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In memoriam prof. dr. Ioan A. Silberg

SPECTROPHOTOMETRIC STUDIES OF DIAZEPAM - β-CYCLODEXTRIN COMPLEX FORMATION

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ABSTRACT. Solid state interactions between a bioactive substance-Diazepam and β-cyclodextrin (β-CD) - the so called inclusion compounds (obtained by different preparation methods: kneading, co-precipitation and freeze-drying) were investigated. The obtained compounds were investigated by FTIR spectroscopy to evidence their formation. The results revealed that the solid state inclusion compound, such as Diazepam/β-cyclodextrin has a good stability. The stoichiometry of the inclusion complexes of diazepam with β-CD is 1:1 was investigated in solution. The association constant of the complex was determined by UV spectrophotometry. The encapsulation might improve Diazepam stability and bioavailability of the drug.

Keywords*: FTIR, UV-Vis, stoichiometry, association constant, inclusion compound, β-cyclodextrin*

INTRODUCTION

An antidepressant bioactive substance, diazepam (DZP)(7-Chloro-1,3-dihydro-1-methyl-5-phenyl-2H-1,4-benzodiazepin-2-one,) (Fig. 1), is used in the treatment of severe anxiety disorders, as a hypnotic in the short-term management of insomnia, as a sedative and premedicant, as an anticonvulsant, and in the management of alcohol withdrawal syndrome.

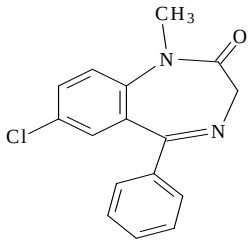

Fig. 1. Diazepam molecule

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In order to improve the solubility in water and the bioavailability this compound is encapsulated in cyclodextrins. Cyclodextrins (CDs) - cyclic oligosaccharides, are natural products obtained by enzymatic reaction of starch. Upon the addition of the CGT-ase enzyme to an aqueous solution of starch, every sixth or seventh of the eight α-1, 4-glycosilic linkages is split, reacting with their own non-reducing end. It results a six-, seven- or eight-membered macro-ring. These cyclic maltodextrins are called α-, β-, and γ-cyclodextrins [1]. The aim of the paper was to prepare and to evidence by different spectrophotometric methods some inclusion compounds of diazepam with β-CD.

Two methods, FTIR and UV spectroscopy [2, 3] were employed to confirm inclusion compound formation.

Materials and methods

The inclusion compound was obtained by different methods: physical mixture (*pm*), kneading (*kn*) - grounded the amounts of diazepam and β-cyclodextrin in an agate mortar for 60 min, using ethanol 33% as the wetting agent and the paste thus obtained was dried at 38^{o}C; co precipitation (*co*) - mixing the amounts of diazepam and β-cyclodextrin (15mM alcoholic solution 3:1 v/v) in 1:1 molar ratio, stirring for 48 hours at 40^{o}C succeeded by evaporation and drying at room temperature; freeze-drying (*fd*) - the amounts of diazepam solved in β-cyclodextrin saturated alcoholic solution in molar ratio 1:1, the product was frozen and dried by immersion in freezer-drier (Alpha 1-2 LD plus) for over 24 hrs.

FTIR spectra were obtained with a JASCO 6100 FTIR spectrometer in the 4000 to 400 cm^{-1} with a resolution of 2 cm^{-1}, using the KBr pellet technique.

UV-Vis absorption spectra were recorded on a V-550 JASCO UV/Vis spectrometer equipped with quartz cells having 1.0 cm optical path length.

RESULTS AND DISCUSSION

Inclusion compound of DZP with β-CD

FTIR spectroscopy

FTIR spectrum of the 1:1 physical mixture (*pm*), see Fig. 2, contains the absorption bands of each component so no inclusion compound was obtained in this case.

In the 4000 to 2000 cm^{-1} spectral region the O-H stretching vibration are located at 3400 cm^{-1} for *pm* and 3384 cm^{-1} for β-CD whereas for *kn* this band is shifted to 3401 cm^{-1} and to 3390 cm^{-1} for *fd* products. One concludes that the hydrogen bonds are implied severely in the inclusion compound formation. In the 1800 to 1550 cm^{-1} spectral region, see Fig. 3, one identify the carbonyl group vibration (located at 1686 cm^{-1}) for pure DZP and *pm* system.

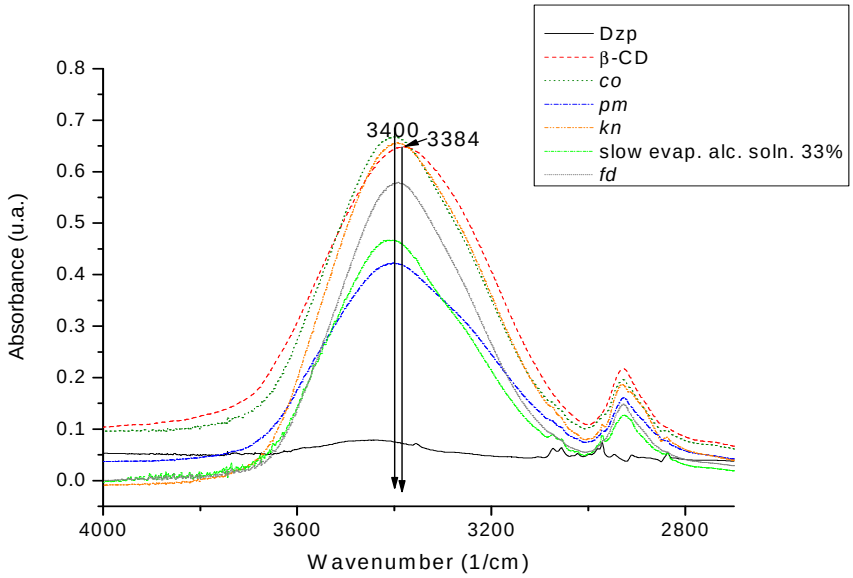

Fig. 2. FTIR spectra of Diazepam and its inclusion compounds obtained by different methods, 4000-2700 cm^{-1} spectral region

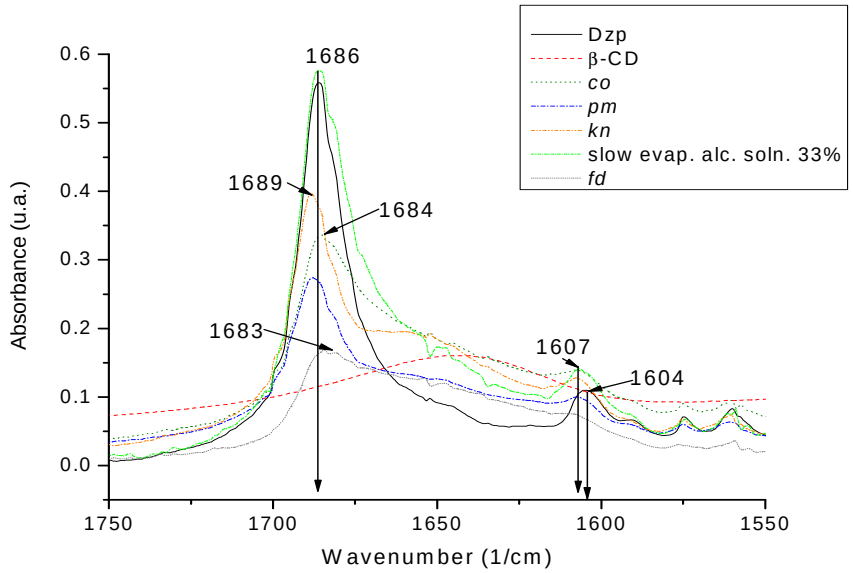

Fig. 3. FTIR spectra of Diazepam and its inclusion compounds obtained by different methods, 1750-1550 cm^{-1} spectral region

UV-Vis spectroscopy

The investigated solutions of DZP at constant concentration $2.5x10^{-5}$ M and β-CD at increasing concentrations (1; 1.5; 2; 2.5; 3; 4; 5; 6; 7; 8)$x10^{-3}$ M have been prepared. The obtained solutions were mixed for 6 hours at 30°C and then left for 24 hours for equilibration. This procedure was replicated in order to obtain two or more absorbance values for each of the β-CD concentration studied. The absorption spectra of the inclusion complex were showed in Fig.4.

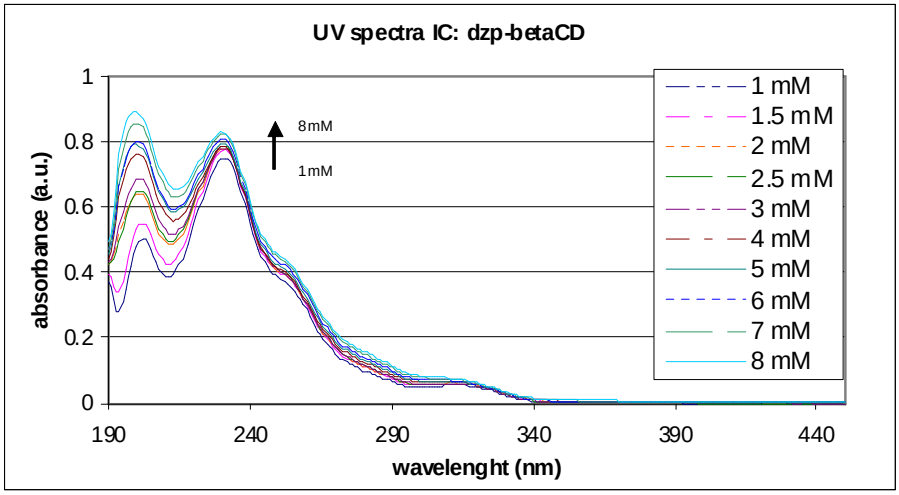

Fig. 4. UV-Vis absorption spectra of 0.025 mM diazepam at different concentration of β-CD: 1, 1.5, 2, 2.5, 3, 4, 5, 6, 7, 8 mM in alcoholic solution (3:1 v/v)

The stoichiometric ratio of inclusion complex of host (β-CD) – guest (DZP) should theoretically be 1:1, according to their chemical reaction equation (1) [4]. A linear relationship should be obtained between 1/dA and 1/[βCD] based on the Benesi-Hildebrand equation [5] (2):

$$DZP + \beta CD \xrightarrow{K} DZP - \beta CD \quad (1)$$

$$\frac{1}{A} = \frac{1}{\varepsilon [G]_o K [CD]} + \frac{1}{\varepsilon [G]_0} \quad (2)$$

where A is the absorbance of the DZP solution at each β-CD concentration; $[G]_0$ the initial concentration of DZP; K the apparent formation constant; [CD] the concentration of β-CD and ε is the molar absorptivity. From the changes in the absorbance, an apparent formation constant value for the

inclusion complex can be determined. The result is shown in Fig. 5, a good linear relationship obtained proved that stoichiometric ratio of inclusion complex of host (β-CD) – guest (DZP) was 1:1. Fig. 6 presents the Scott plot, used as well as the Benesi-Hildebrand plot for the stability (association) constant determination.

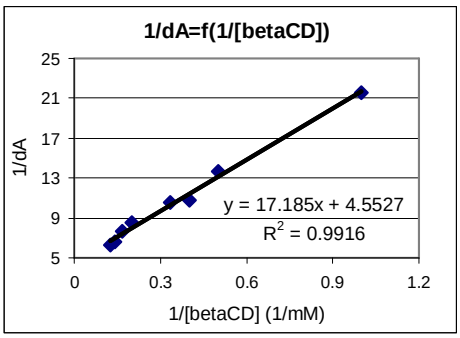

Fig. 5. Benesi-Hildebrand plots for the DZP-βCD systems

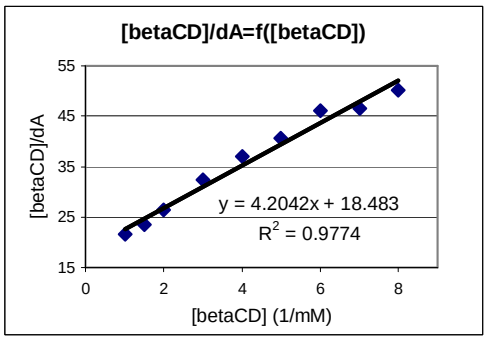

Fig.6. Scott plots for the DZP-βCD systems

The values of the association constants calculated by the two methods [5, 6] (Table 1) differ from each other no more than by their errors. This fact suggests a satisfactory level of correlation between the obtained results.

Table 1.

Association constants for complexation of DZP with β-CD

Method of binding constant calculation	K_a(DZP- βCD) M^{-1}	Correlation coefficient, R^2
Benesi-Hildebrand	264	0.9916
Scott	227	0.9774

CONCLUSIONS

The inclusion compounds of DZP with β-CD were obtained and evidenced by FTIR spectroscopy. The 1:1 stoichiometry and the stability constants of approx. 264 M^{-1} and 227 M^{-1} were determined for aqueous solutions with UV-vis spectroscopy by using Benesi-Hildebrand and Scott methods, respectively. These association constant values show that the encapsulation might improve Diazepam stability and bioavailability of the drug.

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