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Study of the dissolution characteristics of oxazepam via complexation with β-cyclodextrin

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Abstract

Complex formation of oxazepam and β-cyclodextrin in solution was studied by phase solubility and spectral shift methods. The value of the apparent stability constant, K_c, calculated using these techniques, was 205 and 498 M^{-1}, respectively. Solid complexes of oxazepam and β-CD were prepared using the kneading and spray-drying methods. These complexes led to an improvement in the dissolution rate over free oxazepam, spray-drying being the most efficient technique. These complexes were characterized by differential scanning calorimetry (DSC), scanning electron microscopy (SEM) and X-ray diffraction.

Keywords: Oxazepam; β-Cyclodextrin; Kneading; Spray-drying; Inclusion complex

1. Introduction

Cyclodextrins (CD) or cycloamyloses are cyclic oligosaccharides containing six (α-CD), seven (β-CD) or eight (γ-CD) α-(1,4)-linked glucose units. The most important structural feature of these compounds is their toroid or doughnut shape, with a hydrophobic interior cavity and hydrophilic faces.

In recent years, pharmaceutical modification of drug molecules by inclusion complexation has been extensively developed to improve their solubility, dissolution rate, chemical stability and bioavailability (Szejtli, 1988; Duchene and Wouessidjewe, 1990a,b; Bekers et al., 1991). From a pharmaceutical viewpoint, drug-CD solid inclusion complexes turned out to be very convenient for oral administration.

Oxazepam (7-chloro-1,3-dihydro-3-hydroxy-5-phenyl-2*H*-1,4-benzodiazepin-2-one) is a congener of chlordiazepoxide and diazepam, which is applied in therapy as an anxiolytic and hypnotic. However, it has poor aqueous solubility, with a slow dissolution rate and absorption, whist its pharmacological actions require a rapid plasma appearance. The inclusion of oxazepam in β-CD would serve to reduce these drawbacks.

The present study was undertaken to determine whether the solubility and dissolution rate

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of oxazepam can be enhanced by formation of a inclusion complex with β-CD. In addition, these experiments were also carried out to evaluate different methods for preparing a solid complex of oxazepam with β-CD for potential use in the development of a suitable oral formulation (Moyano, 1993).

2. Experimental

2.1. Materials

Oxazepam was supplied by Boehringer-Ingelheim (Barcelona, Spain) and β-CD by Roquette (Lestrem, France). All other materials were analytical reagent grade.

2.2. Phase solubility studies

Solubility studies were performed according to the method reported by Higuchi and Connors (1965). Oxazepam, in amounts that exceeded its solubility (10 mg), were accurately weighed in 50 ml Erlenmeyer flasks to which were added 10 ml of water containing various concentrations of β-CD (0.002–0.014 M). These flasks were sealed and shaken at 20°C for 1 week. This amount of time is considered sufficient to reach equilibrium. Subsequently, the samples were filtered with a syringe through a 1.2 μm Sartorius cellulose nitrate membrane filter and appropriately diluted. A portion of the sample was analyzed spectrophotometrically at 230 nm. The presence of trace amounts of β-CD did not interfere with the assay.

The apparent 1:1 stability constant K_c was calculated from the initial linear portion of the phase solubility diagram, according to the equation:

$$K_c = \text{slope}/[\text{intercept}(1 - \text{slope})]$$

2.3. Spectroscopic studies

Complex formation between oxazepam and β-CD was also studied by the spectral shift method (Connors and Mollica, 1966). The concentration of oxazepam in these studies was 1.85×10^{-6} M, whereas the CD concentration was increased from 8×10^{-4} to 7.5×10^{-3} M and the UV spectra of oxazepam were recorded on an Hitachi U-2000 UV-Vis spectrophotometer. The change in absorbance of the substrate (oxazepam) on the addition of various concentrations of the ligand (β-CD) was measured at 230 nm to evaluate the stability constant of the complex. The blanks were prepared in the same concentrations of β-CD in water so as to cancel any absorbance that may be exhibited by the CD molecules.

2.4. Preparation of solid complexes

The preparation of solid oxazepam-β-CD complexes was performed using two techniques: kneading and spray-drying. Furthermore, the raw materials (oxazepam and β-CD) were separately spray-dried and kneaded. We have also prepared physical mixtures of oxazepam and β-CD, in order to serve as reference.

2.5. Kneading method

β-CD was put in a mortar, wetted with a few drops of purified water and then kneaded. Subsequently, oxazepam was added in stoichiometric proportions (1:1 or 1:2). The mixture was then kneaded with the addition of a few drops of water. This process was continued for 45 min and the product was dried at 37°C for 48 h (Selecta, model 204).

2.6. Spray-drying method

Spray-drying was performed in a Büchi 190M miniSpray-Dryer. Oxazepam was dissolved in 400 ml of 96% ethanol. The required amount of β-CD was dissolved in 400 ml of purified water. The molar ratio of oxazepam and CD was 1:1 or 1:2. The solutions were then mixed for 20 min by sonication, to produce a clear solution, which was subsequently spray-dried. The drying conditions were as follows: flow rate, 1000 ml/h; inlet temperature, 168°C; outlet temperature, 90°C; air flow rate, 400 Nl/h.

2.7. Scanning electron microscopy

The morphological features of oxazepam (commercial sample and kneaded and spray-dried samples), β-CD (commercial, kneaded and spray dried samples), and the oxazepam-β-CD systems (physical mixture, kneaded and spray-dried systems) were analyzed by SEM (ISI SS-40), employing 20 kV tension.

2.8. Differential scanning calorimetry (DSC)

DSC analysis was carried out with a Mettler FP85 furnace, FP80 HT temperature control unit and FP89 HT software. Samples of 10 mg were put into aluminum pans. The pans were pierced in order to permit gas to leave during the heating process. These processes were performed under a static air atmosphere, at a heating rate of 10°C/min, over the temperature range of 30–400°C.

2.9. X-ray diffractometry studies

Diffractograms of different samples were obtained using a Siemens Kristallofex D-500 X-ray diffractometer. The measuring conditions were as follows: Ni-filtered CuKα radiation; voltage, 36 kV; current, 26 mA. The scanning speed was 1° (2θ)/min, the chart speed 1 cm/min and adequate sensitivity, routinely 2×10^4 cps.

2.10. Dissolution rate studies

Dissolution rate studies were performed according to the USP XXII rotating basket method (Turu Grau model D-6). The samples, corresponding to 10 mg of oxazepam, were placed into hard gelatin capsules. The dissolution medium was artificial gastric medium without enzymes; 0.1 N HCl at pH 1.2 and 2 g/l NaCl. The stirring speed employed was 50 rpm and the temperature was maintained at 37 ± 0.5°C. The samples (3 ml) were withdrawn at various time intervals using a syringe and analyzed spectrophotometrically at 230 nm.

3. Results and discussion

3.1. Phase solubility studies

The phase solubility diagram for the complex formation between oxazepam and β-CD is presented in Fig. 1. This plot shows that the aqueous solubility of the drug increases linearly as a function of β-CD concentration. It is clearly observed that the solubility diagram of oxazepam in the presence of β-CD can be classified as the A_L type of Higuchi. The linear relationship may be described to the formation of a 1 : 1 (oxazepam-β-CD) complex. Since the phase solubility diagram was of the A_L type, solid complexes could not be prepared by co-precipitation, which is only possible for complexes showing a B_S type phase solubility diagram. In those cases, other reported methods can be applied, such as kneading (Rajagopalan et al., 1986), freeze-drying (Yamamoto et al., 1992), spray-drying (Conte et al., 1993), sealed heating (Rahman et al., 1993), coevaporation (Sanghavi et al., 1993) amongst others. In this case, the selected techniques are the kneading and spray-drying methods.

The apparent stability constant can be estimated for the linear portion of the solubility diagram according the equation mentioned above (Higuchi and Connors, 1965). The formation constant was calculated using the linear regression analysis method and was found to be 205 M^{-1}. This value for the stability constant may be explained on the basis that β-CD had a rigid struc-

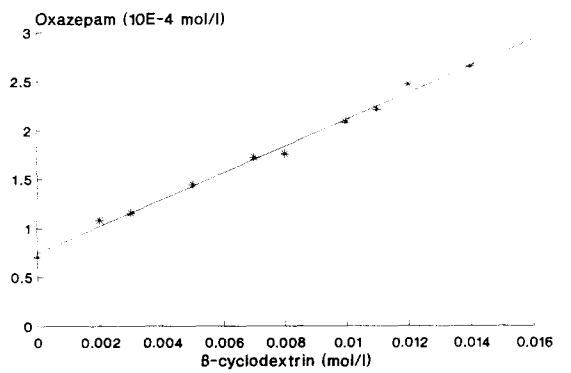

Fig. 1. Phase-solubility diagram of oxazepam-β-CD system at 20°C.

ture due to the presence of intramolecular hydrogen bonds within the hydroxyl groups of C2 and C3 of adjacent glucopyranose units. This structure was found to interfere with the fit of the oxazepam molecule into the CD cavity. Therefore, the K_c value was in good agreement with those obtained by others (Andersen and Bundgaard, 1982; Uekama et al., 1983).

3.2. Spectroscopic studies

The effect of different molar concentrations of β-CD on the absorption spectrum of oxazepam is illustrated in Fig. 2.

A bathochromic effect is evident in the absorption maxima of oxazepam, with diminution in the absorbance. These induced changes in absorbance are attributed, primarily, to the formation of a inclusion complex. The changes in peak intensity are assumed to result from changes in the solvent microenvironment upon inclusion of the solute. The observed reduction in peak intensity may result from the transfer of the guest molecule from water to the CD cavity. This is reasonable in light of the fact that there are no proton-donating groups within the cavity of the CD molecule (Ismail, 1991).

The spectral data were analyzed according to a double-reciprocal plot as shown in Fig. 3. A plot

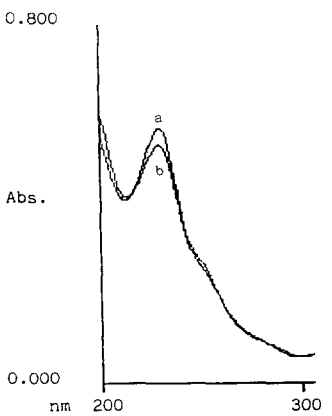

Fig. 2. Effect of β-CD concentration on UV absorption spectra of oxazepam in water. The concentration of drug was 1.85×10^{-6} M: (a) oxazepam alone; (b) in the presence of $7.5 \cdot 10^{-3}$ M β-CD.

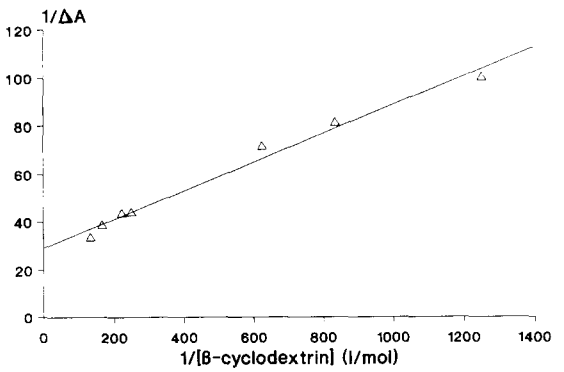

Fig. 3. Benesi-Hildebrand plot for the oxazepam-β-CD system (ΔA denotes the change in absorbance at $\lambda_{max} = 230$ nm and [CD] the cyclodextrin concentration).

of $1/\Delta A$ vs $1/[CD]$ is linear, indicating the presence of a 1:1 complex.

The apparent 1:1 stability constant was determined according to the Benesi-Hildebrand equation (Benesi and Hildebrand, 1949):

$$\frac{1}{\Delta A} = \frac{1}{[D]\, K_c\, \Delta\epsilon} \cdot \frac{1}{[CD]} + \frac{1}{[D]\, \Delta\epsilon}$$

where ΔA is the difference of absorbance at 230 nm, [CD] denotes the concentration of CD, [D] is the total drug concentration (constant) and $\Delta\epsilon$ represents the difference in molar absorptivities between the complexed and free drug. The stability constant, K_c, was determined from the intercept/slope ratio, the value being 498 M^{-1}, i.e., approximately of the same order as that calculated from the solubility studies.

The above K_c value fell within the range of 200–5000 M^{-1}, considered by various authors to be adequate for the formation of a inclusion complex which may contribute to improving the bioavailability of poorly water-soluble drugs (Blanco et al., 1991).

3.3. DSC studies

The DSC curves for all the systems assayed are represented in Fig. 4 and 5. As shown in Fig. 4 and 5, oxazepam exhibits a characteristic endothermic fusion peak at 215°C;, hence no polymorphs of oxazepam could be found. In the range

of 290°C a broad exothermic effect is apparent, which could be due to the beginning of drug decomposition. Furthermore, β-CD shows a broad endothermic effect in the range of 140°C, which may be attributed to a dehydration process.

The DSC thermograms for the 1:1 oxazepam-β-CD systems (Fig. 4) show the persistence of the endothermic peak of oxazepam for the physical mixture and the kneaded product.

For the spray-dried system, this peak is slightly shifted to low temperatures; this result can be explained on the basis of a major interaction between the drug and CD. Furthermore, the characteristic endothermic effect of β-CD disappeared for the spray-dried system.

In contrast, the 1:2 spray-dried system (Fig. 5) does not show the melting endotherm of the drug, indicating that oxazepam has complexed with β-CD.

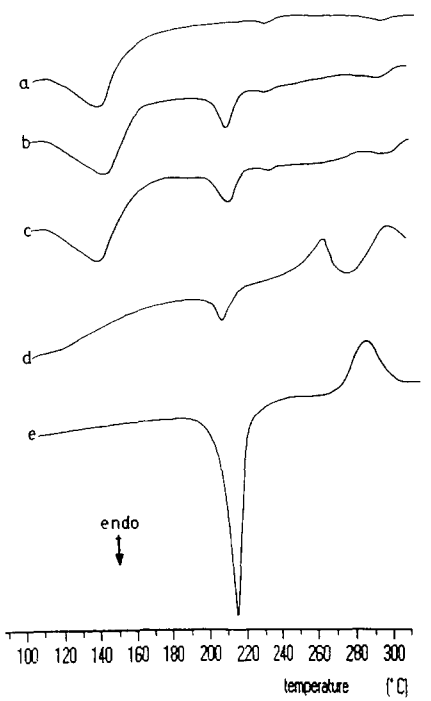

Fig. 4. DSC curves of 1:1 oxazepam-β-CD systems: (a) β-CD; (b) physical mixture; (c) kneaded mixture; (d) spray-dried; (e) pure oxazepam.

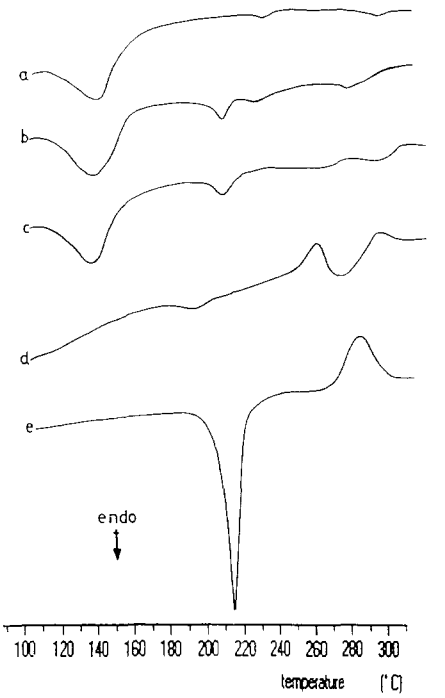

Fig. 5. DSC curves for the 1:2 oxazepam-β-CD systems: (a) β-CD; (b) physical mixture; (c) kneaded mixture; (d) spray-dried; (e) pure oxazepam.

3.4. X-ray diffraction studies

The X-ray diffraction patterns for the oxazepam-β-CD systems are presented in Fig. 6. As a consequence of the coincidence of diffraction peaks between oxazepam and β-CD, we have selected as characteristic peaks of oxazepam those situated at 6 and 30° (2θ), for confirmation of the nature of oxazepam for these studies. The diffraction patterns of the physical mixture and kneaded systems show simply the sum of each component, indicating the presence of oxazepam in the crystalline state. In contrast, the spray-dried system exhibits considerable diminution of the diffraction peaks, suggesting that it is less crystalline than the physical mixture and kneaded systems. The reduction in crystallinity attributed to the spray-drying treatment is clearly evident for pure β-CD, while oxazepam does not show this effect. These results suggest that oxazepam and β-CD form an inclusion complex in the solid

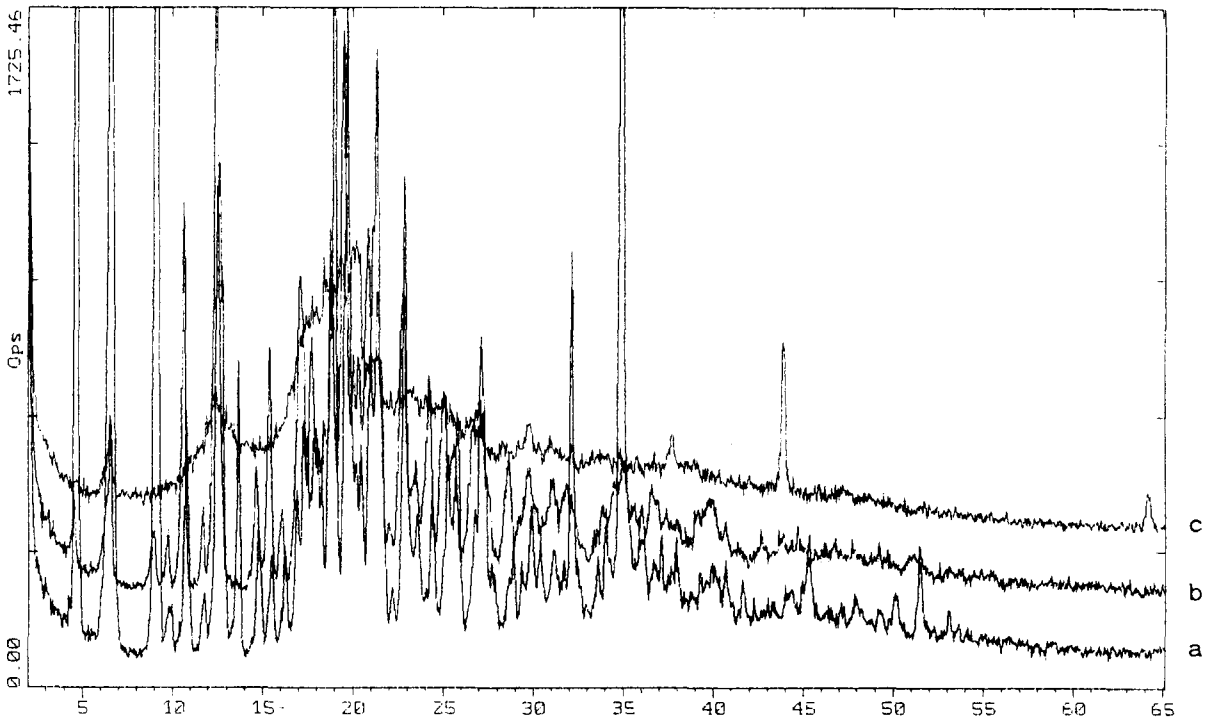

Fig. 6. X-ray diffraction patterns for the following products: (a) physical mixture; (b) kneaded mixture; (c) spray-dried.

state, demonstrating that a new solid phase is formed in the spray-dried product.

3.5. Scanning electron microscopy

The micrographs of oxazepam-β-CD systems are shown in Fig. 7–9. The physical mixture is characterized by the presence of crystals of both components (oxazepam and β-CD), without modification in shape or size. In contrast, the micrograph of the kneaded mixture reveals the effect of the kneading technique, with a significant diminution in particle size, which is now more homogeneous, where it is impossible to differen-

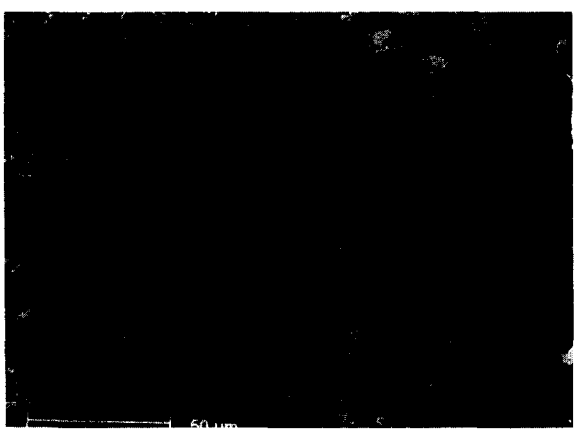

Fig. 7. Micrograph of the 1:1 physical mixture.

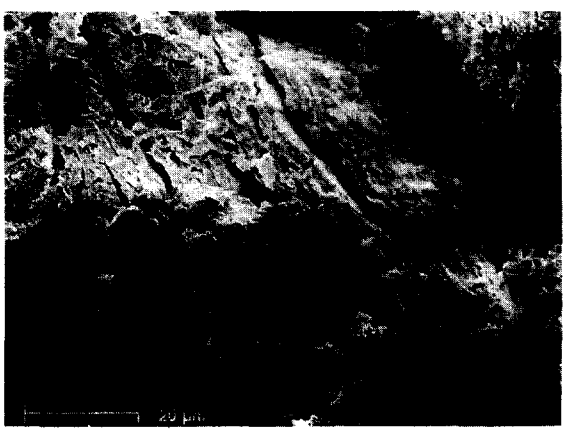

Fig. 8. Micrograph of the 1:1 kneaded mixture.

Fig. 9. Micrograph of the 1 : 1 spray-dried system.

tiate between the crystals of both components. Finally, the system prepared by spray-drying is characterized by the presence of very small particles, with a strong tendency to aggregation and agglomeration, while revealing a clear difference after spray-drying. This image is demonstrative of the amorphousness and homogeneity of this system. These observations are in good agreement with the X-ray diffractometry studies.

3.6. Dissolution rate studies

The dissolution profiles of oxazepam and oxazepam-β-CD systems are reported in Fig. 10. In Fig. 10, it is evident that whereas the physical mixtures do not show a significant improvement in dissolution rate with respect to the free drug,

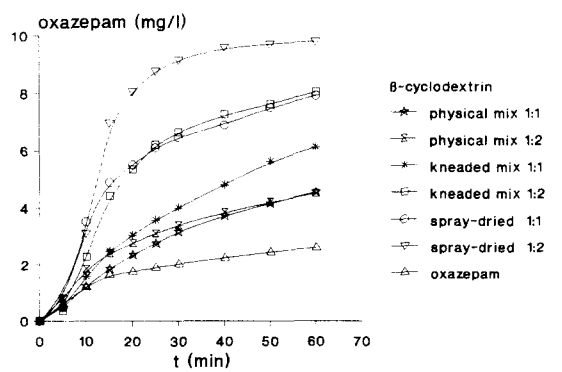

Fig. 10. Dissolution curves of oxazepam-β-CD binary systems.

Table 1
Dissolution efficiency values at 15 and 60 min from oxazepam and the different oxazepam-β-CD systems

Binary systems	Elaboration method	DE_{15}	DE_{60}
Oxazepam-β-CD			
	Physical mixture 1 : 1	0.0869	0.2810
	Kneaded mixture 1 : 1	0.1081	0.3665
	Spray-dried 1 : 1	0.2145	0.5567
	Physical mixture 1 : 2	0.1306	0.3039
	Kneaded mixture 1 : 2	0.1601	0.5520
	Spray-dried 1 : 2	0.2313	0.7478
Oxazepam		0.0870	0.2211

the kneaded and spray-dried products exhibit faster dissolution than the free drug. The dissolution profiles were evaluated on the basis of the dissolution efficiency (DE) parameter (Khan, 1975) at 15 and 60 min. These values are reported in Table 1. It is clear that the extent of the enhancing effect was found to be dependent on the method adopted for the preparation of inclusions, where the spray-dried technique exhibits the highest dissolution rate. In this case, the enhancement of dissolution rate may be attributed to the reduction in crystallinity of the product, confirmed by SEM and X-ray diffraction studies, and a greater extent of complexation by this technique.

Furthermore, the kneaded preparations also increased the dissolution rate of oxazepam. On the other hand, the physical mixtures do not show a significant enhancement in dissolution rate. This behaviour may be explained according to the stronger interaction between drug and CD for the kneaded systems as compared with the physical mixtures.

The influence of the β-CD molar proportion on the dissolution rate of oxazepam is variable according to the method employed. In the case of spray-drying, this influence is not very significant, due to the considerable enhancement of dissolution profiles from the 1 : 1 system, which cannot be improved by increasing the proportion of β-CD. For the physical mixtures, the enhancement in dissolution rate is only due to the wetting effect of β-CD, to which the CD contributed to an equal extent for both drug-CD ratios (1 : 1 and

1:2). In contrast, the molar proportion of β-CD is an important factor in the kneaded products. This phenomenon can be explained on the basis of the characteristic of the kneading method of employing a semisolid medium, where the interactions between oxazepam and β-CD may be hindered; in addition, we have considered the possibility of formation of 'outer-sphere compounds' drug-CD, where the drug molecule is not placed into the cavity and this interacts with the exterior groups of CD (Gelb et al., 1978; Szjetli, 1988). Consequently, an increment of the CD proportion can improve the drug-CD interactions and the proportion of complexed drug, with enhancement of its dissolution rate.

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