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Study on Inclusion Complex of Fenbufen with β-Cyclodextrin

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Inclusion complex formation of fenbufen with β-cyclodextrin in water and in solid state was comfirmed by solubility method, ultra violet absorption, circular dichroism and infra-red spectroscopies, differential thermal analysis, and X-ray diffractometry.

A solid complex of fenbufen with β-cyclodextrin in 1 : 1 molar ratio was prepared by the freeze-drying method, its dissolution characteristics in water and its analgesic and antiinflammatory effect in mouse or rat were examined.

The apparent release of fenbufen from the inclusion complex was significantly improved, but no significant difference in its analgesic and antiinflammatory effect was found.

Fenbufen (γ-biphenyl-4-yl-γ-oxobutyric acid), one of the phenylalkanoic acid derivatives, is widely used orally to reduce pain and inflammation in patients with rheumatoid arthritis.

However, the compound is insoluble in water and, may result in poor absorption characteristics.

Inclusion complexes of β-cyclodextrin with various drugs have been extensively applied to enhance the solubility,[1] dissolution rate,[2] membrane permeability,[3] and bioavailability[4] of slightly soluble drugs.

Thus, this investigation was done for obtaining information on inclusion complexation of fenbufen with β-cyclodextrin, anticipating an improved dissolution characteristic and bioavailability of the drug.

Study on the analgesic and antiinflammatory effect of the complex was conducted according to the Hendershot's and Winter's method by oral administration in mouse or rat, respectively, in comparison with fenbufen itself.

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Experimental

Materials—Fenbufen was favored by YuHan Corporation. β-cyclodextrin was obtained commercially from Sigma Chemical Co. Ltd., and recrystallized from water and dried with P_2O_5 in vacuo. All other chemicals and solvents were analytical reagent grade. Deionized double distilled water was used.

Solubility Studies—Fenbufen 500mg was added to water or β-cyclodextrin (varied from 1.0 to 10×10^{-3}M) in glass stoppered flask and then sealed and shaken at 30± 0.5°C.

After equilibration was attained (about 1 week), an aliquot was filtered through millipore filter(0.45μ).

The sample solution was suitably diluted with 0.1M phosphate buffer (pH 11.0) and assayed by ultra violet(UV) spectrophotometry at 285nm.

Spectral Measurements—The circular dichroism(CD) and UV spectra were taken by a Jasco 20°C recording spectropolarimeter and a Varian 634 spectrophotometer, respectively, in 0.1M phosphate buffer (pH 11.0) at 25°C.

The CD spectra were expressed in terms of molar ellipticity, (θ).

Preparation of Inclusion Complex—The inclusion complex was prepared by the freeze-drying method,[6] as shown in chart 1.

According to the method, β-cyclodextrin and drug with molecular ratio 1 : 1 were dissolved in aqueous ammonium solution, because the drug is acidic and insoluble in water, and then freeze-dried, no ammonium ion being detected in the product by the qualitative analysis using Nessler's reagent.

Infrared Studies—This was done using Perkin-Elmer 467 infrared spectrophotom-

5×10^{-4}M drug
- add 5×10^{-4} M of β-cyclodextrin
- dissolve in 35ml of water and 7 drops of 28% aqueous ammonium water
- freeze-dry
- dry at 50°C
- wash with acetone or ethyl ether

residue
- dry in vacuo. at room temperature

Inclusion Complex

Chart 1—Method for preparation of inclusion complex by freeze-dry.

eter according to the KBr disk method.

Differential Thermal Analysis—This was conducted using a Tracon R.L. Stone LB 202 differential thermal analyzer, in the sample pan for solid sample at the scanning speed 10°/min from 150 to 500°C.

X-Ray Diffraction Studies—Powder X-ray diffractometry was carried out using Rigaku Denki 2037 diffractometer by Ni-filtered Cu-Kα radiation.

Dissolution Rate Studies—The dissolution rate was determined using Sartorius solubility simulator[6] in 100m*l* of water at 30±2°C.

Corrections were applied for cumulative dilution caused by replacement of sample by equal volume of the original medium in same temperature.

At every 5 minutes interval, 4.0m*l* of solutions were collected, diluted with 0.1M phosphate buffer (pH 11.0), and assayed by UV spectroscopy method.

Determination of Analgesic Effect in Mouse—Male mice weighing 20±2g were kept on standard diet and made to fast for about 24 hr. prior to experiments.

According to the Hendershot's method[7], the analgesic effect of the complex was determined in comparison with fenbufen itself.

The drugs were administered orally as fresh suspensions, and 0.2m*l* of 0.02% phenylquinone solution as writhing agent was administered intraperitoneally.

Determination of Antiinflammatory Effect in Rat—Male rats weighing 180±20g were pretreated the same way as mouse prior to experiments.

The antiinflammatory effect was dedermined according to the Winter's method[8]. The drugs were administered orally as fresh suspensions containing 50mg/kg as fenbufen, and 0.1m*l* of 1% carragenin solution as an edema inducer was administered subcutaneously.

Results and Discussion

Phase Solubility Diagram—Complex formation of fenbufen with β-cyclodextrin was studied by solubility method. Figure 1 shows an equlibrium phase solubility diagram obtained for fenbufen-β-cyclodextrin system in water.

The solubility of fenbufen increased by the addition of β-cyclodextrin showing a feature of A_L type phase diagram[9].

Further Evidence of Inclusion Complexation—Solubility study suggested that fenbufen forms soluble complex with β-cyclodextrin in water.

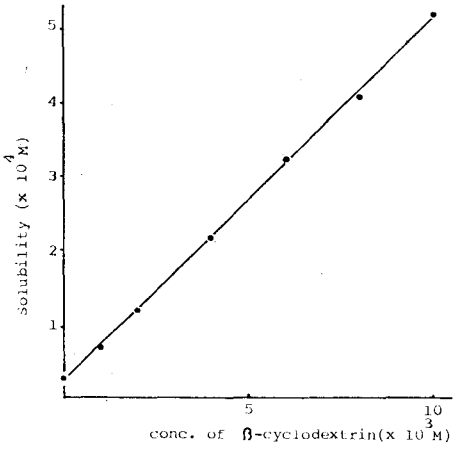


Figure 1—Phase solubility diagram of fenbufen-β-cyclodextrin system in water at 30°C.

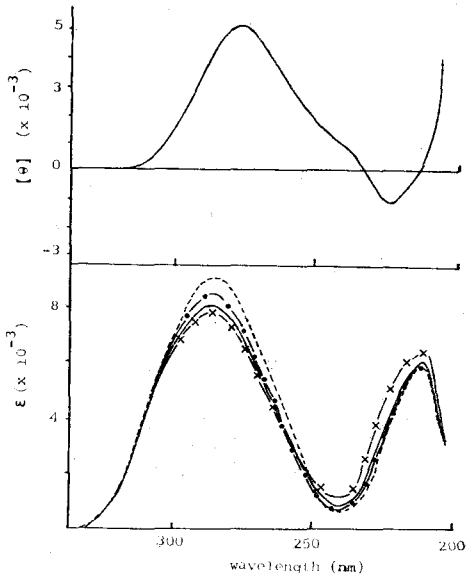


Figure 2—Circular dichroism(upper) and UV absorption spectra(lower) of fenbufen-β-Cyclodextrin system in 0.1M potassium phosphate buffer (pH 11.0).
Key : ……, fenbufen(4.0×10^{-5}N) alone; —·—·—·—, fenbufen+β-cyclodextrin(0.5×10^{-3}M); ——, fenbufen +β-cyclodextrin (5.0×10^{-3}M); —×—×—×—, fenbufen+β-cyclodextrin (10×10^{-3}M).

This interaction was further examined by circular dichroism and UV absorption studies. Since β-cyclodextrin has a large asymmetric cavity, various compounds have been shown to generate the extrinsic Cotton effects by the formation of inclusion complexes[10].

In fenbufen-β-cyclodextrin system, new CD bands were induced with positive sign peak, as shown in figure 2, where distinct UV spectral change was also accompanied.

As β-cyclodextrin has neither CD nor absorption band at longer wave lengh than 220nm[11], this may indicate that the drug chromophore was located within an asymmetric cavity of β-cyclodextrin.

Figure 3 shows the differential thermogram of the complex in comparison with that of physical mixture in the same molar ratio.

The sharp endothermic peak around 184°C, which was observed in fenbufen and the physical mixture, disappeared in the freeze-dried fenbufen-β-cyclodextrin.

Figure 4 shows the IR spectra of the physical mixture and the freeze-dried product

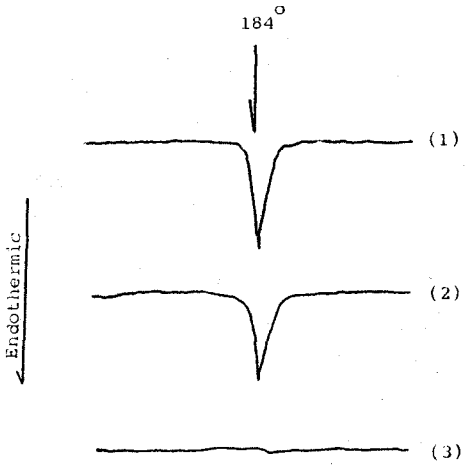


Figure 3—DTA thermogram of fenbufen-β-cyclodextrin complex made by freeze-drying method.
Key : (1), fenbufen (original and freeze-dried); (2), physical mixture (1 : 1); (3), fenbufen-β-cyclodextrin complex (1 : 1).

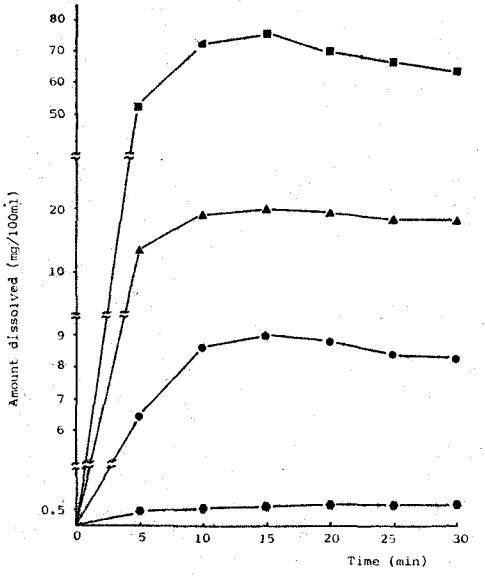


Figure 6—Dissolution rate of fenbufen from fenbufen-β-cyclodextrin comple x under non sink conditions at 30°C.
Key : ◆—◆, fenbufen (original); ●—●, fenbufen (freeze-dried); ▲—▲, physical mixture, fenbufen (freeze-dried) (1 : 1); ■—■, fenbufen-β-cyclodextrin complex (1 : 1).

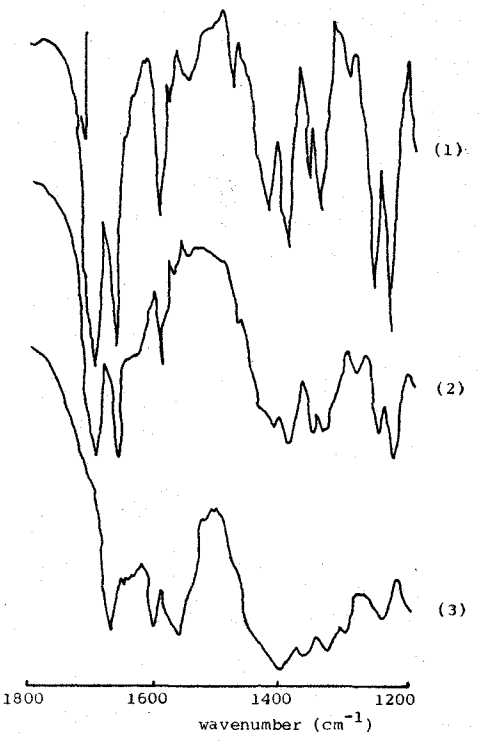


Figure 4—IR absorption spectra of fenbufen-β-cyclodextrin complex made by freeze-drying method.
Key : (1), fenbufen (original and freeze-dried); (2), physical mixture (1 : 1); (3), fenbufen-β-cyclodextrin complex (1 : 1).

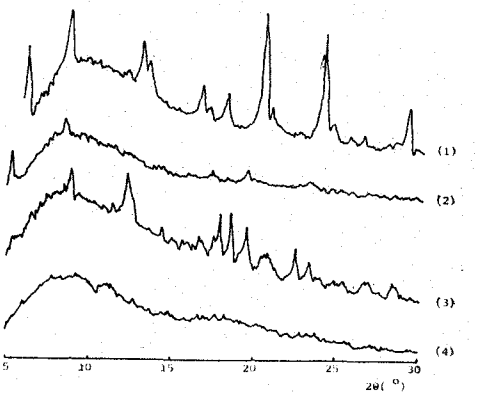


Figure 5—Powder X-ray diffraction patterns of fenbufen-β-cyclodextrin complex made by freeze-drying method.
Key : (1), fenbufen (original); (2), fenbufen (freeze-dried); (3), physical mixture (1 : 1); (4), fenbufen-β-cyclodextrin complex (1 : 1).

of fenbufen-β-cyclodextrin, being different each other.

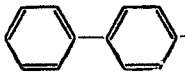
The band at 1710 cm^{-1} due to  , which was observed in the physical equimolar mixture, shifted to the lower wavenumber or hided itself by the broadening in the freeze-dried sample, suggesting the existance of some interactions between the drug and β-cyclodextrin.

Figure 5 shows the X-ray diffraction pattern of fenbufen-β-cyclodextrin, the complex in comparison with that of physical mixture in the same molar ratio.

Diffraction pattern of the physical mixture was found to be simply made up by the superposition of each component, while that of the complex was apparently different from the constituents to give new solid phase, that is, transformed into an amorphous state.

Above results indicate that fenbufen interacts with β-cyclodextrin both in solution and in solid state to form inclusion complex.

Dissolution Behavior of the Complex—The relative rate of dissolution of fenbufen

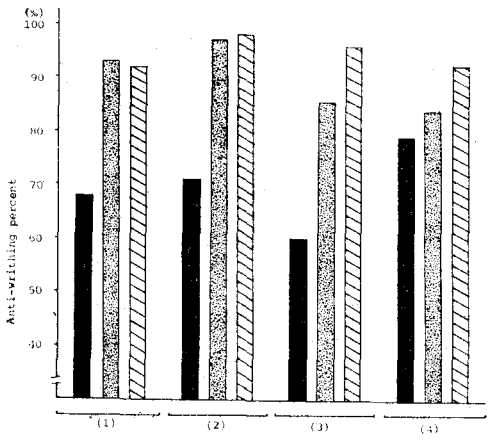


Figure 7—Analgesic effect of fenbufen and its inclusion complex.
Kev : (1), fenbufen (original); (2), fenbufen (freeze-dried); (3), physical mixture (1 : 1); (4), fenbufen-β-cyclodextrin complex (1 : 1); □, 12.5mg/kg; ▥, 25mg/kg; ▭, 50mg/kg.

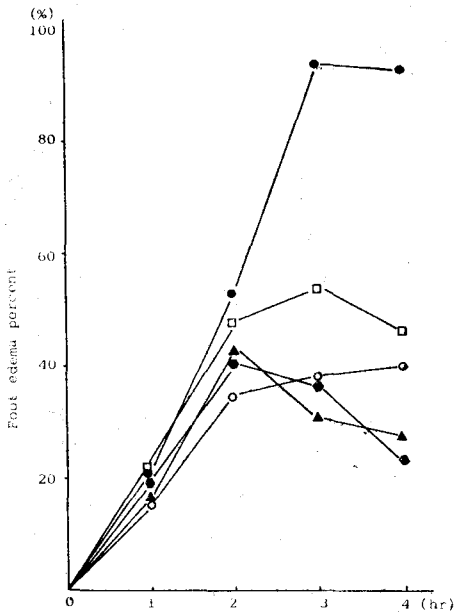


Figure 8—Antiinflammatory effect of fenbufen and its inclusion complex.
Key: ●——●, control; ○——○, fenbufen (original); ◆——◆, fenbufen (freeze-dried); □——□, physical mixture (1 : 1); ▲——▲, fenbufen-β-cyclodextrin complex(1 : 1).

and fenbufen-β-cyclodextrin complex in powder is shown in figure 6. It is evident that the complexed form of fenbufen dissolved much more rapidly than fenbufen itself.

Improved dissolution characteristic of fenbufen by inclusion complexation may be due to the enhanced solubility, as expected from figure 1.

Analgesic and Antiinflammatory Effect—Analgesic and antiinflammatory effect of the complex was compared with that of fenbufen by oral administration in mouse or rat. As shown in figure 7 and 8, the reducing activities to pain and inflammation of the complex were similar to that of fenbufen.

This might indicate that there is no direct relationship between the solubility in water and the absorption of fenbufen in oral administration.

Conclusion

1. It has been confirmed that fenbufen was interacted with β-cyclodextrin in aqueous solution and in solid state.

2. The results obtained from the freeze-dried powder indicated that the chromophore of fenbufen was located within the cavity of β-cyclodextrin due to inclusion complexation.

3. The dissolution characteristics of fenbufen by inclusion complexation was significantly improved, but no improvement in its analgesic and antiinflammatory effect was found.

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