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## Stabilization of AD-1590, a Non-steroidal Antiinflammatory Agent, in Suppository Bases by $\beta$ -Cyclodextrin Complexation<sup>1)</sup>

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Stabilization of AD-1590 in an oleaginous suppository by complexation with  $\beta$ -cyclodextrin ( $\beta$ -CyD) was investigated. Suppositories were stored at 37°C and 75% relative humidity. AD-1590 was rapidly decomposed by autoxidation in the case of the suppository containing only AD-1590. On the other hand, no decomposition was observed in the case of the suppository containing AD-1590- $\beta$ -CyD complex. The stabilizing effect of complexation is considered to be attributable to insolubilization of AD-1590 in the oleaginous base. The release rate was also promoted by complexation. These results indicate that complexation with CyDs is a promising means of improving the bioavailability and the stability of drugs which are very unstable when solubilized in an oleaginous base.

**Keywords**—AD-1590;  $\beta$ -cyclodextrin; inclusion complex; suppository; stability; release rate

### Introduction

2-[8-Methyl-10,11-dihydro-11-oxodibenz[*b,f*]oxepin-2-yl]propionic acid (AD-1590)<sup>2)</sup> (see Fig. 1) is a new acid non-steroidal anti-inflammatory agent having an extraordinarily strong antipyretic action.<sup>3)</sup> AD-1590, as well as other non-steroidal anti-inflammatory agents, causes gastrointestinal lesions (GI-lesions) although its ulcerogenic activity is not as strong as that of indomethacin.<sup>3,4)</sup> In general, attempts have been made to change the dosage form or the route of administration as a means of reducing GI-lesions, especially the local side effect. We adopted the rectal suppository as a dosage form for reducing the local side effect of AD-1590 on the gastric mucosa. However, it has been found that AD-1590 in an oleaginous base is readily oxidized by oxygen gas, and its autoxidation can not be inhibited by additives such as hydroquinone or similar antioxidants. Uekama and coworkers have succeeded in improving the chemical stability, the solubility and the bioavailability of various drugs by means of cyclodextrin (CyD) complexation.<sup>5-8)</sup> Uekama *et al.* recently reported that the oxidation of benzaldehyde was completely inhibited in CyD complexes.<sup>9)</sup> Thus, the present study was undertaken to investigate the stabilization of AD-1590, which is poorly water-soluble and oxygen-sensitive, in an oleaginous base by means of  $\beta$ -CyD complexation.

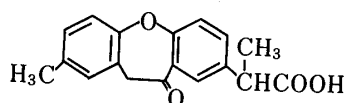


Fig. 1. Structural Formula of AD-1590

### Experimental

**Materials**—AD-1590 (Dainippon Pharmaceutical Co., Ltd.), which is a racemic compound, was pulverized using a Sample Mill (Hosokawa Micro Co., Ltd.) to obtain a fine powder. The specific surface diameter of the fine

powder particles measured by the air permeability method was about  $10\ \mu\text{m}$ . Witepsol H-15 (Dynamit Nobel Chemicals), which is not irritant to the rectal mucosa, was used as an oleaginous base. The  $\alpha$ -,  $\beta$ - and  $\gamma$ -CyDs were purchased from Sanraku Ocean Co., Ltd. and were not especially purified. All other materials were of analytical reagent grade. Deionized double-distilled water was used throughout the study.

**Solubility Studies**—Solubility was measured according to the solubility method of Higuchi and Lach.<sup>10)</sup> Excess amounts of AD-1590 were added to aqueous CyD solutions saturated with nitrogen and were shaken at  $25^\circ\text{C}$  for 7–10 d in the dark. After equilibration had been attained, an aliquot was filtered through a Millipore filter of  $0.4\ \mu\text{m}$  pore size. The sample was analyzed spectrophotometrically after suitable dilution with water. No degradation of AD-1590 was observed under these experimental conditions.

**Preparation of Solid Complexes**—The solid complex was obtained by mixing appropriate amounts of AD-1590 and the CyD in water. Amounts were calculated from the descending portion of the phase solubility diagram (see Fig. 2). For example, 0.48 g of AD-1590 and 10.22 g of  $\beta$ -CyD were added to 300 ml of distilled water, sealed in a container after the air had been replaced by nitrogen, and stirred at  $25^\circ\text{C}$  for 2 weeks in the dark. The complex precipitated as a microcrystalline powder, which was filtered off and then dried under vacuum at room temperature for 24 h. Subsequently the dried complex was washed with ether to remove intact AD-1590 and dried again under vacuum at room temperature for 24 h. This powder corresponded to 1:2 AD-1590- $\beta$ -CyD complex. This complex was used to prepare the suppositories after being pulverized (specific surface diameter, about  $10\ \mu\text{m}$ ).

**X-Ray Diffractometry**—The powder X-ray diffraction patterns were obtained using a Geiger Flex 2012 X-ray diffraction analyzer (Rigaku Denki Co., Ltd.) with Ni-filtered  $\text{Cu-K}\alpha$  radiation.

**Thermal Analysis**—Differential scanning calorimetry (DSC) was carried out using a heating rate of  $5^\circ\text{C}/\text{min}$  on a Thermo Flex 8085DI TG-DSC (Rigaku Denki Co., Ltd.).

**Preparation of Suppositories**—Suppositories were prepared by the fusion method. AD-1590 or AD-1590- $\beta$ -CyD complex was added to the molten base under vigorous stirring at  $40^\circ\text{C}$ . The mixture was poured into a polypropylene suppository mold to give a suppository weight of 1.2 g and then allowed to stand for 30 min at  $20^\circ\text{C}$  to solidify. Suppositories were prepared as follows: suppositories containing 5, 10, 15, 30 and 50 mg of AD-1590; suppositories containing AD-1590- $\beta$ -CyD complex (10 mg as AD-1590).

**Storage of Suppositories**—Each suppository was put in a glass vial and stored in a chamber (Tabai Espec) at controlled temperature and relative humidity ( $30^\circ\text{C}$ – $75\%$  RH) in the dark. After a suitable interval, a suppository was dissolved in mixed solvent (methanol:chloroform, 4:1) and the solution was filtered through a Millipore filter of  $0.2\ \mu\text{m}$  pore size. Intact AD-1590 in the sample was quantitatively analyzed by high-performance liquid chromatography (HPLC). The peroxide value of the suppository was determined according to the usual method.<sup>11)</sup>

**Solubility in Bases**—Excess amounts of AD-1590 or AD-1590- $\beta$ -CyD complex were added to the molten bases and vigorously stirred at  $40^\circ\text{C}$  for 3 h. An aliquot was filtered through a Millipore filter of  $0.2\ \mu\text{m}$  pore size. AD-1590 in the sample was quantitatively analyzed by HPLC. No degradation of AD-1590 was observed under these experimental conditions.

**Release Rate**—Release of AD-1590 from suppositories was measured using an apparatus for measuring release rate, model TMS-103 (Toyama Sangyo Co., Ltd.), according to the procedures reported by Muranishi *et al.*<sup>12)</sup> Isotonic phosphate buffer of pH 7.3 (300 ml) was employed as a release medium. The temperature of the medium was kept at  $39^\circ\text{C}$ , since AD-1590 may be administered to a patient having a fever. A Millipore filter, SSWP 04700 (pore size  $3.0\ \mu\text{m}$ ; Nihon Millipore Co., Ltd.), was employed. The rotation rate of the steel rod was 25 rpm. At predetermined intervals, one ml of sample solution was pipetted through a Millipore filter of  $0.4\ \mu\text{m}$  pore size and its absorbance was measured at 250 nm after suitable dilution with methanol.

**HPLC Analysis**—HPLC was performed using an LC4A instrument (Shimadzu) equipped with a Develosil ODS-7 column  $250\ \text{mm} \times 4\ \text{mm}$  i.d.; 0.1 M sodium citrate (pH 3.0)–methanol–acetonitrile (30:30:40) as the mobile phase; detection at 250 nm. Diphenyl was used as an internal standard and peak height ratios were used for quantitation.

## Results and Discussion

### Inclusion Complex of AD-1590 with CyDs

Figure 2 shows the phase solubility diagrams obtained for AD-1590 with the 3 CyDs in water at  $25^\circ\text{C}$ . The differences among the solubility curves are clear. The  $\alpha$ -CyD system showed a typical  $A_L$ -type<sup>13)</sup> solubility curve with linearly increasing solubility of AD-1590. On the other hand, the  $\beta$ - and  $\gamma$ -CyD systems showed typical  $B_s$ -type<sup>13)</sup> solubility curves with the microcrystalline complex precipitating at higher CyD concentration. The stoichiometries of the complexes in the solid phase were analyzed on the basis of data in the plateau region of the solubility diagrams, and were estimated to be 1:2 for AD-1590- $\beta$ -CyD and AD-1590- $\gamma$ -CyD. These results are in good agreement with those obtained by isolation and analysis of the solid

complexes. The apparent 1:1 stability constants of AD-1590- $\alpha$ -CyD, AD-1590- $\beta$ -CyD and AD-1590- $\gamma$ -CyD calculated from the initial rising portion of the solubility curve according to the method of Higuchi and Connors<sup>13)</sup> were 140, 1900 and 760  $M^{-1}$ , respectively. These values indicate that  $\beta$ -CyD has the most suitable cavity to accommodate the AD-1590 molecule.

In order to confirm the complexation of AD-1590 with  $\beta$ -CyD in the solid state, the microcrystalline complex was examined by X-ray diffractometry and DSC. Figure 3 shows the powder X-ray diffraction pattern of the complex in comparison with those of a physical mixture in the same molar ratio and AD-1590 alone. The diffraction pattern of the complex could not be duplicated by superposition of those of the components, but appeared to represent the formation of a new solid phase. Figure 4 shows the DSC thermograms of the complex in comparison with those of the physical mixture and AD-1590. AD-1590 alone and the physical mixture showed an endothermic peak at around 130 °C owing to melting. However the endothermic peak disappeared with formation of the complex. These results indicate that AD-1590 interacts with  $\beta$ -CyD in the solid state to form an inclusion complex.

### Stability of AD-1590 and AD-1590- $\beta$ -CyD Complex

Figure 5 shows the time courses of decomposition of AD-1590 and the peroxide value (POV) when suppositories containing AD-1590 or AD-1590- $\beta$ -CyD complex were stored at

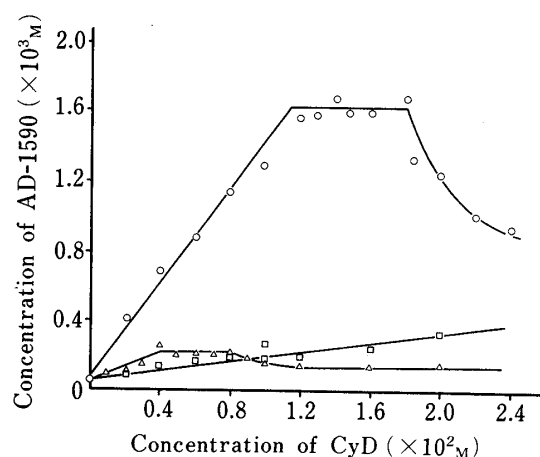


Fig. 2. Phase Solubility Diagrams of AD-1590-CyD Systems in Water at 25 °C

□,  $\alpha$ -CyD; ○,  $\beta$ -CyD; △,  $\gamma$ -CyD.

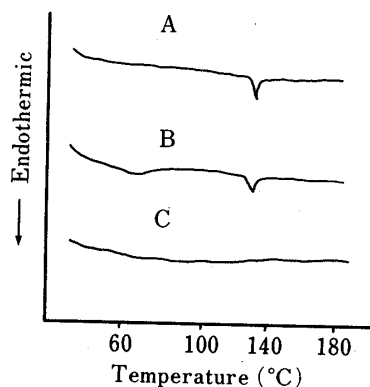


Fig. 4. DSC Thermograms of AD-1590- $\beta$ -CyD Complex

A, AD-1590 alone; B, physical mixture; C, complex.

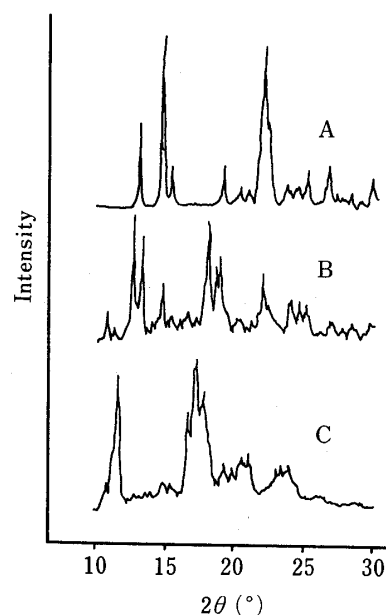


Fig. 3. Powder X-Ray Diffraction Patterns of AD-1590- $\alpha$ -CyD Complex

A, AD-1590 alone; B, physical mixture; C, complex.

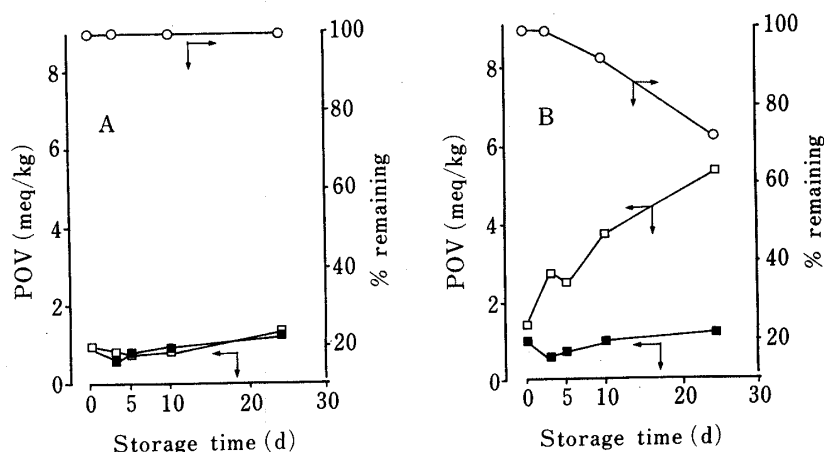


Fig. 5. Time Courses of Decomposition of AD-1590 and Peroxide Value in Oleaginous Suppositories with AD-1590 or AD-1590- $\beta$ -CyD Complex at 30°C-75% RH

Each point represents the mean for three suppositories. A, suppository with complex (10 mg as AD-1590); B, suppository with 10 mg of AD-1590;  $\circ$ , % remaining;  $\square$ , POV of suppository;  $\blacksquare$ , POV of base alone.

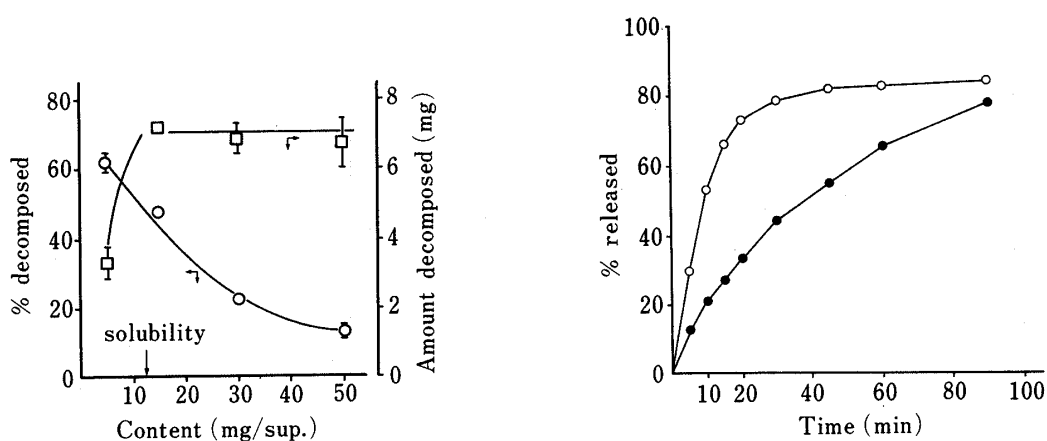


Fig. 6. Relationship between Decomposition and Content of AD-1590 in Oleaginous Suppositories

Suppositories were stored at 30°C and 75% RH for 6 weeks. Each point represents the mean and S.D. for three suppositories.

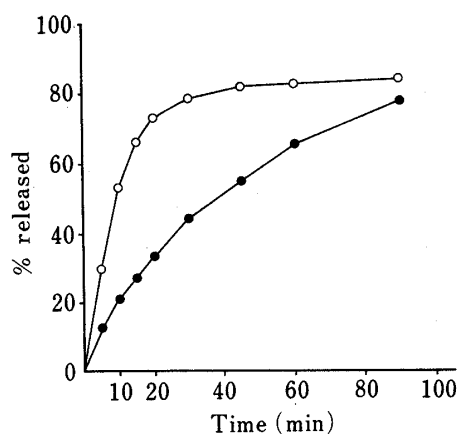


Fig. 7. Release Rate of AD-1590 from Oleaginous Suppositories with AD-1590 or AD-1590- $\beta$ -CyD Complex

Each point represents the mean for three suppositories.  $\bullet$ , suppository with 10 mg of AD-1590;  $\circ$ , suppository with AD-1590- $\beta$ -CyD complex (10 mg as AD-1590).

30°C and 75% RH. In the case of the suppository containing only AD-1590, AD-1590 was rapidly decomposed and its decomposition was accompanied by an increase of POV (a usual index employed in measuring oxygen-dependent decomposition). The increase of POV was concluded to arise from the decomposition of AD-1590 because POV of the base alone did not increase. In another experiment, we found that the decomposition was inhibited under nitrogen. Therefore we consider that AD-1590 in an oleaginous base is oxidized by atmospheric oxygen. In the case of the suppository containing the complex, no increase of POV was observed and AD-1590 was stable. Thus, the oxidation of AD-1590 was completely inhibited by complexation. However, it is not clear whether the stabilizing effect is attributable to the prevention of diffusion of oxygen through the host structure or to some other mechanism.

Figure 6 shows the relationship between the content of AD-1590 and the stability during

storage at 30 °C and 75% RH. The percent decomposed decreased with increasing content of AD-1590 in the suppository and higher contents were apparently more stable. However, the amount decomposed was approximately constant at contents of over 15 mg. Since the solubility of AD-1590 in one Witepsol H-15 base suppository was 12.5 mg (see the arrow in Fig. 6), the solubilized amount is constant in suppositories having higher contents. These results indicate that AD-1590 in the solid state is very stable, and only the solubilized fraction of AD-1590 is susceptible to oxidation. On the other hand, AD-1590- $\beta$ -CyD complex was found not to be dissolved in Witepsol H-15. Thus, it is considered that the stabilizing effect of complexation is attributable to insolubilization of AD-1590 in the oleaginous base.

### Release Rate

Figure 7 shows the release profiles of AD-1590 from the suppository containing AD-1590- $\beta$ -CyD complex in comparison with that from the suppository containing only AD-1590. The release of AD-1590 was considerably promoted by complexation. The release rate of AD-1590 from the suppository containing only AD-1590 may be dependent on the concentration of AD-1590 in the base and the partition coefficient of AD-1590 between the base and the aqueous phase, while that from the suppository containing the complex may be dependent on the dissolution of AD-1590 from the complex, because the complex is released into the aqueous phase immediately after the base melts. Accordingly, the enhanced release rate of AD-1590 from the suppository containing the complex is considered to be attributable to alteration of the release process and enhancement of the dissolution in the aqueous phase by complexation with  $\beta$ -CyD.

The present data indicate that the stability of AD-1590 in and the release of AD-1590 from an oleaginous suppository are improved by  $\beta$ -CyD. Therefore, the complexation of AD-1590 with  $\beta$ -CyD is a promising means of improving the bioavailability and overcoming the problem of prolonged storage of AD-1590.

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### References and Notes

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