# Effect of Cyclodextrin Derivation and Amorphous State of Complex on Accelerated Degradation of Ziprasidone

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**ABSTRACT:** Inclusion complexes of ziprasidone with several β-cyclodextrins [β-CDs; sulfobutylether-β-cyclodextrins (SBEβCD), hydroxypropyl-β-cyclodextrins (HPβCD), methyl-β-cyclodextrins (MβCD), and carboxyethyl-β-cyclodextrins (CEβCD)] were prepared and solution stability was evaluated at elevated temperature. Solid-state stability was assessed by subjecting various CD complexes of ziprasidone, spray-dried dispersion (SDD), partially crystalline ziprasidone–SBEβCD salts, and the physical mixture of ziprasidone–SBEβCD to γ-irradiation. Degradant I was formed by oxidation of ziprasidone, which upon aldol condensation with ziprasidone formed degradant II in both solution and solid states. In the solution state, CD complexes with electron-donating side chains, such as SBEβCD and CEβCD, produced the highest oxidative degradation followed by HPβCD with 6, 3, and 4 degrees of substitution. In the solid state, crystalline drug substance and physical mixture of crystalline drug–SBEβCD showed very little to no degradation. In contrast, amorphous βCD, MβCD, CEβCD, and SBEβCD complexes as well as the amorphous SDD exhibited greatest extent of oxidative degradation. Results suggest that electron-donating side chains of the derivatized CD interact with transition state of the oxidation reaction and catalyze drug degradation in solution, However, higher mobility in the amorphous state of CD–drug complexes promoted chemical instability of ziprasidone under accelerated conditions irrespective of the chemical nature of the side chain on CD.© 2011 Wiley-Liss, Inc. and the American Pharmacists Association J Pharm Sci 100:2703–2716, 2011

**Keywords:** chemical stability; cyclodextrin; complexation; kinetics; oxidation; solid-state NMR

## INTRODUCTION

Cyclodextrin (CD) has been used in pharmaceutical formulation as a solubilizer for drug molecules with low aqueous solubility. CD, with its hydrophobic cavity and hydrophilic exterior, effectively complexes with many lipophilic compounds and thereby increases the aqueous solubility of these compounds.[1–4] Many chemical modifications have been made on CD's basic structure to improve its ability to maximize solubility both in food and drug industry applications. Many of these modifications involve the introduction of hydrophilic side chains to the exterior of CD.[3–4] Some noticeable examples of modified CDs are hydroxypropyl-β-CD (HPβCD) and sulfobutylether-β-CD (SBEβCD).[3]

In addition to solubilization, CD complexation has been claimed to stabilize drug by protecting the molecule in the CD cavity and preventing access of reactive species such as water in hydrolysis of aspirin.[5] Jarho et al.[6] reported that the deacetylation of spironolactone in CD complex is pH and CD dependent, shedding light on the catalytic capability of derivatized CD on drug degradation. However, the effects of side-chain electronic and/or steric catalytic or protective effect on reaction rate of the complexed drug have not been studied systematically.

Cyclodextrin complexation could affect drug molecules in many profound ways. When a drug molecule is complexed in the CD cavity, the physical environment is fundamentally different from either its native crystalline solid state or its simple solvated state in solution. In addition, complexation restricts freedom of the bound molecule compared with the bulk drug in solution, and with side chains in a strategic position on CD that can facilitate the

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interaction between the guest molecule and the side chains of the host molecule as in enzyme catalysis or as in neighboring group participation. This type of interaction could have beneficial or detrimental impact on the chemical stability of the guest molecule.

Another significant impact of complexation is on the physical or solid state of the guest molecule when isolated as a complex. The complex may be isolated in dry form by a process such as lyophilization or spray drying. Complexation of the host molecule with chemically modified amorphous CDs often promotes amorphous state when the complex is lyophilized. The resulting change in the solid-state form of drug from crystalline to amorphous could have a major impact on the chemical stability of the drug molecule by changing the drug molecule's mobility and environment. Thus, complexation with CD may stabilize or destabilize the included drug in the solid state indirectly.

Ziprasidone is an antipsychotic drug for the treatment of schizophrenia and schizoaffective disorder. Ziprasidone has a strong crystallization tendency due to extensive intramolecular hydrogen binding and π–stacking interactions. As the result, ziprasidone free base has very low solubility in water. An injectable formulation has been developed utilizing SBEβCD as a solubilizer.[7] Because of the potential degradation of ziprasidone in an aqueous solution, namely the formation of oxidative degradants I and II (Fig. 1), the drug product was developed as a lyophilized form of the complex.

In this study, the impact of different side chains of CD derivatives and the effect of various solid-state forms on the stability of ziprasidone in complexes was explored in both solution and solid states. Several derivatized CDs were employed to form complexes with ziprasidone. Because of the relatively thermally stable nature of crystalline ziprasidone in solid state, irradiation was used to assess stability of drug in complexed form to rapidly evaluate the impact of solid form on stability.

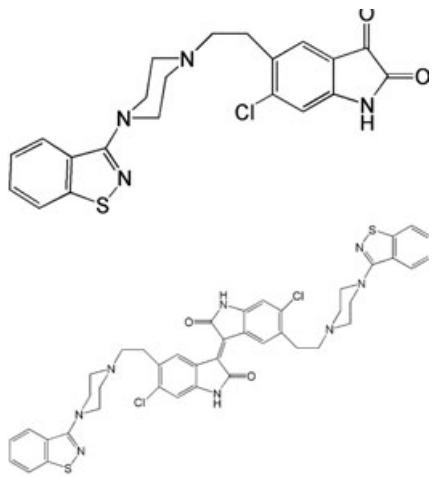

**Figure 1.** Structures of ziprasidone and its oxidative deagradant I and secondary degradant II.

## Experimental

### Materials

With the exception of βCD and SBEβCD, all of the CD derivatives used in both studies were purchased from CyDex, Inc. (Overland Park, Kansas). These included Trappsol®, randomly methylated β-CD (MβCD, degree of substitution = 1.8), Trappsol® hydroxypropyl-β-CD (HPβCD, degree of substitution = 4.2 and 6.3), and Trappsol® carboxyethyl-β-CD (CEβCD, degree of substitution = 3.0). Sulfobutylether-β-CD (SBEβCD) was obtained from Pfizer Inc. (degree of substitution = 6.5). The unsubstituted β-CD was obtained from Sigma–Aldrich (St. Louis, Missouri). Ziprasidone mesylate was sourced from Pfizer Global Research and Development (PGRD), and the spray-dried dispersion (SDD) of ziprasidone in HPMCAS was a gift from Dr. S. Shamblin (PGRD). SBEβCD salts of ziprasidone were obtained from Dr. S. Walinsky and Mr. T. Sinay of the Chemical Research and Development Department of PGRD. High-performance liquid chromatography (HPLC) methods and reference standards of degradants I and II were obtained from Analytical Research and Development, PGRD. Millex®-GV PVDF 0.22 μm membrane syringe filter was obtained from Millipore (Billerica, Massachusetts). The container/closure, storage, and packaging materials for each study were obtained from Pfizer, Inc. and included 2.5 mL Flint Type I vials sealed with 13-mm Omniflex stopper, and 10 mL Flint Type I vials sealed with 20-mm Omniflex stopper. All other compounds were reagent grade from commercial sources and used without further purification.

### X-ray Powder Diffraction

X-ray powder diffraction patterns of the test articles were obtained with a D5000 Siemens Diffractometer (Siemens, Munich, Germany) with voltage of 50 kV and a current of 40 mA. Alignment was verified with an aluminum standard before each measurement. Samples were prepared by placing powders in a quartz zero background sample holder and scanned from 3° to 40° 2θ at a rate of 1°/s.

### Morphology by Microscopy

Microscopic analysis of ziprasidone active pharmaceutical ingredient (API) and salts, lyophilized complexes, and salt forms was performed on an Olympus BH-2 microscope (Olympus, Tokyo, Japan) under both bright and dark field to assess crystallinity and shape.

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### Nuclear Magnetic Resonance

Inclusion complexation of ziprasidone with SBEβCD at a molar stoichiometry of 1:1 was confirmed using proton nuclear magnetic resonance (NMR) of ziprasidone solubilized in SBEβCD in dimethyl sulfoxide (DMSO) previously by Kim et al.[7] In the current study, solid-state NMR was used to characterize the lyophile and determine whether molecular level interaction is maintained as an inclusion complex between SBEβCD and ziprasidone in the solid state. Approximately 300 mg of each sample was tightly packed into a 7 mm ZrO spinner for each sample that was analyzed. One-dimensional $^{13}C$ spectra were collected at ambient pressure using $^{1}H$–$^{13}C$ cross-polarization magic angle spinning (CPMAS) at 295 K on a Bruker 7-mm BL CPMAS probe positioned into a wide-bore Bruker Avance DSX 500 MHz NMR spectrometer (Bruker BioSpin Corporation, Billerica, Massachusetts). The samples were spun at 7000 Hz corresponding to the maximum specified spinning speed for the 7-mm spinners. The fast spinning speed minimized the intensities of the spinning side bands. To optimize signal sensitivity, cross-polarization contact time was adjusted to 1 ms. A total of 600 scans were acquired, unless sensitivity limited, resulting in approximately 30-min acquisition times. The spectra were referenced using an external sample of hexamethylbenzene with the most upfield methyl signal set to 17.3 ppm.

### Purity Assay for Stability in Solution and Solid State

The purity assay was accomplished on an HP 1100 HPLC system (Agilent Technologies, Santa Clara, California). For analysis of degradant I, a Waters Symmetry C18 150 × 3.9 mm, 5 μm column (Waters Corporatioin, Milford, Massachusetts) was used with the ultraviolet (UV) detector set at 229 nm. Mobile phase was pH 3.0, 0.05 M $KH_2PO_4$ buffer/methanol, 60:40 (v/v) mixture at a flow rate of 1.0 mL/min. Both column and mobile phase were heated to 40°C. For analysis of degradant II, a Waters Symmetry C18 (150 × 3.9 mm, 5 μm) column with UV detection at 229 nm was used. Mobile phase was pH 3.0 and 0.1 M 1-octanesulfonic acid/0.025 M $KH_2PO_4$ buffer/acetonitrile/methanol (50/42/8, v/v/v) mixture at a flow rate of 1.0 mL/min. The column and mobile phase were heated to 35°C.

### Thermal Stability of Ziprasidone–CD Complexes in Solution

#### *Preparation of Ziprasidone–CD Complexes*

Complexes of each β-CD derivative and ziprasidone were prepared in order to compare the effect of the side chains of the CD on ziprasidone degradation. These CD derivatives (Table 1) included MβCD, SBEβCD, CEβCD, and HPβCD-3 (degree of substitution = 3.0), hydroxypropyl β-CD (HPβCD-4, degree of substitution = 4.5), and hydroxypropyl β-CD (HPβCD-6, degree of substitution = 6.3).

The process used to form the complex between ziprasidone mesylate and CD has been reported previously[8] and is briefly described here. To form the complex, CDs were added to 15 mL of sterile water for injection (sWFI) in a small beaker and stirred until fully dissolved. After dissolving, CD solutions were placed in a water bath and heated to 70°C. Because of the low solubility of unsubstituted β-CD, this sample did not dissolve until heated. When the temperature reached 70°C, ziprasidone mesylate was slowly added to the solution and stirred until dissolved. Once ziprasidone–CD solution became clear, it was immediately removed from the heat source and allowed to cool while stirring.

#### *Solution Stability*

The pH of each solution was measured and adjusted with methanesulfonic acid to pH 2.8, approximately. The solution was stirred for 24 h and then filtered into clean 2.5 mL glass vials with a fill volume of 1 mL. In addition to the complexes, two control samples without CD s were prepared. Because of the extremely low aqueous solubility of ziprasidone, these control samples had to be prepared with a water/organic solvent system. The first control was composed of 0.20 g ziprasidone mesylate in 20 mL of 50:50 (v/v) methanol/water solution. The second control was

**Table 1.** Cyclodextrin (CD) and Ziprasidone Amounts Used for 1:1 Molar Complexation in Solution and the Resultant pH

| Sample | Degree of Substitution | MW (g/mol) | Moles of CD | CD (g) | Ziprasidone (g) | Initial pH | Final pH |
|---|---|---|---|---|---|---|---|
| βCD | 0 | 1134 | $4.41 \times 10^{-4}$ | 0.50 | 0.12 | 2.77 | 2.77 (Not adjusted) |
| MβCD | 1.8 | 1161 | $2.58 \times 10^{-3}$ | 3.00 | 0.35 | 3.17 | 2.81 |
| HPβCD-6 | 6.3 | 1506 | $2.58 \times 10^{-3}$ | 3.89 | 0.35 | 3.33 | 2.15 |
| HPβCD-4 | 4.2–4.5 | 1386–1404 | $2.58 \times 10^{-3}$ | 3.57 | 0.35 | 2.87 | 2.87 (Not adjusted) |
| HPβCD-3 | 3.0 | 1309 | $2.58 \times 10^{-3}$ | 3.38 | 0.35 | 3.81 | 2.80 |
| CEβCD | 3.0 | 1350 | $2.58 \times 10^{-3}$ | 3.48 | 0.35 | 3.64 | 2.67 |
| SBEβCD | 6.5 | 2163 | $2.58 \times 10^{-3}$ | 5.58 | 0.35 | 4.02 | 2.83 |
| Control (1:1, v/v, methanol/water ) | 0 | 412.9 (drug only) | 0 | 0 | 0.20[a] | 3.75 | 3.75 (Not adjusted) |

[a] Drug dissolved in 20 mL of 50:50 (v/v) methanol/water.

composed of 0.20 g of ziprasidone mesylate in 20 mL of 10% DMSO/45% $H_2O$/45% methanol solution. The solution pH was adjusted with methanesulfonic acid. The vials were sealed and placed in a 50°C stability chamber. Samples were analyzed in triplicate at 0, 4, 8, and 12 weeks for potency and purity by HPLC.

The concentration of drug and CD used in the study are listed in Table 1. Molecular weight was used to determine the amount of CD in grams used in complexation experiment in order to keep molar concentrations equal or higher to ensure 1:1 molar stoichiometry with the only exception being unsubstituted β-CD. Because of the very low water solubility of underivatized β-CD, the concentration of ziprasidone in this sample was lower than that used for other β-CD –drug complexes.

### Gamma Irradiation of Ziprasidone–CD Complexes to Assess Stability in Solid State

To assess the effect of CD derivative and nature of solid state, the test samples obtained by freeze-drying CD–ziprasidone complexes along with other solid-state forms of ziprasidone were subjected to gamma irradiation. Because of intrinsically greater chemical stability of ziprasidone in solid state, high-energy irradiation was used as an accelerated condition to differentiate the effects of side chain and solid form on oxidative degradation.

#### *Preparation of Lyophilized Ziprasidone–CD Complexes*

Complexes were formed using the same procedure as for the solution-state study except for pH adjustment, which was not performed for these samples. Solution pH for all solutions was below pH 4.0, dictated by the solution pH of ziprasidone mesylate. After filtering 3 mL of CD–ziprasidone complex into clean 10 mL glass vials, the samples were freeze-dried using a U.S. Filter FTS Lyostar® freeze drier. The vials were sealed under ambient conditions in air with a 20-mm Omniflex stopper. This procedure was followed for complexation and drying of ziprasidone with β-CD, MβCD, SBEβCD, CEβCD, HPβCD (substitution = 4.5), and HPβCD (substitution = 6.3).

#### *Preparation of Ziprasidone–SBEβCD Salts*

The free acid form of SBEβCD was prepared in quantitative yield by using an Amberjet 1200H ion-exchange resin to exchange the sodium ion for protons. SBEβCD polysulfonic acid contains approximately six polymethylenesulfonic acid groups per β-CD as determined by acid–base titration. Different stoichiometric salts of ziprasidone with SBEβCD were then prepared by adding the free acid form of SBEβCD to 3–6 molar equivalents of ziprasidone free base. The solid forms of ziprasidone–SBEβCD salts were isolated by removal of reaction medium at reduced pressure and moderate heat. Sample vials were filled with 0.3 g of ziprasidone–SBEβCD salt (4:1), and ziprasidone–SBEβCD salt (6:1) each.

### Spray-dried Dispersion

Spray-dried dispersion of ziprasidone in the polymer hydroxypropyl methyl acetate succinate (HPMCAS) was prepared by solvent spray drying from a methanolic solution. The resulting sample contained 0.5 g of ziprasidone free base at 10% (w/w) in HPCMAS as SDD in glass vials.

### Physical Mixture

As one of the crystalline controls, a physical mixture of ziprasidone and CD was prepared by mixing the components then grinding with a mortar and pestle to form an intimate mixture. Each glass vial contained 1.8 g of a physical mixture of SBEβCD and ziprasidone mesylate in 10:1 (w/w) ratio. Additional crystalline control included 1 mg ziprasidone mesylate in glass vial alone.

#### *Gamma Irradiation*

The freeze-dried complexes, SDD, ziprasidone mesylate drug substance, physical mixture of ziprasidone mesylate and SBEβCD, and ziprasidone–SBEβCD salts were irradiated and received a 22.9–23.8 kGy dose of gamma irradiation at SteriGenics International, Inc. (Charlotte, North Carolina). Each sample was analyzed for potency and purity.

#### *Sample Analysis*

Control samples from each lot were stored and protected from light until analysis on all samples were performed. Analysis of samples included potency and purity analysis by HPLC, and solid form characterization by X-ray diffraction, microscopy, and NMR to confirm the solid form of the test article.

## RESULTS

### Physical State (Crystallinity) of Test Samples

The solid-state form of test samples was assessed by X-ray diffraction and optical microscopy under bright and dark field. Some representative powder XRD spectra and microscope pictures are shown in Figures 2 and 3, respectively, and the results are summarized in Table 2. As indicated by XRD and microscopy, the solid forms of the test materials range from completely crystalline to completely amorphous. Although ziprasidone mesylate and ziprasidone–SBEβCD physical mixtures were completely crystalline, ziprasidone–CD complexes and SDD were entirely amorphous. Ziprasdione–SBEβCD salts exhibited increasing degree of crystallinity with increasing ziprasidone–SBEβCD ratio. Rank order of crystallinity of these materials was as follows:

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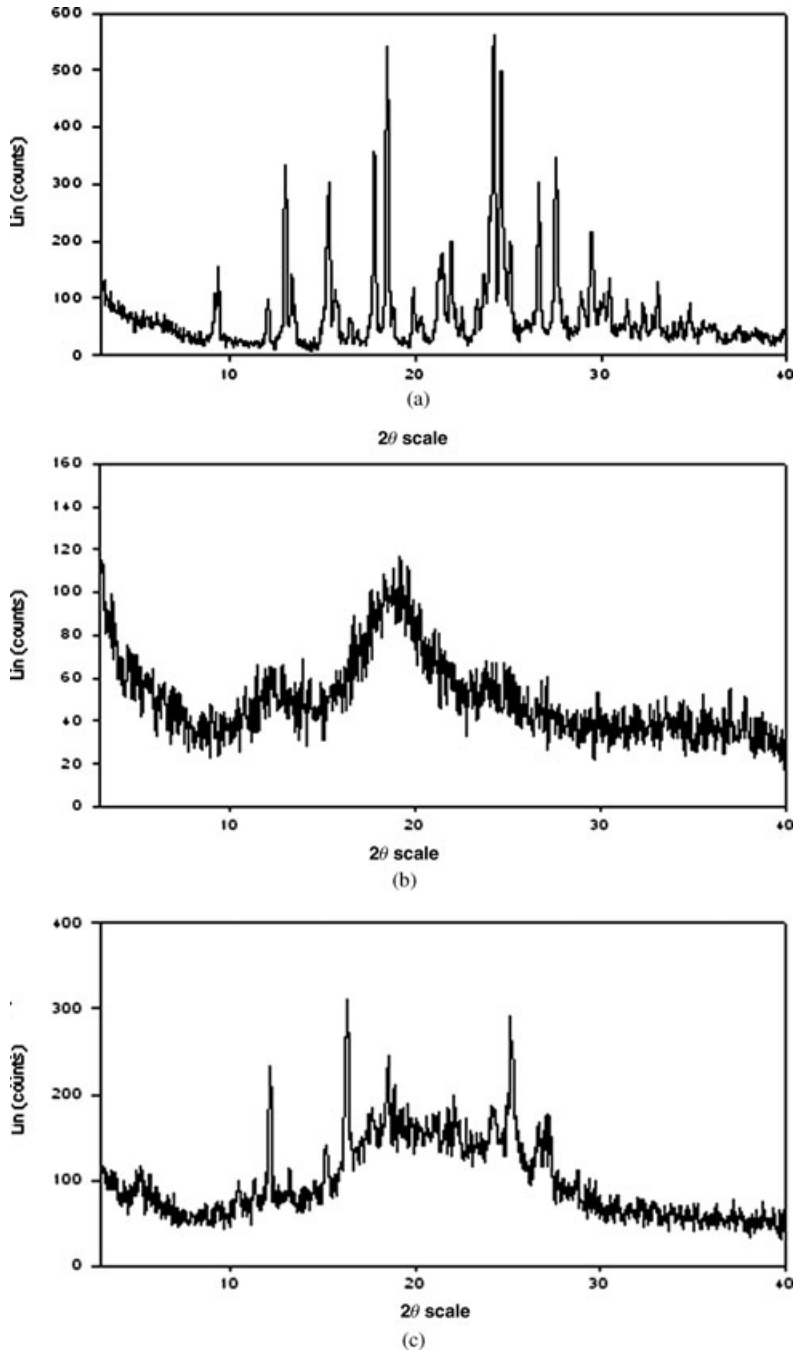


**Figure 2.** X-ray diffraction patterns of various ziprasidone samples in different physical states: (a) crystalline drug substance, ziprasidone mesylate; (b) amorphous nature of ziprasidone–βCD complex; and (c) partially crystalline nature of ziprasidone–SBEβCD salt, 6:1.

ziprasidone mesylate (completely crystalline) ≥ physical mixture of SBEβCD–ziprasidone > ziprasidone–SBEβCD salt 6:1 > ziprasidone–SBEβCD salt 4:1 > ziprasidone–CD complexes = SDD (completely amorphous).

### Characterization of CD Complexes in the Solid Form by NMR

Kim et al.[7] demonstrated the formation of 1:1 ziprasidone–SBEβCD complex by circular dichroism, NMR, phase-solubility study, and Monte Carlo simulation in polar solvents. However, the complex in the solid state was not isolated and characterized. In our study, the solid form of the complex was characterized by NMR to show retention of the inclusion complex in the solid state. Six samples were analyzed by $^{13}C$ CP-MAS solid-state NMR with total suppression of spinning sidebands to minimize an influence of spinning sidebands and to ensure accurate calculation of relaxation times. The samples included pure forms of

**Table 2.** Physical Nature of Ziprasidone in Different Chemical Forms for Solid State-Stability Study

| Sample | Physical State | |
|---|---|---|
| | XRD | Dark Field Microscopy |
| Drug substance (ziprasidone mesylate) | Crystalline | Crystalline |
| Physical mixture of ziprasidone mesylate with SBEβCD | Crystalline | Crystalline |
| Spray-dried dispersion of ziprasidone mesylate with HPMCAS | Amorphous | Amorphous |
| Ziprasidone–SBEβCD salt 4:1 | Partially crystalline | Partially crystalline |
| Ziprasidone–SBEβCD salt 6:1 | Partially crystalline | Partially crystalline |
| SBEβCD–ziprasidone complex | Amorphous | Amorphous |
| MβCD–ziprasidone complex | Amorphous | Amorphous |
| HPβCD-4–ziprasidone complex | Amorphous | Amorphous |
| HPβCD-6–ziprasidone complex | Amorphous | Amorphous |
| CEβCD–ziprasidone complex | Amorphous | Amorphous |
| βCD–ziprasidone complex | Amorphous | Amorphous |

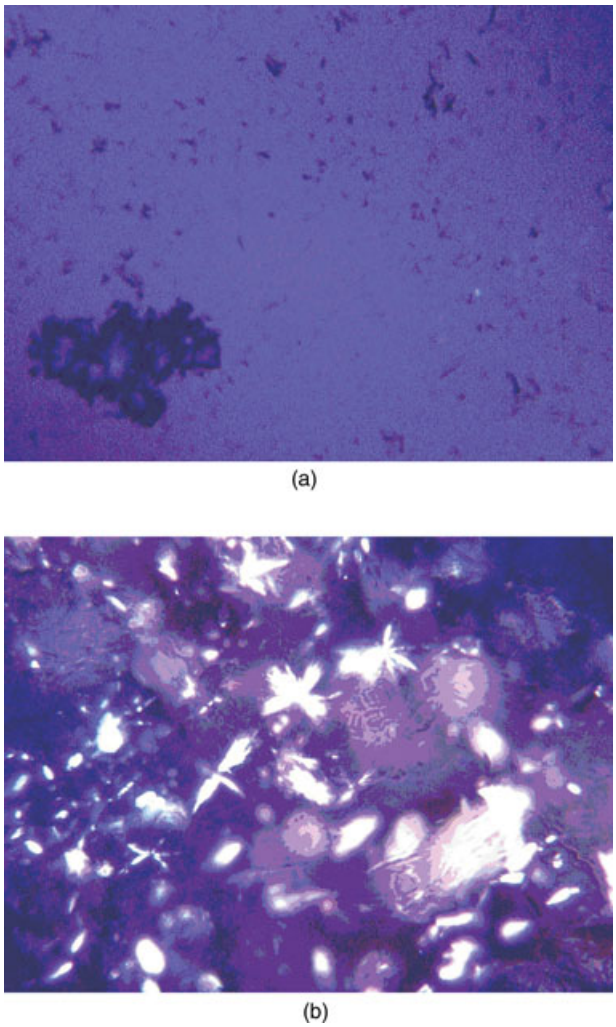


**Figure 3.** Representative microscopic pictures of ziprasidone samples taken under dark field to characterize solid form of the sample: (a) amorphous (ziprasidone/βCD complex) and (b) crystalline (ziprasidone–SBEβCD salt, 6:1).

crystalline and amorphous ziprasidone mesylate and ziprasidone–SBEβCD inclusion complexes at various molar stoichiometric ratios.

The solid-state samples of SBEβCD complex were evaluated using solid-state-nuclear magnetic resonance (SS-NMR) to measure the changes in the $^{1}H$ relaxation times to determine if inclusion complex is maintained during isolation in the solid form. $^{1}H$ relaxation time is a measure of the motion of protons at molecular level. In general, amorphous forms show a shorter relaxation time than the crystalline form due to higher mobility and thus faster relaxation of the proton signal. Also, interaction at the molecular level in the complex in contrast to a simple physical mixture may lead to similar relaxation times due to spin–spin coupling. As seen in Figure 4, in the $^{1}H$ relaxation time study, the amorphous SBEβCD showed a shorter relaxation time (2 s) along with broader peak shapes. Similarly, amorphous ziprasidone peaks were broad with shorter relaxation times. The physical mixture of SBEβCD and crystalline ziprasidone mesylate show two sets of relaxation times, 1.7 and 5 s, associated with the broad and narrow peak characteristics of SBEβCD and ziprasidone mesylate, respectively. The relaxation time of 1.7 s and broad peak shape is likely due to amorphous SBEβCD and the relaxation time of 5 s associated with the narrow peak shape is due to the crystalline form of ziprasidone mesylate. Shift positions of the signal confirm the above conclusion for the physical mixture. Interestingly, the three lyophilized complexes with different ziprasidone to SBEβCD molar ratios (1:1.36, 1:1.63, 1:2.83), all showed uniform relaxation times of 3.7, 3.0, and 2.9 s, respectively, irrespective of whether the peak was associated with ziprasidone or SBEβCD. Also, these relaxation times are significantly higher than that for pure amorphous ziprasidone and lower than that for crystalline form of ziprasidone mesylate, which indicates a different form of ziprasidone in the lyophilized complex. The uniform relaxation time for these peaks that is distinct from the relaxation times for amorphous SBEβCD and crystalline ziprasidone is direct evidence of a coupled interaction at the molecular level such as complexation.

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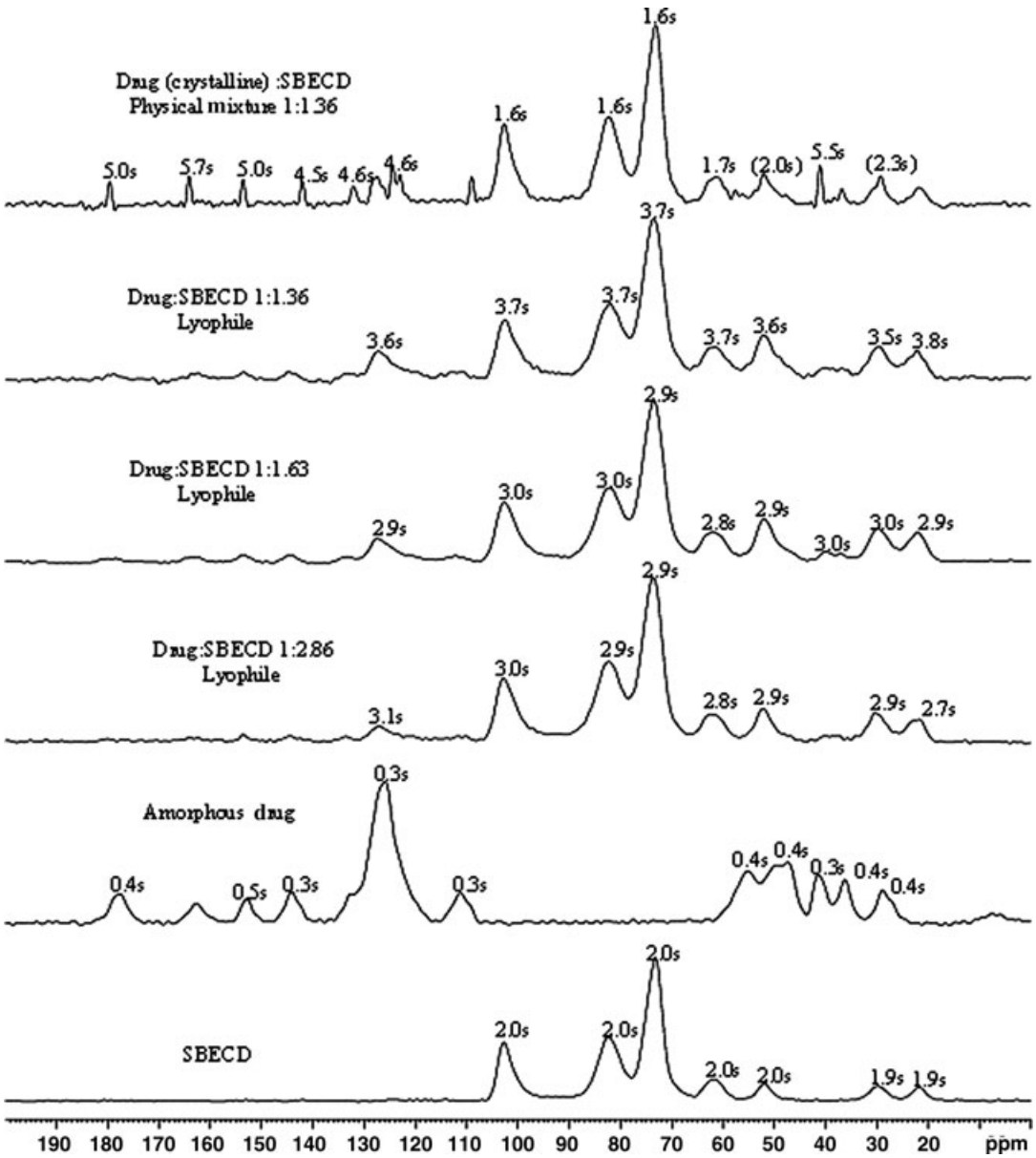


**Figure 4.** Solid-state NMR $^{1}H$ $T_1$ relaxation study for ziprasidone. Direct evidence of the interaction between drug and SBEβCD at the molecular level in amorphous complexes.

An alternative explanation could be the formation of random solid solutions or molecular dispersions of the amorphous form of drug in the carrier with no direct chemical interaction. However, in this scenario, one would not expect similar or coupled relaxation times for the drug and the carrier. The relaxation times for the three lyophile cakes varied only slightly from 2.9 to 3.7 s with increasing ziprasidone to SBEβCD ratio (molar ratio 1:2.86–1:1.36). These lyophile cakes had similar peak shapes and positions, and the significance of these slight differences in relaxation times is unclear. The fact that ziprasidone dissolved at high concentration from the isolated solid into an aqueous solution provided indirect evidence that a complex was formed and the sample remained as a complex in the isolated solid state. This is further supported by the fact that the lyophilized complex can be rapidly reconstituted with water although the formation of the complex required heating the CD solutions to 70°C to complex and dissolve ziprasidone.

In addition, SS-NMR was conducted on ziprasidone–SBEβCD salts at 4:1 and 6:1 molar ratios. In comparing the relaxation properties of the two salts, it was observed that both samples appear to be mixtures of multiple phases. There are at least two different phases in the 4:1 salt and at least three different phases in the 6:1 salt, one phase being crystalline free base. The peaks related to the mesylate salt form that were observed in the physical mixture are missing from the spectra

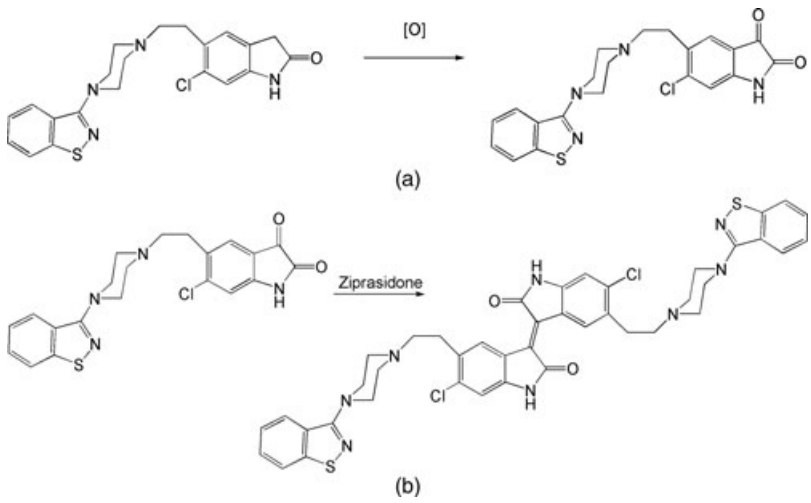


**Figure 5.** Reaction scheme for oxidative degradation of ziprasidone to form degradant I (a) and subsequent aldol condensation with another ziprasidone molecule to form degradant II (b).

of both samples prepared by adding stoichiometric ratios of the ziprasidone free base and the free acid form of SBEβCD. The ziprasidone–SBEβCD 6:1 salt spectrum contained many peaks that are not present in the spectrum for the 4:1 salt. These peaks match exactly with peaks from the crystalline free base, indirectly suggesting that other form(s) such as the ziprasidone–SBEβCD salt is present along with the free base in crystalline form.

## Degradation in Solution

As anticipated, two major degradants were detected in the solution stability study. Degradant I was a product of ziprasidone oxidation at the alpha position of the oxindole ketone moiety (Fig. 5a). The formation of I directly facilitated the formation of degradant II, which is an aldol condensation product of I and ziprasidone (Fig. 5b). Figure 6a shows the growth of degradant I as a function of time for ziprasidone complexes with different CD derivatives in comparison to controls. Ziprasidone complexed with negatively charged CEβCD and SBEβCD exhibited significantly higher oxidative degradant levels than all other ziprasidone–CD complexes, whereas the βCD and MβCD complex with ziprasidone had the least extent of oxidative degradation.

Degradant II is formed from a secondary reaction of ziprasidone with I and Figure 6b shows the kinetics of II formation. It is evident from the formation of II that ziprasidone when complexed with electron-rich CEβCD and SBEβCD is more prone to degradation than ziprasidone in other CD complexes. Ziprasidone complexes in βCD and MβCD showed the least extent of degradation. Because both degradants, I and II, are due to initial oxidation of ziprasidone, Figure 6c shows the kinetics of formation of oxidative degradants by combining the amount of two degradants formed. A clear trend can be seen from this plot that the extent of degradation follows the order of:

$$OCH_2CH_2COO^- \approx OCH_2CH_2CH_2CH_2SO_3^- > OCH_2CHOHCH_3 > OCH_3 \approx OH \approx \text{Control}$$

In addition, a higher degree of substitution on the CD ring increases the probability of interaction between drug and the side chain therefore leading to greater degradation of the complexed drug molecule as seen for HPβCD complexes: $OCH_2CHOHCH_3$ (6) > $OCH_2CHOHCH_3$ (3 and 4). The initial rate of the oxidative degradation calculated from this study is listed in Table 3 and follows the order described above.

Ziprasidone mesylate solution in the absence of CD showed very little degradation, indicating the important role of CD complexation and the resultant catalytic effect of side chain in the oxidative degradation of ziprasidone. It is worth pointing out that due to the extremely low aqueous solubility of ziprasidone, control samples of ziprasidone solution were prepared in mixtures of water and organic solvent instead of pure water, namely 50:50 (v/v) methanol/water and 10% DMSO/45%$H_2O$/ 45% methanol solutions. The fact that oxygen solubility in DMSO (2.2 mmol/L) and in methanol (10.2 mmol/L) is higher than that

**Table 3.** Initial Rate of Degradant II Formation in Solution at 50°C

| Cyclodextrin Used for Complexation | Initiate Rate, $r_0$ (M·week$^{-1}$) |
|---|---|
| CEβCD | $1.85 \times 10^{-4} \pm 1.25 \times 10^{-5}$ |
| SBEβCD | $1.51 \times 10^{-4} \pm 1.04 \times 10^{-5}$ |
| Control-None | $1.17 \times 10^{-5} \pm 3.26 \times 10^{-6}$ |
| HPβCD6 | $3.06 \times 10^{-5} \pm 2.11 \times 10^{-6}$ |
| HPβCD3 | $2.03 \times 10^{-5} \pm 3.91 \times 10^{-6}$ |
| MeβCD | $1.21 \times 10^{-5} \pm 1.58 \times 10^{-6}$ |
| HPβCD4 | $4.12 \times 10^{-6} \pm 6.53 \times 10^{-7}$ |
| β-CD | $3.44 \times 10^{-6} \pm 9.44 \times 10^{-7}$ |

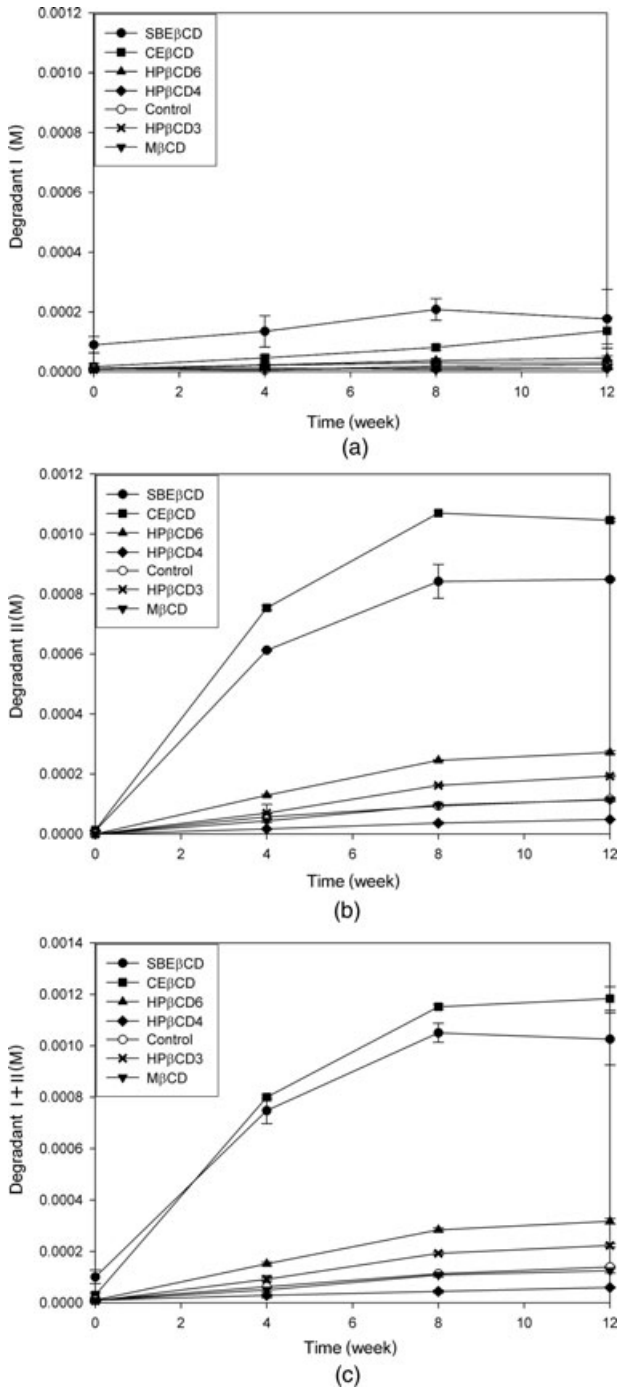


**Figure 6.** Degradation behavior of ziprasidone–cyclodextrin complexes in solution state: (a) degradant I—oxidation; (b) degradant II-–oxidation followed by aldol condensation; and (c) total oxidative degradants.

in water (1.27 mmol/L) suggests that the solvent systems used could be worse-case scenario as controls for the oxidative degradation.[9]

### Degradation in Solid State

Figure 7 shows the formation of degradants I and II after gamma irradiation at 23 kGy. Compared with the degradation of ziprasidone in solution, no apparent trend in degradation as a function of substituent on CD is evident and the effect at first appears random. The rank order for formation of I is as follows:

HPβCD-6 > HPβCD-4 = βCD > MβCD = SBEβCD = CEβCD = SDD > ziprasidone–SBEβCD salts > ziprasidone–SBEβCD physical mixture > ziprasidone mesylate.

The levels of degradant II were too low to draw any conclusion. Lack of distinct rank order as a function of the nature of the side chains on CD suggests a limited impact of electronic interaction on ziprasidone stability to high energy irradiation. However, a careful look at the results suggest an effect of the physical state of the sample on extent of degradation, with all amorphous samples showing high levels of oxidative degradant I with little to low levels of I in crystalline (ziprasidone mesylate control sample, ziprasidone–SBEβCD physical mixture) to partially crystalline samples (ziprasidone–SBEβCD salts). It also appears that all amorphous forms of ziprasidone are equally reactive irrespective of the matrix (CD complexes and SDD) and show similar extents of degradation.

Another interesting observation is that aldol condensation of I with intact ziprasidone occurs only in SDD and SBEβCD–ziprasidone salt to form degradant II. Although it is difficult to draw conclusion due to the very low levels of II formed, it may be that stoichiometric inclusion complexation may have shielded the oxidized ziprasidone (I) from encountering a neighboring ziprasidone molecule so that the secondary condensation reaction is inhibited. The nature of the solid state has a greater effect on the stability of ziprasidone in complexes than the catalytic effect of side chain as apparent from these results.

## DISCUSSION

### Effect of CD on Drug Stability

There have been reports of CD stabilizing drugs as well as catalyzing degradation of drugs.[5–6] However, a systematic study on the effect of side chain on drug stability and the mechanism for catalysis does not exist. Considering that high concentrations of CDs are required to ensure complexation of the guest even at 1:1 stoichiometric ratio, a nonspecific catalytic effect of CDs on the reaction of complexed drug can occur. In addition, charged CDs such as carboxyethyl and sulfobutyl ether ß-CDs can catalyze reactions by general acid–base mechanism or by providing high ionic strength, provided the reaction intermediate involves a charged species. Another mechanism for stabilization was proposed to be the extent of free drug available for degradative reaction because CD sequesters the drug molecule. Hydrolysis of drugs can be

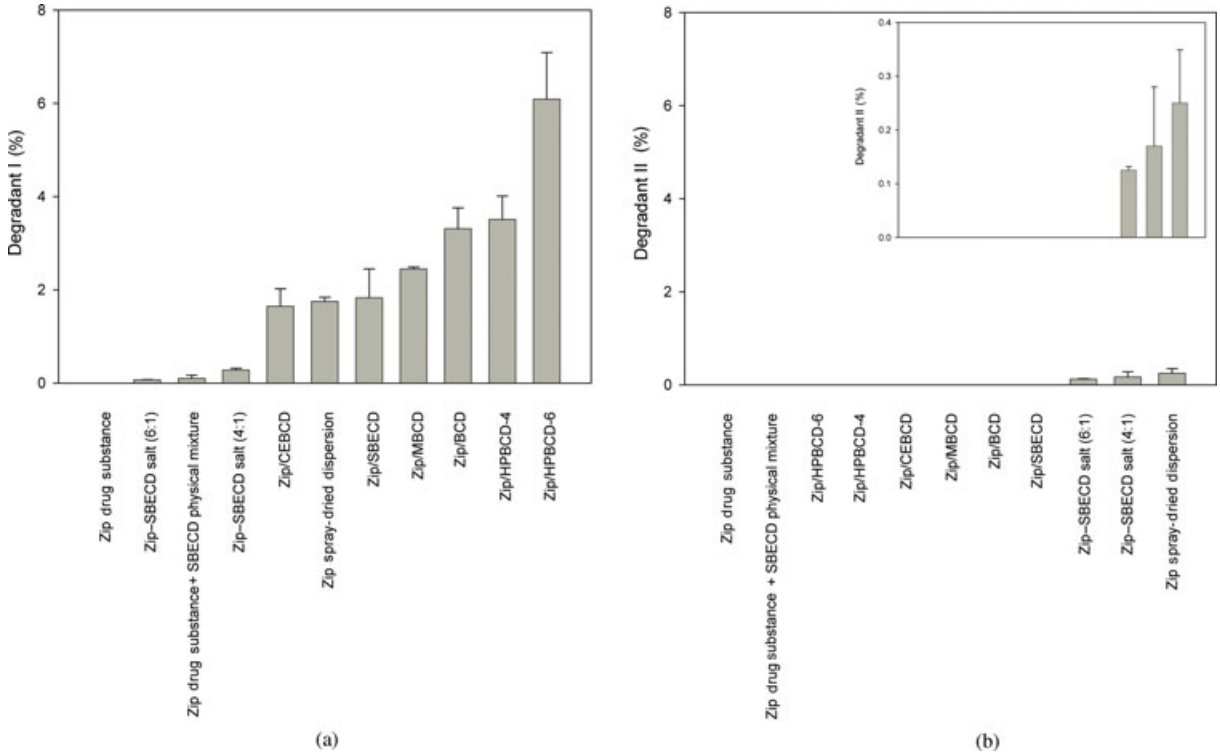


**Figure 7.** Degradation behavior of amorphous ziprasidone–cyclodextrin complexes in solid state compared with amorphous spray-dried dispersion, partially crystalline salts, and crystalline mesylate salt form upon gamma irradiation: (a) degradant I-–oxidation; (b) degradant II-–oxidation followed by aldol condensation.

affected by pH, solvents, general acid-base catalysis, ionic strength and solvent polarity, and hence may not be that easily amenable to mechanistic investigation for catalysis by CDs. In contrast, because the primary pathway for ziprasidone degradation involves oxidation, it is a good model system to investigate the specific interactions between the drug and cylcodextrin, and to study the effect of side-chain ionic and electronic catalytic effects on drug degradation.

## Solution Stability

The oxidative degradation of ziprasidone in CD complexes likely follows electron transfer mechanism and a free radical cation intermediate is expected. Whether ziprasidone molecule undergoes proton extraction or hydrogen atom extraction first to form a cation intermediate is beyond the scope of this study. Nevertheless, as the free radical cation forms, the electron-donating group in the vicinity could provide stabilization if the right interaction could be achieved. The mechanism of neighboring group assistance in catalyzing oxidation by stabilizing the free radical intermediate has been well documented.[6] Such neighboring group participation has been also shown for hydrolysis[10] and dimmerization reactions.[11]

Cyclodextrin complexation mimics an enzyme action by binding and locking the molecule in a strategic, specific conformational position facilitating the interaction of guest molecule and the side chains. When the side chain possesses the catalytic capability by electronic and/or steric interactions, acceleration in degradation rate can be observed. Side chains of CD derivatives interact with drug molecules in the inclusion complex affecting stability by a mechanism resembling the neighboring group assistance. The negatively charged (carboxyethyl and sulfobutylether) and electron-donating (hydroxypropyl) groups can catalyze degradation of ziprasidone by stabilizing the oxidative intermediate as shown in Figure 8 (three radical cation[12]). In this scenario, electron-donating capability of the side chains would directly influence the stability of the shown reaction intermediate resulting in the following rank order for degradation:

$$OCH_2CH_2COO^- > OCH_2CH_2CH_2CH_2SO_3^- > OCH_2CHOHCH_3 > OCH_3 > OH$$

It is also conceivable that with higher degree of substitution on the CD ring, there is an increased probability of interaction between ziprasidone and the side chain causing greater degradation of the drug molecule. This is evident by greater oxidative degradation of ziprasidone in the complex of

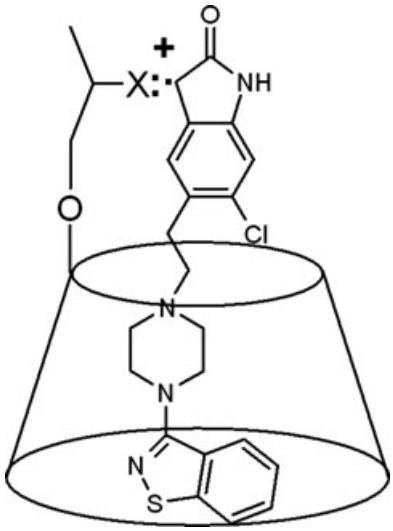


**Figure 8.** Schematic for potential interaction of cyclodextrin side chain with ziprasidone that may facilitate oxidative degradation intermediate formation.

hydroxypropyl-βCD-6 (HPβCD-6) than that of hydroxypropyl-βCD-3 (HPβCD-3 and -4).

To demonstrate the catalytic effects related to the electron-donating tendency of the side chain, the initial rate ($\log r_0$) for degradation is plotted as a function of p*K*a of the side chain, a measure of the electron-donating capability of the side chain. Because the real p*K*a's of side chains of these derivatized CDs were not available, p*K*a's of the parent species, methanesulfonic acid, propionic acid, and isopropylalcohol were used as a reasonable approximation. The initial rates were normalized to an average of 6.5 degrees of substitution for each CD. The linear relationship between $\log k_0$ and p*K*a is a good indicator that the electron-donating capability of the side chain is one of the key parameter in catalyzing the oxidative degradation. The effect of pH and ionic strength on degradation of ziprasidone is not anticipated as initial pH's were very similar for all solution samples.

Several factors might affect the correlation between p*K*a and $\log r_0$ shown in Figure 9. For the proposed catalytic effect by neighboring group, the electron-donating group should be in close proximity to the oxidative reaction intermediate. Kim et al.[7] have proposed an energy minimized structure for the complex based upon Monte Carlo simulation and two-dimensional NMR results suggesting inclusion of benzisothiazole ring in the CD cavity with an oxindole nucleus in close proximity to the side chain substituent. In this case, the length of the side chain can influence the degree of catalytic effect. It likely explains why native β-CD, although possessing multiple -OH groups that are more acidic than the $-OCH_3$ of the methyl β-CD (MβCD), showed very little catalysis for ziprasidone oxidation. The interaction of β-CD OH group with an oxindole nucleus of complexed ziprasidone molecule is simply unachievable due to the spatial distance and the polarity of the aqueous environment significantly screening the effect at a distance. In contrast, -OH substituents on propyl side chain may be at a specific distance close to an oxindole nucleus to influence the degradation of ziprasidone. It has been demonstrated that chain length of the CD derivatives play an important role in facilitating the complexation of drug molecules in CD.[13] It is therefore possible that chain length of different derivatives would dictate the efficiency of side chain interaction with the oxidative degradation intermediate. In the future, a systematic investigation of catalytic effect of different chain lengths of electron-donating groups on ziprasidone oxidative catalysis could provide additional support for this proposed mechanism.

### Solid-state Stability

Reaction mechanisms and kinetics can be different in solid state versus solution state and hence catalytic effects can be very different. The primary interaction between CD and inclusion complexed drug is through hydrophobic interaction in a polar solution environment with the complex in dynamic equilibrium with free drug. Hence, one may not predict any catalytic effect in the solid state because there is no dynamic equilibrium with free drug due to significantly reduced mobility for all species, and neighboring group participation feasible only for spatially close groups. Indeed the solid-state stability behavior of ziprasidone in CD complexes was quite different from that in solution state. The correlation observed that electron-donating side chains accelerate the oxidative degradation of ziprasidone in the solution state, which is not seen in the solid state when one compares the extent of degradation for various complexes in solution state with that of in solid state (Table 4). Apparently, the nature or form of the solid state played a larger role in reactivity rather than the catalytic effect because all amorphous forms (different CD complexes and SDD) showed equally higher oxidation rates to form I than crystalline forms of ziprasidone (control ziprasidone mesylate salt and ziprasidone–SBEβCD salts). XRD, birefringence, and NMR of all the materials tested showed crystallinity in the following order: ziprasidone mesylate salt > physical mixture of SBEβCD–ziprasidone ≈ ziprasidone–SBEβCD salts > ziprasidone–CD complexes ≈ SDD. Figure 7 indicates that the formation of I follows the order of:

HPβCD (6) > HPβCD (4) ≈ βCD > MβCD ≈ SBEβCD ≈ CEβCD ≈ SDD > ziprasidone–SBEβCD salts > physical mixture of ziprasidone–SBEβCD > ziprasidone mesylate.

These combined results suggest that the oxidation of ziprasidone in the CD complexes in solid state is mainly influenced by the higher mobility of

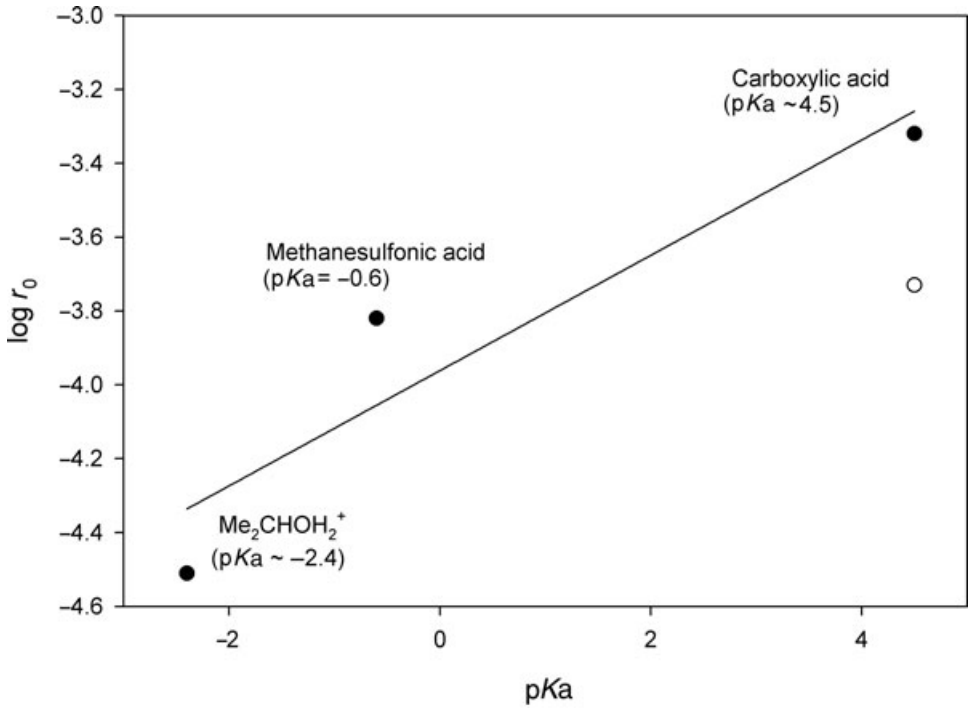


**Figure 9.** The linear relationship between initial rate (log $r_0$) of ziprasidone oxidative degradation in solutions of cyclodextrin complex with cyclodextrin side chain p*K*a suggestive of electron-donating assistance (○: initial rate in CEβCD complex with 3.0 substitution; ●: normalized to 6.5 substitution).

ziprasidone in the amorphous state. Complexed ziprasidone, irrespective of the type of modified cyclodxetrin, showed similar reactivity as the amorphous SDD and reactivity generally followed the order of crystallinity of the sample, that is, higher crystallinity resulting in lower reactivity. The results with various amorphous forms of ziprasidone (6 complexes and SDD) compared with crystalline forms (four test articles) show that the major impact of the solid form is on the extent of degradation (Fig. 7, Table 4). There is no correlation of extent of degradation with chemical nature of CD.

All the complexes are indeed amorphous as confirmed by PXRD and solid-state NMR (Fig. 4) provided for SBECD. However not all amorphous forms are identical due to differences in relaxation state indicative of differences in mobility which can influence reactivity. In addition, because water level can be slightly different in each sample which can further influence mobility and reactivity by plasticization of amorphous state, we did not expect identical extent of degradation. The glass transition temperatures of CDs are not easily characterized because they are fragile glasses but $T_g$ for CDs are known to be greater than 120°C. Hence, at ambient temperatures as well as at accelerated temperatures (50°C–70°C) significantly below $T_g$, it would have been difficult to further differentiate the effect of different degrees

**Table 4.** Total Oxidative Degradation; Comparison in Solution and Solid States

| Sample | Solution (50°C, 12 Week, %) | Solid (25 kGy, %) |
|---|---|---|
| HPβCD-4 complex | 0.82 | 3.51 |
| MβCD complex | 1.05 | 2.44 |
| βCD complex | 1.20 | 3.30 |
| HPβCD-3 complex | 1.47 | N/A |
| Solution control | 1.79 | N/A |
| HPβCD-6 complex | 3.08 | 6.09 |
| SBEβCD complex | 6.77 | 1.83 |
| CEβCD complex | 8.55 | 1.65 |
| Ziprasidone–SBEβCD salt 6:1 | N/A | 0.24 |
| Solid ziprasidone SBEβCD physical mixture | N/A | 0.10 |
| Ziprasidone–SBEβCD salt 4:1 | N/A | 0.40 |
| Spray-dried dispersion | N/A | 1.99 |

of mobility and hence different amorphous forms on reaction rates in these amorphous solids. Only SDD and ziprasidione–SBEβCD salts exhibited detectable amounts of II, which is the product of aldol condensation between ziprasidone and its oxidative degradant I. The formation reaction for II is essentially a second-order reaction and should be concentration dependent and slower in solid state due to lack of mobility. As a result, complexation with an excess amount of CD and resultant dilution may have prevented the close encounter between ziprasidone and I for facile formation of II. These results are understandable because complexation restricts the mobility of ziprasidone further precluding colliding with each other; therefore, reducing the probability for the condensation reaction.

These results clearly demonstrated the different factors governing the chemical degradation reactions in the solid and solution state for CD complexed drug. The formation of I and II showcased the influence of these factors with catalytic effect of derivatives a primary parameter in solution-state reactivity, and mobility as the primary cause for instability in the solid state. In the solid state, degradation I level is closely related to the amorphous nature of the drug, suggesting the pivotal role that molecular mobility and lack of crystallinity play in the access of oxidative species to the drug molecule. Whereas in solution, due to high mobility for all species, the key parameter influencing the oxidative degradation shifted to the electronic catalytic effect of side chains of CD derivatives due to their close proximity with complexed ziprasidone. Clearly, the electronic characteristics of the charged side chains participated in the oxidative degradation of ziprasidone, presumably via interaction with free radical intermediate to accelerate the degradation reaction.

Cyclodextrins in native and modified forms have been used for drug solubilization and resultant drug products can be a solution or solid form (lyophile, spray-dried powder). Although derivatives of CDs can enhance aqueous solubility to a greater extent than unmodified CDs, effect of side chains on the stability of drug may allow preferential selection of a CD derivative. In addition, the selection of CD derivative may depend upon the desired form of drug product and its related stability.

## CONCLUSION

In solution, derivatization of the host molecule of a drug–CD complex can have a profound impact on the stability of the guest molecule by electronic interaction with the degradation intermediate. The groups that can readily donate electrons had the most significant catalytic effect in this case for oxidation. The restrictive nature of the complexation makes the interaction more effective such that a catalytic acceleration was realized. In the solid state, the amorphous characteristic of the complex had a major impact on the stability of the guest molecule, whereas electronic nature of the side chain had no impact. The mobility of the molecule in the complex or the sample was the deciding factor on the oxidative degradation of the drug molecule. It is conceivable that the side chain derivatization influences the solid state of the complex by promoting amorphous or crystalline state and therefore has an indirect impact on drug stability. This study illustrates that CDs can have a detrimental impact on the stability of complexed drug in both solution and solid state and a systematic mechanistic investigation may allow selection of appropriate CD derivative for drug product development. Selection of derivatized CD and final form of drug dosage form should take into consideration the potential adverse impact of side chain substitution and solid-state nature of the complex on stability of the complexed drug in addition to solubility enhancement.

## ACKNOWLEDGMENTS

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