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Original Article

# STUDY OF THE INCLUSION BEHAVIOUR OF B-CYCLODEXTRIN WITH ZIPRASIDONE AND ITS PHARMACEUTICAL APPLICATION

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**ABSTRACT**

**Objective:** To study the inclusion complex of the slight water solubility ziprasidone (ZIP) with β-cyclodextrin (β-CD).

**Methods:** The inclusion interaction was investigated by spectrofluorimetry. Influence of pH and β-CD concentration on the fluorescence spectra was reported. The inclusion complex formation was also confirmed by scanning electron microscopy SEM.

**Results:** The obtained results exhibited that the ZIP was encapsulated in β-CD cavity to form a 1:1 stoichiometry host-guest complex. The inclusion constant was confirmed by Benesi-Hildebrand method. Based on the remarkable enhancement of the fluorescence intensity of ZIP in the presence of β-CD anew spectrofluorimetric method for the determination of ZIP was developed. In order to obtain the maximum fluorescence several experimental conditions were optimized. The linear range was 0.5-100 ng $mL^{-1}$and correlation coefficient was 0.9992. The detection limit was 0.2 ng $mL^{-1}$.

**Conclusion:** The study demonstrates the inclusion complex formation between ziprasidone and β-cyclodextrin. By inclusion complexation the aqueous solubility and stability of ZIP were significantly improved. The proposed spectrofluorimetric method was successfully applied to the analysis of ZIPin pure and pharmaceutical dosage forms with the satisfactory results.

**Keywords:** Ziprasidone, β-cyclodextrin, Inclusion complex, Spectrofluorimetry.

## INTRODUCTION

β-cyclodextrin (β-CD) is cyclic oligosaccharides, consisting of seven-(+) glucopyranose units linked by α-1,4-glucosidic bonds. With a wide variety of molecules with suitable polarity and dimention β-CD forms inclusion complexes[1,2].During the inclusion process of pharmaceutical molecules with cyclodextrins a modulation of the physicochemical and pharmaceutical properties of guests molecules, such as increased solubility, improved chemically stability and bioavailability, and reduced toxicity controlled rate release[3-5]isobserved. CDs can be represented asa truncated cone structure. A relatively hydrophobic cavity in the center and a hydrophilic outer surface due to external hydroxyl faces are shown[6-8]. If a lipophilic guest molecule has an appropriate shape and size, it can be hosted in the cavity with a significant increase in its aqueous solubility. Cyclodextrins can form inclusion complexes in aqueous solution where a lipophilic guest molecule or moiety locates in the inner cavity[9]. Host-guest inclusion complex formation with CDs usually express increased solubility in aqueous solutions, as well as improved stability and bioavailability of the guest molecule[10-12]. The non-polarity of the interior cavity of cyclodextrin makes it ideal for solubilizing non-polar solutes. The polarity of its exterior helps increase water solubility of the guest. Native and modified β-CD have been extensive application in many fields such as food supplements, phytochemical preparations and medicinal chemistry[13-17].

Ziprasidone (ZIP) (5-[2-[4-91,2,-benzisothiazol-3-yl)-1-piperazinyl] ethyl]-6-chloro-1,3-dihydro-2H-indol-2-one)(Fig.1) is one of a new generation ofatypical antipsychotic drugs. It has potential application also as an anxiolytic and as an antidepressant drug. Ziprasidone has unique pharmacological activity, because it acts both on serotonin and on dopamine receptors, but it exhibits much more affinity for serotoninthan for dopamine receptors[18].The drug was introduced in the USA during 2001 for the treatment of schizophrenia, bipolar mania or other psychotic syndromes. Ziprasidone is available in Europe, it is used in Sweden,

Austria and in some other countries for both indications. The main advantages with respect to the other atypical antipsychotics is that Ziprasidone was shown to have a very low liability for inducing weight gain. The adverse effects are dyspepsia, somnolence, nausea, dizziness and extrapyramidal symptoms (rarely). The drug is not suitable in patients with cardiac problems. Ziprasidone is usually administrated at daily dose of 80-160 mg. It should be taken during meals because food can doubly its bioavailability.

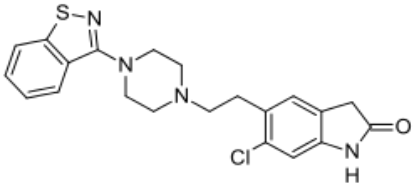



**Fig. 1: The chemical structure of ziprasidone**

Ziprasidone is low water soluble drug. For this reason the application and determination of ZIP is limited. It is quite meaningful to develop new methods for determination of ZIP in aqueous media. In the literature several chromatographic and spectrophotometric methods for determination of ZIP have been reported[19-22]. Among this studies there was no spectrofluorimetric method for ZIP determination in the presence of β-CD. This proposed method's dominance displayed with wide linear range, high sensitivity and low analytical cost.

In this paper, the host-guest interaction between β-CD and ZIP was explored by spectrofluorimetry. A series of conditions during the formation of the inclusion complex were investigated. The inclusion complex formation was also confirmed by scanning electron microscopy SEM. Based on the great enhancement of the fluorescence intensity of ZIP, a novel method was developed to determine ZIP in pharmaceutical formulations. It will provide some basis for developing new methods for determination of ZIP in pharmaceuticals. The proposed method for ZIP determination is rapid, fairly simple and has high selectivity and wide linear range, which is of significance for analytical determination.

## MATERIALS AND METHODS

### Materials and reagents

Reagent-grade ziprasidone hydrochloride was purchased from Pfizer Central Research (Groton, CT, USA). Its capsules (20 mg, 80 mg/capsule) were obtained from the company. Analytical-grade β-cyclodextrins from Sigma Chemical Co., Germany, was used without further purification, except for vacuum drying. All of other chemicals were from Merck (Darmstadt, Germany) and used as received. Doubly distilled deionized water was used throughout.

A stock solution of ziprasidone hydrochloride $10^{-3}$ mole $L^{-1}$ was prepared by dissolving an appropriate amount in methanol in 10 mL calibrated volumetric flask.

A $10^{-2}$ mole $L^{-1}$ of β-CD solution was prepared by dissolving appropriate amount of pure solid in water in a 10 mL volumetric flask. The solution was diluted to the mark by deionized water. Bufferof pH 6.0 was used.

All reagents were of analytical grade.

### Apparatus

Fluorescence spectra were recorded by a Shimadzu RF-5301 spectrofluorometer with a 1 cm path length quartz cell. The excitation and emission slit widths were both 5 nm each.

Microscopic morphological structure measurements were performed with a Joel JSM-5900 scanning electron microscope SEM. Particles were fixed on a brass stub using double-sided tape and vacuum-coated gold.

pH was measured by pH meter (Lei-ci pH S-25, Shanghai, China). A thermostatic water bath kettle was used for maintaining temperature.

### General procedures

### Fluorescence spectra

The experiment procedurewas carried out as follows:In a 10 mL color comparison tube, 1 mL of 2 x $10^{-5}$ mole$L^{-1}$ZIP, 1 mL buffer solution (pH 6) and the varied amounts of β-CD (1, 2, 3, 5, 6 mL of $10^{-4}$ mole $L^{-1}$) were added in this order. Then the mixed solution was diluted to the mark with doubly distilled water and ultrasonically oscillated for 25 min at room temperature. The fluorescence spectra was measured at $\lambda_{ex}/\lambda_{em}$ = 320 nm/410 nm. The stoichiometry and inclusion constant of ZIP/β-CD was gained from Benesi-Hildebrand method.

### Determination of ziprasidone

An aliquot of solution containing 0.5-100 ng $mL^{-1}$ of ZIP were added in colorimetric tube, respectively, then 1 mL pH 6.0 buffer solution and 3 mL of $10^{-4}$ mole $L^{-1}$ β-CD were added sequentially. The mixture was diluted by doubly distilled water to 10 mL and ultrasonically oscillated for 25 min at room temperature. The fluorescence intensities were measured at 410 nm.

## RESULTS AND DISCUSSION

### Fluorescence studies

### Influence of pH and buffer solution

Cyclodextrin is instability at very low pH and the use of strongly acidic solution containing β-CD was avoided. pH effect on the system was studied over the range of 4.0-8.0. It was observed, the fluorescence intensity was relatively high and remained constant in the pH range of 5.0-7.0. Among Tris-HCl, $H_3BO_3$-KCl-NaOH, Britton-Robinson and $KH_2PO_4$-NaOH buffer solution systems, it was more sensitive for the reaction system in Britton-Robinson buffer solution. In further experiments the acidity of solution was adjusted to pH 6.0 withBritton-Robinson buffer solution.

### Effect of β-CD concentration

The effect of β-CD concentration on the fluorescence intensity of ZIP was investigated by keeping its concentration constant at 5.0 x $10^{-6}$mole $L^{-1}$ and varying the β-CD concentration from 0.0 to $10^{-4}$ mole$L^{-1}$. It was demonstrated that the fluorescence intensity of β-CD increased withincreasing concentration of β-CD in solution. The fluorescence intensity reached its maximum when the concentration of β-CD up to $10^{-5}$ mole

$L^{-1}$, and there is slight change in fluorescence intensity by further addition of β-CD. So, concentration of 5x$10^{-5}$ mole $L^{-1}$ of β-CD was chosen for further experiments.

### Influence of order addition and reaction time

The effect of sequence of adding reagentson the fluorescence intensity of ZIP was investigated. The order:β-CD, ZIP,Britton-Robinson buffer solution was proved to be best. Reaction time was also analyzed. The obtained results exhibited that the fluorescence intensity of the complex was relatively high after the mixture of reagent solutions, had been ultrasonically oscillated for 25 min and remained constant for at least 2h. The reaction time of 25 min was used.

### Excitation and emission spectra studies

In optimum conditions, the fluorescence spectra of ZIP in solutions in the absence and presence of β-CD was performed and exhibited emission maxima at 410 nm. The intensity of fluorescence increased with a concomitant increase concentration of β-CD. The enhancement of fluorescence intensity could be rationalized by the increased micro environmental hydrophobicity and /or stericshielding around the fluorophore arising from the cooperative interactions between the host and guest.

Fluorophores were partially or wholly encapsulated within the CD's cavity could be better protection from quenching and other processes that occur in the bulk solvent. It would be the hindered rotation of the guest molecules as well as a considerable decrease in the relaxation of the solvent molecules. The effects can result in a decreased vibrational deactivation of the excited guest molecules and in increased fluorescence intensity of the system. The phenomena revealed that ZIP molecule was moved into β-CD's cavities, an obvious inclusion process occurred.

### Determination of stoichiometric ratio and formation constants of inclusion complexes

For a quantitative investigation on the host-guest inclusion system the fluorescence enhancement of ZIP with increasing the concentration of β-CD was used. The apparent formation constants (K) for ZIP/β-CD complexes and the stoichiometry were calculated by Benesi-Hildebrand method.

Assuming that the composition of the complex was 1:1, the inclusion reaction can be written:

ZIP + β-CD ↔ β-CD-ZIP

After ZIP incorporated by β-CD the fluorescence intensity enhanced, and the complexation constant (K) was given by:

$$K = \frac{[\beta-CD-ZIP]}{[ZIP]\,[\beta-CD])}$$

Where [β-CD], [ZIP] and [β-CD-ZIP] were equilibrium concentrations. The K value for the inclusion complex could be obtained by the following expression:

$$\frac{1}{(F - F0)} = \frac{1}{(F\infty - F0)\ K\ C(\beta - CD)} + \frac{1}{(F\infty - F0)}$$

F was the fluorescence intensity of the ZIP at each β-CD concentration tasted;$F_0$ was the tested fluorescence intensity of ZIP in the absence of β-CD;$F_\infty$ denoted the fluorescence intensity when all the ZIP molecules were essentially complexed with β-CD and K was the inclusion constant. A good linear relationship was observed when $1/(F\text{-}F_0)$ was plotted against $1/C_{\beta\text{-}CD}$in Fig. 2, which indicated of a 1:1 stoichiometry for ZIP/β-CD at pH 6.0. Its apparent association constant was calculated to be K= 1496L/moleat room temperature and RSD was 0,86% (n=5) through the experimental data.

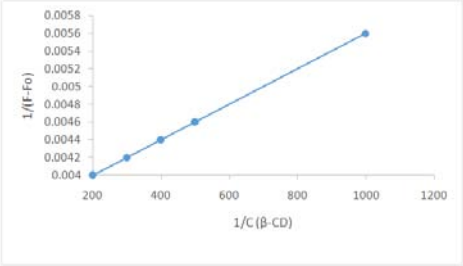



**Fig. 2: Double reciprocal plot obtained from $1/(F-F_0)$ plotted against $1/C_{\beta\text{-CD}}$**

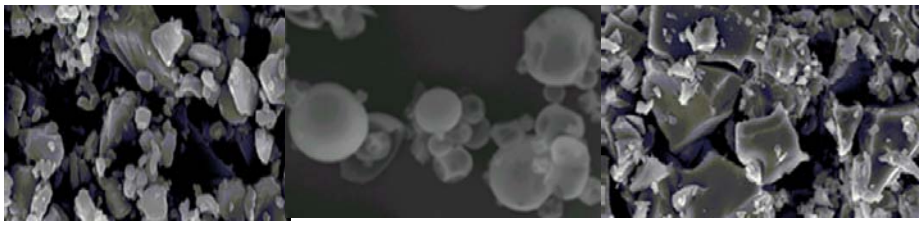
**Characterization of the inclusion complex through the scanning electron microscopy SEM**

Ziprasidone and β-CD were separately powdered and the structure of the particles in the powders were observed first, in the scanning electron microscopy SEM. Then the particles of the powdered form of the inclusion complex was also studied. Some results are presented in Fig. 3.

The photomicrographs observation, presented in Fig.3, revealed the presence of irregular shape crystals of ZIP and spherical forms characteristics of β-CD. The inclusion complex photomicrograph shows a change in particles morphological appearance, with loss of the β-CD spherical shape and loss of the ZIP typical shown. Morphological changes can be used as evidence to verify interactions between molecules. The structure of the inclusion complex is different from that of ZIP and β-CD and it can be assumed as proof of the formation of a new inclusion complex. The data obtained from SEM are added to previous results, suggesting the inclusion complex formation from the processing of the ZIP by inclusion into β-CD cavity.

**Analytical results**

Due to the increase of the fluorescence intensity of ZIP in presence of β-CD, a simple spectro fluorimetric method for the determination of ZIP in bulk aqueous solution was developed. Under the optimum experimental conditions, a good liner relationship (R= 0.9992) was obtained between the fluorescence intensity and the concentration of ZIP ranging from 0.5-100.0 ng/ml. The detection limit, as defined by IUPAC[23] was calculated to be 0.2 ng/ml and RSD was 0.92%. The proposed method had high sensitivity, reproducibility and selectivity at the same temperature.



**(a) (b) (c)**

**Fig.3: Photographs of scanning electron microscopy SEM of (a) ZIP, (b) β-CD and (c) ZIP/ β-CD inclusion complex**

**The studies of foreign interferences**

The effects of the foreign interferences on the determination of5.0x $10^{-6}$ mole $L^{-1}$ of ZIP was carried out by a systematic study. A 1000 – fold mass excess of each interference over ZIP was investigated first. If interferences occurred, the ratio was reduced gradually until the interferences ceased. The criterion for interference was fixed at a ±5% variation of the average fluorescence intensity calculated for the established level of ZIP. The obtained results were given in table 1 and it was obvious that the proposed method had good selectivity.

**Application to analysis of pharmaceutical preparations**

The proposed method was employed to determine ZIP in pharmaceutical preparations – Geodon (Pfizer, capsules 20 mg and 80 mg). The results were shown in table 2. The recoveries agreed with the certified content and the values obtained for the methods and the precision is quite satisfactory. There is a satisfactory agreement between the results of the proposed and official methods [24] as well the labeled amounts of the pharmaceutical products. Good accuracy and precision, a wide linear range of determination demonstrate that the proposed method is suitable for routine analysis of ziprasidone for use in pharmaceutical industries and in institutes for drug control.

**Table 1: Effect of interference on determination of ziprasidone by proposed method**

| Tolerance limit* | Interference |
|---|---|
| 100 | $Na^+$ |
| 100 | $Cl^-$ |
| 50 | Glucose |
| 50 | Glycin |
| 100 | Saccharose |
| 30 | Lactose |
| 20 | Tween 20 |
| 25 | β-alanine |

*To ZIP mole ratio

**Table 2: Determination of ziprasidone in pharmaceuticals (n=3).**

| Sample | Labelled claim | Amount found (mg) | | Recovery (%) | |
|---|---|---|---|---|---|
| | | P M* | OM ** | PM | OM |
| Geodon | 20 | 19.80 | 20.12 | 99.00 | 100.60 |
| (Pfizer,mg/capsule) | 80 | 80.10 | 78.90 | 100.12 | 98.62 |

*PM- Proposed method, ** OM- official method (European Pharmacopoeia, 2011)

**CONCLUSION**

The inclusion complex ZIP/β-CD with host-guest ratio 1:1 has been reported. The aqueous solubility and stability of ZIP by complexation with β-CD were significantly improved. The fluorescence spectra confirmedZIP was able to form inclusion complexes with β-CD in solution. ZIP, β-CD and ZIP/β-CD inclusion complex were also studied by scanning electron microscopy SEM.

Basis on the significant enhancement of fluorescence intensity of ZIPin the presence ofβ-CD, a spectrofluorimetric method for determination of ZIP was established. Under optimized experimental conditions, the method was applied to determination of ZIP in pharmaceuticals. A wide linear range of determination, good accuracy and precision (RSD, 0.6%) demonstrate that the proposed method is suitable for routine analysis of ziprasidone.

**CONFLICT OF INTERESTS**

Declared None

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