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A study of the inclusion complex of naproxen with β-cyclodextrin

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Summary

The aim of this study was to increase the solubility and dissolution rate of naproxen (NAP) by inclusion complex formation with β-cyclodextrin (β-CD). The solubility of NAP with β-CD in aqueous solution was determined. The apparent stability constant, K_c, was calculated from the slope and intercept of the AL solubility diagram as 568 M^{-1}. The solid complexes of NAP with β-CD in 1 : 1 molar ratio were prepared by the freeze-drying and neutralization method. The formation of an inclusion complex with β-CD in solid state was confirmed by X-ray diffractometry, IR spectroscopy and differential scanning calorimetry (DSC). The dissolution rate of NAP from the inclusion complex was much more rapid than of NAP alone. The amount of NAP released from the tablet surfaces was determined for tablets pressed under 3500 kg/cm^2. It was seen that the total released amount of NAP from the NAP/β-CD complex was greater than that of intact NAP.

Introduction

"Molecular encapsulation" by means of monomolecular inclusion complex formation has offered promise for the development of new dosage forms, and its importance in pharmaceutical formulations has been fully realized (Uekama and Otagiri, 1987). Generally, the inclusion complex involves the spatial entrapment of a single guest molecule in the cavity of one host molecule without the formation of any covalent bonds. This is the essence of the so-called molecular encapsulation (Saenger, 1980; Szejtli, 1982).

The molecular encapsulation of many compounds can be performed with cyclodextrins (CDs). This process often advantageously modifies the various physical and chemical properties of the guest molecules (Andersen and Bundgaard, 1984; Uekama et al., 1982a; Tokumura et al., 1985). This method is simpler and cheaper than most other methods of encapsulation.

In the pharmaceutical field, the number of papers and patents dealing with CDs have increased in the past two decades (Saenger, 1980; Jones et al., 1984). β-Cyclodextrins (β-CDs) and their derivatives have been used in pharmaceutical formulations to enhance solubility (Cohen and Lach, 1963), dissolution rate (Corrigan et al. 1982; Uekama et al. 1983, 1984), membrane permeability (Uekama et al. 1985; Okamoto et al. 1986) and bioavailability (Nambu et al. 1978; Vila-Jato et al. 1986; Iwaoku et al. 1982) of slightly soluble drugs.

By taking advantage of CD complexation, many attempts have been made to reduce the untoward side effects associated with drugs (Uekama and

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Otagiri, 1987). It is well established that CDs reduce the ulcerogenic potencies of several acidic anti-inflammatory drugs (Szejtli et al., 1981a and b). Ulceration of the stomach may be caused by slowly dissolving drugs which provide high local drug concentrations, which may cause mucosal damage. In such situations, enhanced dissolution and absorption of the complex may result in a reduction of drug-induced gastric ulceration.

Several studies have reported the interactions of β-CD with non-steroid anti-inflammatory agents (Hamada et al. 1975; Kurozumi et al. 1975; Ikeda et al. 1975).

Naproxen (NAP; D-2-(6-methoxy-2-naphthyl)-propionic acid) is a non-steroidal anti-inflammatory agent with anti-inflammatory, analgesic and antipyretic properties, frequently used in the treatment of rheumatic diseases (Mahler et al., 1976; Calvo et al., 1987) and which, more recently, has been used as an analgesic (Sevelius et al., 1980).

NAP is very slightly soluble in water and when administered orally, it causes gastric irritation. Therefore, the aim of the present study was to increase the solubility and dissolution rate of NAP in artificial gastric medium by inclusion complex formation with β-CD

Materials and Methods

Materials

NAP and β-CD were kindly supplied by Bilim Pharmaceutical Co. Ltd. (Turkey) and Chinoin Pharmaceutical and Chemical Works (Hungary), respectively. All other materials and solvents were of analytical reagent grade.

Apparatus

An ultraviolet (UV) spectrophotometer (Perkin-Elmer Hitachi 200), IR spectrophotometer (Perkin-Elmer 1330 Japan), X-ray diffractometry (Jeol JDX-SP, Japan), differential scanning calorimetry (DSC) (Shimadzu, DT 40, Japan), lyophilizer (Virtis 10-146 MRBA U.S.A.), shaker (Nel, ST 400, Turkey) and a dissolution tester (modified USP XX paddle method, Turkey) were used.

Phase–solubility studies

The phase–solubility studies were carried out according to the method reported by Higuchi and Connors (1965). Excess amounts of NAP were weighed into 20 ml tubes, to which were added 10 ml of aqueous solutions containing various concentrations of β-CD (0.001–0.016 M) and shaken at $37 \pm 0.5\,^{\circ}C$. At equilibrium after 2 days, an aliquot was filtered through a Whatman 45 filter. A portion of the sample was adequately diluted and analyzed spectrophotometrically. The experiment was carried out in triplicate.

Complex formation between NAP and β-CD was also studied using the spectral shift method (Connors and Mollica, 1966). The change in absorbance of the substrate (NAP) by the addition of various concentrations of ligand (β-CD) was measured at 331 nm.

The apparent formation constant, K_c was calculated from the straight line portion of the phase solubility diagram according to the following equation (Higuchi and Connors, 1965):

$$K_c = \frac{\text{slope}}{\text{intercept}\,(1-\text{slope})}$$

indicating that the complex stochiometric ratio is 1 : 1 (guest : host), as in β-CD inclusion complexes (Otagiri et al., 1983, Nakajima et al.; 1984, Uekama et al., 1984).

Preparation of NAP / β-CD solid complex

Solid complex of NAP with β-CD was prepared using conditions derived from the solubility diagram (Fig. 1). The solid complex of NAP with β-CD in 1 : 1 molar ratio was prepared by two different methods, which were freeze-drying and neutralization.

Freeze-drying method A solid complex of NAP with β-CD was prepared by lyophilizing a solution of the complex. 1 g of NAP and 5 g of CD were added to 200 ml of water, sealed in a flask, and the mixture was stirred at 25 °C for 2 days and lyophilized.

Neutralization method 1 g of NAP was dissolved in 50 mol of 1 N NaOH and 5 g of β-CD was added. This mixture was stirred until a clear solution was obtained and then 1 N HCl was

added with vigorous stirring. The preparation was filtered under vacuum and washed repeatedly with water and dried in vacuum at room temperature for 48 h.

Preparation of the physical mixture

The mode of preparation of the physical mixture was the simplest. The calculated and exactly weighed (1 : 1 molar ratio) amounts of NAP and β-CD were pulverized in a ceramic mortar and carefully mixed.

Infrared spectroscopy (IR)

The IR spectra of NAP and complexes were measured as potassium bromide discs. For comparison, the IR spectra of physical mixtures of NAP-β-CD and β-CD were carried out using the same procedures.

X-ray diffractometry

The powder X-ray diffraction patterns were taken by an X-ray diffractometer. The operation data were as follows: X-ray, Ni-filtered Cu-K_α radiation; voltage, 40 kV; current, 20 mA; time constant, 2 s; scanning speed, 2°/cm.

Differential scanning calorimetry (DSC)

The differential scanning calorimetry (DSC) scans were recorded on a DSC apparatus equipped with a low temperature cell and nitrogen as the purging gas. Each sample was subjected to DSC at scanning speed of 20°/min from 0°C to 275°C.

Dissolution rate

The dissolution rate studies were done according to the USP XX paddle method in buffer solution pH 1.2 at 37 ± 0.5°C. A certain amount of each sample of NAP (passing through a 0.125 mm sieve) was placed in the dissolution medium. The stirring rate was 50 rpm. At appropriate intervals, 4 ml of solution was sampled and filtered rapidly through a filter (Whatman 45), diluted and assayed spectrophotometrically. A correction was applied for the cumulative dilution caused by replacement of the sample by equal volumes of the original medium. The experiment was carried out in triplicate. The raw data were evaluated using a computer program written for this purpose (Ağabeyoğlu, 1984).

Dissolution study, disc method

The released amount of NAP from the tablet surfaces was determined. The sample powder was compressed under 3500 kg/cm^2 pressure. The release of NAP was measured using a rotating disc apparatus in 500 ml of buffer solution (pH 1.2) at 50 rpm and 37 ± 0.5°C.

At appropriate intervals, 4 ml samples were removed and replaced by fresh test fluids. Samples were assayed spectrophotometrically for NAP content. The experiment was carried out in triplicate.

Results and Discussion

Inclusion complexation in solution

The phase solubility diagram obtained for naproxen with β-CD is shown in Fig. 1. In the case of β-CD, the solubility of naproxen increased linearly as a function of β-CD concentration and the solubility curve can be generally classified as type AL (Higuchi and Connors, 1965).

Calculation of the stoichiometry of the complex based on the data in Fig. 1 was in good agreement with that obtained by isolation and analysis of the crystalline complex. Solid complex of NAP with β-CD was also obtained in the molar ratio of 1 : 1 (guest : host).

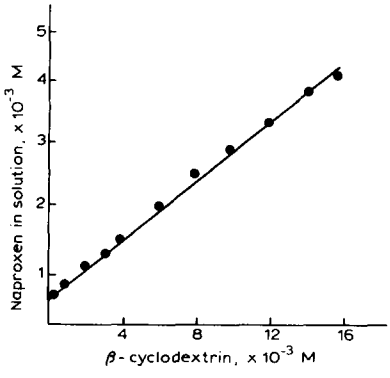

Fig. 1. Solubility of naproxen as a function of β-cyclodextrin concentration in water at 37°C.

On the other hand, with α-CD and γ-CD, no appreciable increase in the solubility of naproxen was observed. This might be due to the smaller cavity size of β-CD, therefore allowing very little penetration of the bulky NAP molecule.

The apparent formation constant (K_c) for the complex was calculated from the straight line of solubility diagram according to the previously mentioned equation. K_c was found to be 568 M^{-1}.

The hydrophobic guest molecules such as progesterone, triamcinolone acetate, dexamethasone acetate and fluocinolone acetonide exhibited the greatest binding (largest K_c values), as would be expected from their partition coefficients. These findings imply that the hydrophobic nature of the guest molecule and steric factors between the host and guest molecules were responsible for these interactions (Uekama et al., 1982b).

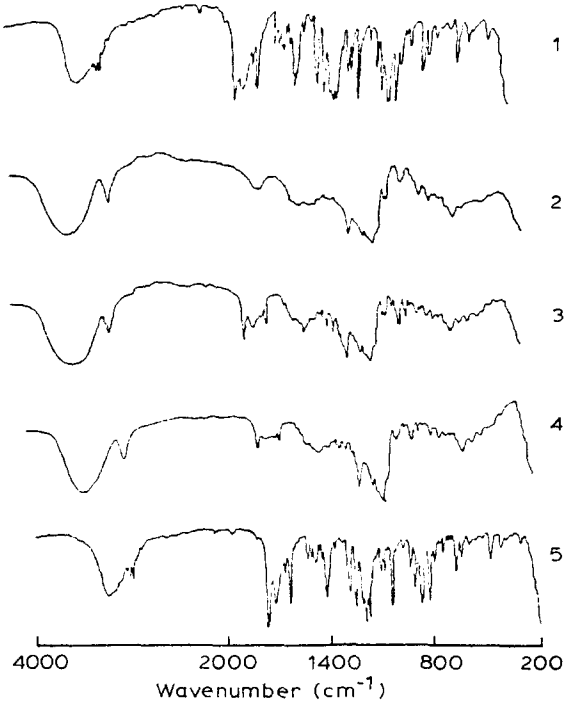

Fig. 2. IR spectra of the NAP-β-CD system. (1) Intact NAP; (2) β-CD alone; (3) physical mixture of NAP and β-CD; (4) NAP/β-CD complex (freeze-drying method); (5) NAP/β-CD complex (neutralization method).

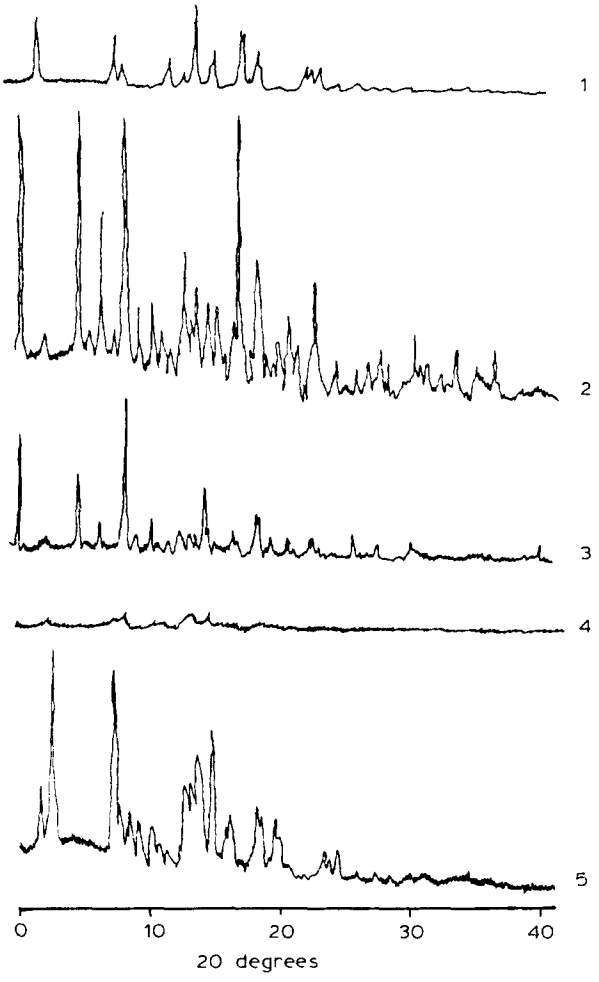

Fig. 3. Powder X-ray diffraction patterns of the NAP-β-CD system. (1) Intact NAP; (2) β-CD alone; (3) physical mixture of NAP and β-CD; (4) NAP/β-CD complex (freeze-drying method); (5) NAP/β-CD complex (neutralization method).

Inclusion complexation in the solid state

The complex of NAP with the β-CD was examined by IR spectroscopy, X-ray diffractometry and differential scanning calorimetry (DSC) measurement, and compared with the corresponding physical mixture in the same molar ratio.

Fig. 2 shows IR spectra of NAP, physical mixture, NAP/β-CD complexes and β-CD. As shown in the figure, NAP has a carbonyl band of 1725–1685 cm^{-1}. In the IR spectrums of the physical mixture and the complex prepared by the neutralisation method, there weren't any changes.

Whereas, in the IR spectrum of the complex which was prepared by freeze-drying method, there was a change in its carbonyl band and a significant decrease was observed in its intensity.

These spectral changes can be explained by the dissociation of the intermolecular hydrogen bonds of NAP through inclusion complexation.

The observed decrease in intensity of the carbonyl band may have resulted from its restriction within the β-CD cavity.

The X-ray diffraction analysis of powder samples revealed less crystallinity in the complex system as evidenced by fewer and broader peaks of lower intensity. The diffraction patterns obtained for the complexes and physical mixture are shown in Fig. 3.

The diffraction pattern of the physical mixture was simply the superposition of each component, while that of β-CD complex was apparently different from the constituents and constitutes a new solid phase.

It was observed that there was an amorphous structure in the complex prepared by the freeze-drying method (Fig. 3), whereas there was little change in the intensity of the X-ray pattern of the complex which was prepared by the neutralization method.

More evidence of complex formation was obtained from the DSC thermograms (Fig. 4).

While the peak of the NAP was at 156 °C, its peak was at 180 °C in the physical mixture. The

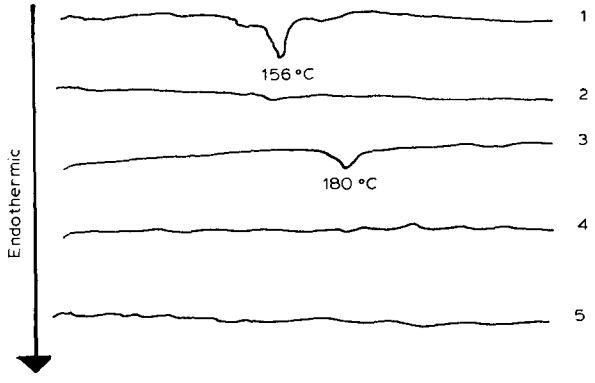

Fig. 4. Differential scanning calorimetry curves of NAP-β-CD system. (1) Intact NAP; (2) β-CD alone; (3) physical mixture of NAP and β-CD; (4) NAP/β-CD complex (freeze-drying method); (5) NAP/β-CD complex (neutralization method).

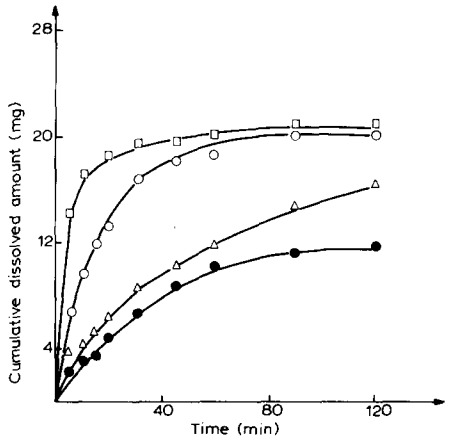

Fig. 5. Dissolution profiles of NAP and its β-CD complex in pH 1.2 buffer solution at 37 °C. ● Intact NAP; △ physical mixture of NAP and β-CD; □ NAP/β-CD complex (freeze-drying method); ○, NAP/β-CD complex (neutralization method).

fact that the peak of the mixture changed, showed that there was a weak interaction. The interaction of NAP with β-CD was accompanied by the disappearance of this endothermic peak, as would be expected.

The above results clearly indicate that the NAP/β-CD complex, which was prepared by the freeze-drying method, exists in the solid state.

The dissolution rate profiles of complexes, physical mixture and intact NAP are shown in Fig. 5. As seen, the freeze-dried complex exhibits a faster drug dissolution rate than intact NAP.

30 min later, the dissolved amount of NAP was 27% while the same amounts were 35% and 78%, respectively, in the physical mixture and the freeze-dried complex. In freeze-dried complex, an increase was observed in the dissolution rate within 30 min. Later, it reached an asymphtotic level (Fig. 5).

In the case of the complex prepared by the neutralisation method, 37% of NAP was dissolved in 30 min. This percentage was significant compared to the physical mixture.

With the disc method when the dissolution profiles of NAP and freeze-dried complex were examined, it was observed that dissolution of NAP from the fixed surface area was linear for 60 min. From that point on, 1.92 mg was dissolved from

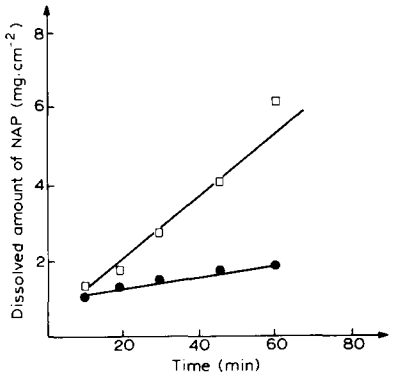

Fig. 6. Dissolution profiles of NAP from unit surface area. ●, intact NAP; □, NAP/β-CD complex (freeze-drying method).

the compressed intact NAP and 6.06 mg was dissolved from the compressed freeze-dried product (Fig. 6).

In the freeze-drying method, since a complex was formed and a porous structure was obtained, the amount of NAP dissolved from a unit area was found to be 6.06 mg. With the disc of the complex prepared by the neutralisation method, the rate of dissolution was not carried out, since the inclusion complex was not formed.

Conclusion

From these results, the freeze-drying method was suitable for obtaining inclusion complex of NAP with β-CD.

The improved dissolution rate may be due to the increase in solubility, as well as a decrease in the crystallinity of NAP, brought about by complexation.

Thus, the present study indicates that the aqueous solubility and dissolution rate of NAP can be significantly increased by forming an inclusion complex with β-CD. This enhancement in solubility brought about by complexation, may be of potential use in developing a suitable oral dosage form of NAP. This conclusion should be investigated by further in vivo experiments with these dosage forms.

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