik P - Solubility study of nimodipine inclusion complexation with α and β -cyclodextrin and some substituted cyclodextrins; *Journal of Inclusion Phenomena and Macrocyclic Chemistry* 2001; 39:215–217.
- Hirose K - A Practical Guide for the Determination of Binding Constants; *Journal of Inclusion Phenomena and Macrocyclic Chemistry* 2001; 39:193–209.
- Voet D, Voet J. *Biochemistry*; 1st edn; New York: John Wiley & Sons; 1990.
- Benesi H A, Hildebrand J H - Spectrophotometric Investigation of the Interaction of Iodine with Aromatic Hydrocarbons; *Journal of the American Chemical Society* 1949; 71:2703-2706.
- Rose N J, Drago R S - Determination of equilibrium constants for weakly bound complexes; *Journal of the American Chemical Society* 1959; 81:6138-6145.