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Effects of ethanol on formation of inclusion complexes of hydroxypropylcyclodextrins with testosterone or with methyl orange

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Summary

Gradual additions of ethanol decreased and eventually abolished the formation of inclusion complexes of testosterone with hydroxypropylcyclodextrins in aqueous solutions. With hydroxypropyl-β-cyclodextrin this occurred through two mechanisms. At low concentrations of ethanol (< 30%), the solvent primarily acted as a competing guest compound; at higher concentrations the dissociation primarily occurred through non-specific solvent effects. With hydroxypropyl-γ-cyclodextrin only the dissociation through nonspecific solvent effects was observed. Surprisingly, when ethanolic solutions containing fully dissociated complexes were evaporated, the solid residues had properties characteristic of complexed species, i.e., they showed the rapid and complete dissolution characteristic of complexes prepared by freeze drying of aqueous solutions. That inclusion complexes were formed during the final stages of evaporation of ethanolic solution of components was confirmed by measurements of circular dichroic spectra of a methyl orange : hydroxypropyl-β-cyclodextrin combination. In this combination the spectra of included species were highly characteristic and were recorded both in aqueous solutions and in solid state after the evaporation of ethanolic solutions but not in concentrated ethanolic solutions.

Introduction

Since amorphous inclusion complexes of drugs with cyclodextrin derivatives have multiple pharmaceutical uses, their preparation is of practical interest (Pitha and Pitha, 1985; Pitha et al., 1988; Loftsson et al., 1991; Uekama et al., 1992). These complexes have traditionally been prepared by co-dissolution of the components in water, a process which is straightforward but often time-consuming and risky, since the drug can hydrolyze (Pitha et al., 1988). Mechanistically, this preparation of complexes consists of two steps which differ strongly in character. The first one involves a time-consuming transfer of the drug from the solid phase into the aqueous solutions of cyclodextrin derivatives. The second step consists of the formation of the inclusion complex which

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occurs through an equilibrium process which is established in solutions very rapidly (Saenger, 1980; Kempfle et al., 1987).

If the preparation of complexes is to be improved, it is sensible to attempt to increase the speed of the phase-phase transfer of the first step. Adding solvents or manipulating temperature are the principal ways to facilitate the phase-phase transfer. We developed a method using volatile co-solvents. In this work, we present the physico-chemical background of the co-solvent method using, as an example, ethanol as a co-solvent, testosterone and methyl orange as guests and hydroxypropyl derivatives of β- and γ-cyclodextrins as hosts. In the accompanying paper, it is shown that the co-solvent method is applicable to a variety of drugs (Pitha et al., 1992).

Materials and Methods

Materials

Hydroxypropylcyclodextrins were purchased from Pharmatec, Inc., Alachua, FL, and had an average degree of substitution of about 5.5. Other chemicals were purchased from Sigma Chemical Co., St. Louis, MO. USP ethyl alcohol used throughout the study was Pharmaco™ brand (190 proof).

Spectral measurements

Ultraviolet and visible spectra were measured using a Perkin Elmer Lambda 3B Spectrophotometer; circular dichroism (CD) spectra were recorded using a CD spectropolarimeter Jasco J500C. For measurements in the solid state, thin films of materials were prepared by techniques developed for the measurement of infrared spectra of salts of deoxyribonucleic acid (Pitha, 1971). A concentrated solution of components in ethanol (190 proof) or water, as specified, was spread on a microscope slide and left to evaporate slowly at room temperature. Amounts and rate of evaporation were empirically adjusted until satisfactory homogeneous films of uniform and distinct color were obtained. This technique is applicable for casting of films from materials which are principally amorphous and unsuitable for crystalline compounds.

Calorimetry measurements

A Perkin Elmer DSC-7 differential scanning calorimeter was used. Samples were sealed in aluminum pans and measured at a scanning speed of 10°C/min. Measurements were carried out by L.J. Broutman & Assoc., Ltd (Chicago, IL).

Powder X-ray diffraction measurements

A Siemens 50 automated diffractometer was used. The instrument was set up with a radiation of wavelength $\lambda = 1.54$ Å and a graphite monochromator. The measurements were performed by Oneida Research Services (Whitesboro, NY).

Solubility studies

An excess of testosterone was added to the aqueous-ethanolic solution of the respective hydroxypropylcyclodextrin mixture, briefly sonicated and stirred for 2 days at room temperature (21–23°C). Then the sample was filtered through a membrane filter (Millex-GS Millipore 0.22 μm) and the testosterone content measured by spectrophotometry at 238 nm (Pitha and Pitha, 1985). Solid complexes for dissolution studies were prepared by evaporation of the solution in 190 proof ethanol at room temperature in vacuo or at atmospheric pressure and a warm water bath in a stream of nitrogen.

Dissolution studies

Tablets (200 mg each) were made using a single station hand-operated press (Parr Instrument Co., Pellet Press, 0.8 cm diameter). In the dissolution experiments, a tablet was placed in a basket made out of stainless-steel mesh (40 × 40 mesh, 34% open area) which was then submerged into a water bath (400 ml) at room temperature (21–23°C) followed by stirring rapidly at a constant rate. At the intervals given, samples were withdrawn from the bath, filtered through the membrane filter and the content of testosterone measured by spectrophotometry.

Measurement of ethanol

Ethanol content was determined by an alcohol (ethanol) diagnostic kit (Sigma Chemical Co., St. Louis, MO) which is based on ethanol oxidation by alcohol dehydrogenase; hydroxypropylcyclodextrins were found not to interfere in this quantitation.

Results

The solubility of testosterone in water is very low (30 μg/ml; Brotherton, 1976), but can be increased considerably through the addition of ethanol. The logarithm of solubility of many drugs is known to increase linearly with the volume fraction of a solvent in water : solvent mixtures, the slope being denoted as σ (Yalkowsky et al., 1972). This log-linear solubility relationship was later derived from the extended Hildebrand solubility approach (Martin et al., 1982). For the testosterone and water-ethanol solvent system, the linearity in the log-linear solubility relationship was found to be sustained up to the volume fraction 0.5; the slope σ was calculated to be 5.6 (Fig. 1).

Testosterone can also be solubilized into water by hydroxypropyl-β-cyclodextrin or hydroxypropyl-γ-cyclodextrin as described previously (Pitha and Pitha, 1985). The solubility of testosterone then was linearly proportional to the concentration of solubilizer (Fig. 1). The linear-linear solubility relationship, in this case, follows from consideration of the reversible formation of 1 : 1 complexes (Szejtli, 1988; Uekama et al., 1992). Association constants of testosterone : hydroxypropylcyclodextrin complex formation could be calculated from the slopes of these lines; they were 13 000 and 11 000 M^{-1}, respectively, for the β and γ derivatives. It should be noted that hydroxypropylcyclodextrins are mixtures of compounds, each of which may be expected to have a slightly different association constant. Thus, the association constants presented here should be termed compound apparent association constants, since they have not been corrected for non-ideal behavior.

When ethanol and hydroxypropylcyclodextrins were used simultaneously to solubilize testosterone into water, these solubilizers did not act in a synergic way (Fig. 2). The effects observed with hydroxypropyl-β-cyclodextrin and hydroxypropyl-

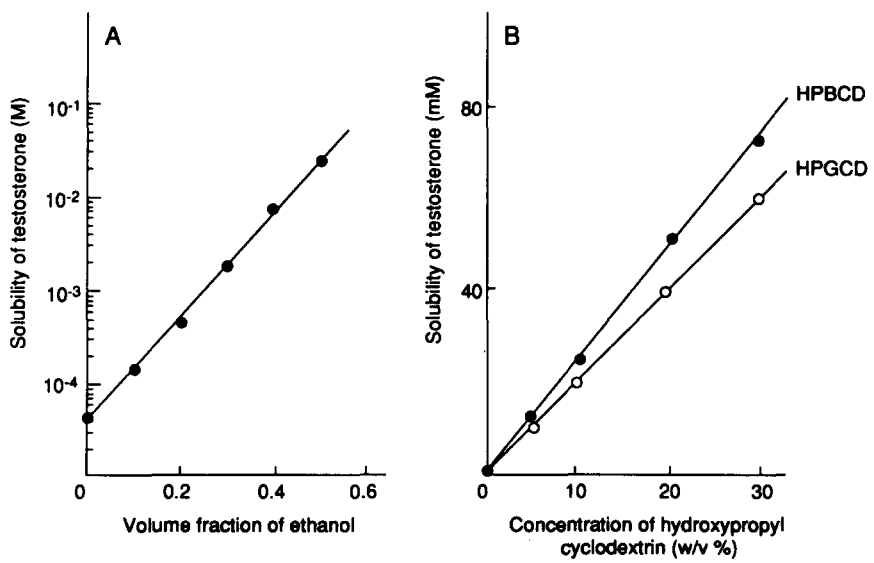

Fig. 1. Solubilization of testosterone into aqueous solution by ethanol (A) and by hydroxypropyl-β-cyclodextrin (HPBCD) or hydroxypropyl-γ-cyclodextrin (HPGCD) (B).

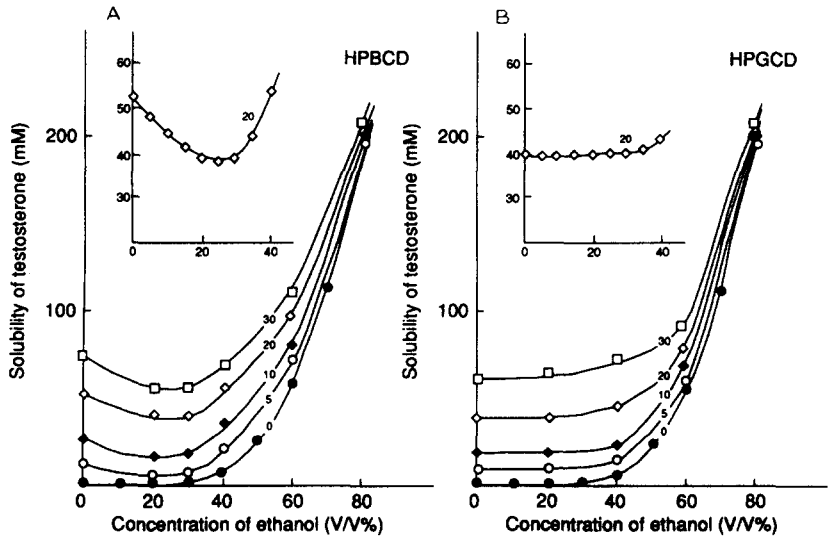

Fig. 2. Effects of ethanol on solubilization of testosterone into aqueous solution by hydroxypropyl-β-cyclodextrin (A) or by hydroxypropyl-γ-cyclodextrin (B). Numbers on the curves indicate the respective concentration (% w/v) of hydroxypropylcyclodextrin in the solution. Insets show magnified sections of the main graphs.

γ-cyclodextrin were somewhat different but had a common gross feature suggesting a decreased role of inclusion complexation as ethanol concentration increased. Hydroxypropylcyclodextrins, unlike unsubstituted cyclodextrins, are readily soluble in ethanol, and thus the association constants for formation of the testosterone : hydroxypropylcyclodextrin complex could be determined over a wide range of ethanol concentrations. Logarithms of association constants were found to decrease linearly with the volume fraction of ethanol for both hydroxypropyl-β-cyclodextrin and hydroxypropyl-γ-cyclodextrin (Fig. 3).

Thus, there was a clear similarity between the effects of ethanol on solubilization by hydroxypropyl-β-cyclodextrin and hydroxypropyl-γ-cyclodextrin when examined grossly; nevertheless, when these were studied in detail, distinct differences emerged at low ethanol concentrations (insets Fig. 2). A gradual addition of ethanol to testosterone solubilized by large amounts of hydroxypropyl-β-cyclodextrin initially decreased the solubility of testosterone, reaching a minimum value at about 25% of ethanol. It then started again to rise as would be expected on the basis of testosterone being soluble in ethanol. The observed decrease suggested that ethanol and testosterone directly compete for the cavity of hydroxypropyl-β-cyclodextrin. When the slope of the initial decrease of testosterone solubilization and the value of the association constant for testosterone : hydroxypropyl-β-cyclodextrin com-

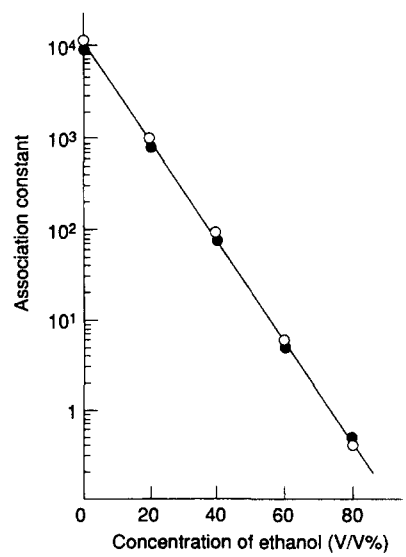

Fig. 3. Effects of ethanol on association constant (M^{-1}) of testosterone with hydroxypropyl-β-cyclodextrin (●) or with hydroxypropyl-γ-cyclodextrin (○). Association constants were calculated from the data in Fig. 2 by the initial slope method.

plexes were used, the association constant for ethanol : hydroxypropyl-β-cyclodextrin could be calculated and was found to be 0.05 M^{-1}. Previously, the association constant for the ethanol : β-cyclodextrin complex was evaluated as 0.9 M^{-1} when using very dilute solutions, i.e., conditions closer to those when the behavior of the compound may be expected to be ideal (Matsui and Mochida, 1979; cf. also Gelb et al. (1982) and Suzuki et al. (1988)). Detailed examination of the effects of ethanol on the solubilization of testosterone by hydroxypropyl-γ-cyclodextrin produced rather different results (Fig. 2B, inset). In the same region as that where ethanol decreased solubilization by hydroxypropyl-β-cyclodextrin, only a slight effect on solubilization by hydroxypropyl-γ-cyclodextrin was observed. Obviously, ethanol cannot effectively compete with testosterone for the larger cavity of hydroxypropyl-γ-cyclodextrin.

The above results show that addition of ethanol to the aqueous solutions of testosterone : hydroxypropylcyclodextrin complexes leads to their gradual dissociation into the components and, that at concentrations above 75% ethanol, no complexes in solution are detectable. These observations are in contrast with the conclusions drawn on the basis of evaluations of the solid residues after evaporation of such ethanolic solutions. All of these results indicate that the residues are true inclusion complexes of testosterone with hydroxypropylcyclodextrin. When testosterone and hydroxypropylcyclodextrins were dissolved in azeotropic 190 proof ethanol and the solutions evaporated, solid residues were obtained. Soon after solidification these solids contained about 5% ethanol; on prolonged drying in vacuo at room temperature, the ethanol content could be decreased less than 0.5% (for additional data see the following paper (Pitha et al., 1992)). These solids, even when left for over 30 days to equilibrate, showed amorphous patterns in powder X-ray diffraction (results not shown); furthermore, no crystalline phase of testosterone could be detected in such equilibrated samples by DSC (Fig. 4). Evaporation of ethanolic solution of free testosterone invariably yields crystalline preparations and any metastable amorphous phase of

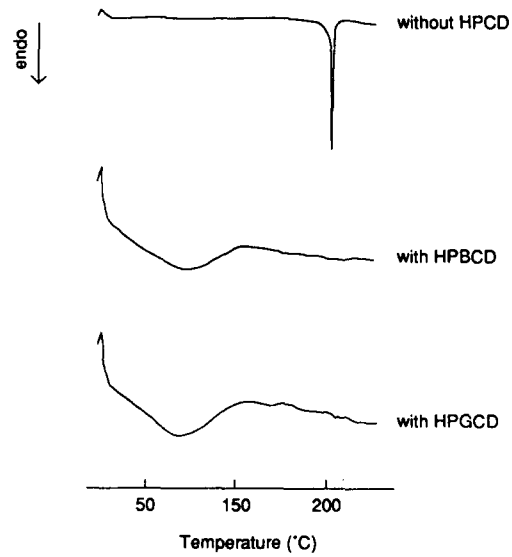

Fig. 4. Differential scanning calorimetry of crystalline testosterone (top), of the solid residue after evaporation of ethanolic (190 proof) solution of testosterone with hydroxypropyl-β-cyclodextrin (center) and of the solid residue after evaporation of ethanolic (190 proof) solution of testosterone with hydroxypropyl-γ-cyclodextrin (bottom); drug to hydroxypropylcyclodextrin weight ratios of 1:20 were used. In order to attain equilibrium the measurements were carried out 30 days after preparation of the samples.

testosterone may be expected to convert rapidly into the stable crystalline form.

Further information on the phase of testosterone in the residues after evaporation was obtained from dissolution studies. When a dispersion of crystalline testosterone in water or a solution of testosterone in ethanol was evaporated onto microcrystalline cellulose, the tablets prepared from such solids were found to release the testosterone only at a diminutive rate (Fig. 5), this behavior being expected for the dissolution of crystalline testosterone. When testosterone solutions in aqueous hydroxypropyl-β-cyclodextrin or hydroxypropyl-γ-cyclodextrin were evaporated, the tablets prepared from these residues dissolved fully within a few minutes in water – such dissolution properties may be expected for amorphous inclusion complexes (Pitha and Pitha, 1985). The tablets from the residues obtained by evaporation of the ethanolic solutions of the testosterone : hydroxypropylcyclodextrins dis-

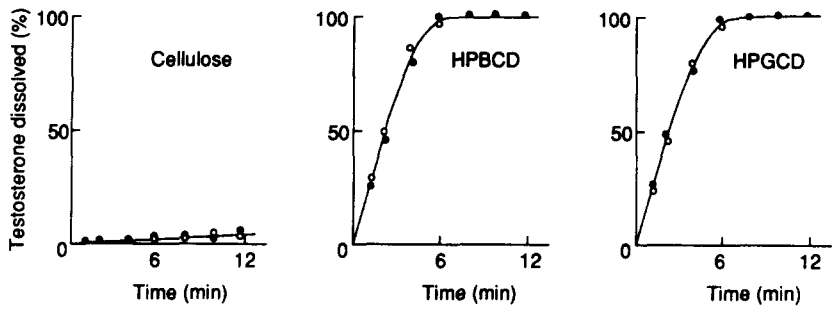

Fig. 5. Rate of dissolution of tablets made from testosterone : cellulose (left), from testosterone : hydroxypropyl-β-cyclodextrin (HPBCD) (center) and from testosterone : hydroxypropyl-γ-cyclodextrin (HPGCD) (right). Weight ratios of drug to excipient were 1 : 20. Tablet made from solid residues after evaporation of the components from either water (○) or 190 proof ethanol (●).

solved showing practically undistinguishable kinetics from those obtained from water. Study of the stoichiometry of the solubilization also yielded results suggesting the presence of inclusion complexes. About 2 moles of hydroxypropylcyclodextrins were needed to solubilize 1 mole of testosterone when the complex was prepared by evaporation of ethanolic (95%) solution (Fig. 6), i.e., the stoichiometry was similar to that observed when equilibrium dissolution by water was used (Pitha and Pitha, 1985). Thus, it may be concluded that evaporation of ethanolic solutions of testosterone : hydroxypropylcyclodextrin mixtures yields a highly stabilized amorphous phase of

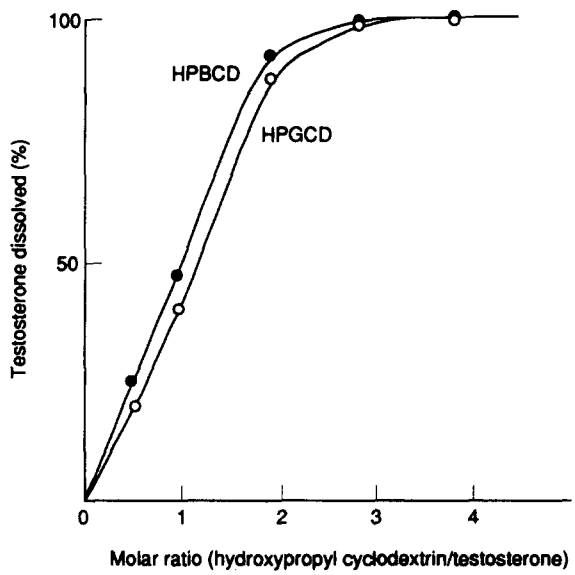

Fig. 6. Effects of molar ratio of testosterone to hydroxypropyl-β-cyclodextrin (HPBCD) or hydroxypropyl-γ-cyclodextrin (HPGCD) on the solubilization of testosterone into water. The samples were prepared by evaporation of components from solutions in ethanol (190 proof).

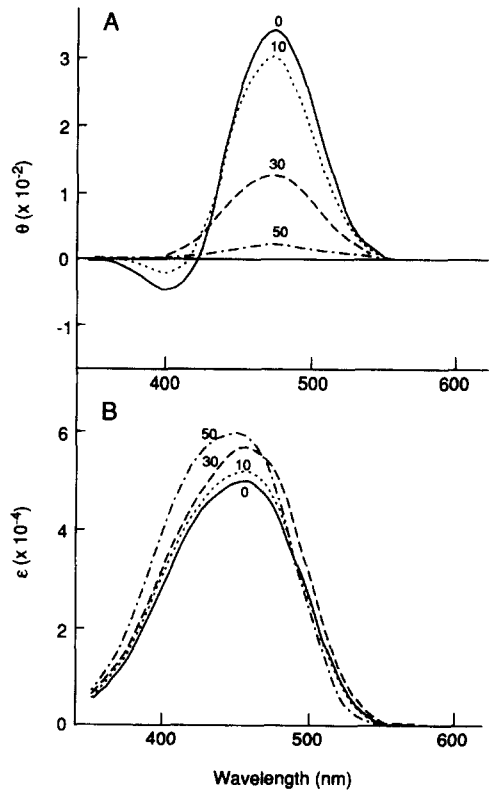

Fig. 7. Effects of ethanol on the CD spectra (A) or absorption spectra (B) of methyl orange in aqueous solutions of hydroxypropyl-β-cyclodextrin (1 : 20 weight ratio). Numbers adjacent to curves denote the respective final concentration of ethanol.

testosterone. We attempted to obtain direct proof that this stabilization is due to inclusion complexation, i.e., that the residues after evaporation of ethanolic solutions of testosterone : hydroxypropylcyclodextrins contain inclusion complexes. Unfortunately, this was not possible, since the differences observed in the infrared and CD spectra of various forms of testosterone were too small to be of use in demonstrating inclusion complexation. Another guest compound had to be used.

Direct proof that solid amorphous inclusion complexes can be prepared by coevaporation of components in azeotropic ethanol was obtained for the methyl orange : hydroxypropyl-β-cyclodextrin combination. Methyl orange is an achiral azo dye which forms complexes both with α- or β-cyclodextrins (Matsui and Mochida, 1979; Szejtli, 1988). Since the cavities of cyclodextrins are chiral, the molecules of dye contained therein are exposed to the chiral environment. This may induce optical activity in the absorption spectra of this azo dye (Szejtli, 1988) which can be detected with great sensitivity. At first, we established that the effects of ethanol on complexes of methyl orange in solutions were similar to those seen with testosterone. The visible and corresponding CD spectra of methyl orange : hydroxypropyl-β-cyclodextrin inclusion complexes in aqueous solutions were recorded (Fig. 7). While the addition of ethanol exerted only minor effects on the absorption spectra, the intensities in the corresponding CD spectra were strongly diminished, until finally at 50% of ethanol, circular dichroism was no longer detectable. These results suggest that in aqueous solutions inclusion complexes were formed, but that they were completely dissociated in azeotropic ethanol. The solutions of methyl orange : hydroxypropyl-β-cyclodextrins could be evaporated to yield thin films, the spectra of which could be measured. The absorption and CD spectra of such films differed only slightly according to whether their preparation was carried out by evaporation from either aqueous or ethanolic solutions (Fig. 8). Comparison of the results in solution and in solid phase, thus, clearly shows that the inclusion complex of methyl orange with hydroxypropyl-β-cyclodextrin formed in water could be completely dissociated by ethanol

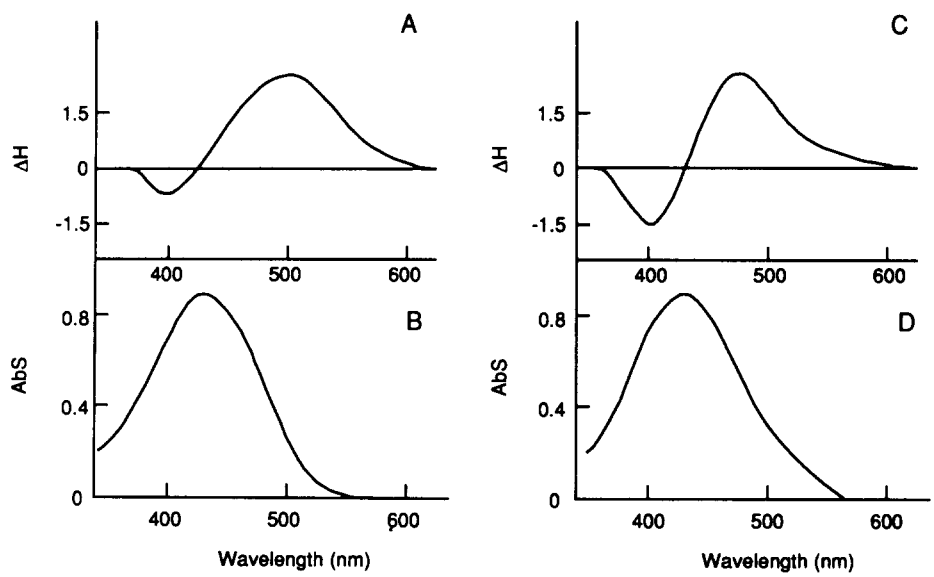

Fig. 8. CD spectra (A,C) and absorption spectra (B,D) of solid films composed of methyl orange and hydroxypropyl-β-cyclodextrin in a weight ratio of 1 : 20. Preparation of the films was by evaporation of the components from (A,B) aqueous solutions and (C,D) ethanolic solutions (190 proof).

and that, in the solution of azeotropic ethanol, no inclusion complexation occurred. Nevertheless, when such solutions in azeotropic ethanol were evaporated to dryness, a true inclusion complex between the components was obtained.

Discussion

Formation of inclusion complexes with cyclodextrins as hosts is a relatively non-specific process and even lipophilic molecules, which are considerably smaller than the host cavity, may form such complexes. The association constants of ethanol with α- and β-cyclodextrin were found to be quite small, (5.6 and 0.9 M^{-1}, respectively; Matsui and Mochida, 1979) when compared to the association constants of drugs, which are often 3–4 orders of magnitude higher (Saenger, 1980; Uekama et al., 1992). Nevertheless, complexes are formed and data on the crystal structure of the β-cyclodextrin : ethanol complex show that ethanol is indeed contained in the cavity of the host: i.e., complexes are of the guest : host type (Tokuoka et al, 1980). For γ-cyclodextrin, which has an even larger cavity, the complexes with solvents of low molecular weight can be expected to be even less stable. Consequently, only those associations in which the rest of the cavity is filled by a larger lipophilic molecule could be measured, e.g., ternary complexes of γ-cyclodextrin, pyrene and 1-butanol (Kano et al., 1982). The low stability of complexes of γ-cyclodextrin with solvents is also obvious from the fact that in order to prepare an inclusion complex of γ-cyclodextrin with 1-propanol, the crystal structure of which is known, a medium containing 60% of 1-propanol had to be used (Lindler et al., 1980).

Addition of ethanol may thus be assumed to lead to dissociation of drug : hydroxypropylcyclodextrin complexes simply through the competition between ethanol and drug, both functioning as guests for the hydroxypropylcyclodextrin host. The present results confirmed this process to be effective for ethanol : testosterone : hydroxypropyl-β-cyclodextrin systems. No such process could be detected for the system ethanol : testosterone : hydroxypropyl-γ-cyclodextrin; no evidence was obtained for the formation of ternary complexes.

There is another mechanism which leads to the dissociation of inclusion complexes by solvents, a non-specific solvent effect which is based on changes in energy required to form a cavity for the guest molecule in the solvent (e.g., Connors and Sun, 1971; Harrison and Efting, 1982; Orstan and Ross, 1987). Ethanol is known to be very effective in lowering this energy and thus in easing the dissociation of inclusion complexes (e.g., Orstan and Ross, 1987; Kralova and Mitterhauszerova, 1989). This effect may be expected to be the same for both the β- and γ-cyclodextrin derivatives and perhaps this mechanism is responsible for the linear decrease in the logarithm of association constants with ethanol concentration observed in Fig. 3. In the case of hydroxypropyl-γ-cyclodextrin, this non-specific solvent effect appears to be the main mechanism leading to the dissociation of inclusion complexes.

Thus, all the evidence collected here and elsewhere (cf. above) shows that in aqueous ethanolic solutions where ethanol predominates, only very little if any drug is complexed with cyclodextrin derivatives. Nevertheless, when such solutions were evaporated, even in the case of azeotropic ethanol-water mixtures, the solid residues contained hydroxypropylcyclodextrin : drug complexes. Formation of inclusion complexes is known to be a very rapid process (Saenger, 1980; Kempfle et al., 1987) and may provide an opportunity for the observed reversal to occur. Two reasons for the occurrence of such a reversal may be suggested. At the time of solidification, the concentration of ethanol in the system is decreased and thus the solvent may be expected to compete less effectively with a more tightly bound drug for the host. Also, the non-specific solvent effect at the stage near solidification again starts to favor the formation of inclusion complexes. The polarity of the spaces outside the cavity at that stage is bound to increase, since the role of external polarity regarding the cyclodextrin host becomes dominant and such an increase may drive the lipophilic drug into the cyclodextrin cavity.

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References

Brotherton, J., *Sex Hormones Pharmacology*, Academic Press, New York, 1976, p. 36.

Conners, K.A. and Sun, S., The stability of some molecular complexes in aqueous mixed solvents. Correlation with solvent surface tension. *J. Am. Chem. Soc.*, 93 (1971) 7239–7244.

Gelb, R.I., Schwartz, L.M., Radeos, M., Edmonds, R.B. and Laufer, D.A., Cyclohexamylose complexation with organic solvent molecules. *J. Am. Chem. Soc.*, 104 (1982) 6283–6288.

Harrison, J.C. and Efting, M.R., Cyclodextrin-adamantanecarboxylate inclusion complexes: A model for the hydrophobic effect. *Biopolymers*, 21 (1982) 1153–1166.

Kano, K., Takenoshita, I. and Ogawa, T., γ-Cyclodextrin – enhanced excimer of Pyrene and effects of *n*-butyl alcohol. *Chem. Lett.*, (1982) 321–324.

Kempfle, M.A., Muller, R.F., Palluk, R. and Winkler, H.A. The binding of fluorescent 4,6,8(14)-triene-3-one-steroids to cyclodextrins as a model for steroid-protein interactions. *Biochim Biophys. Acta*, 923 (1987) 83–87.

Kralova, K. and Mitterhauszerova, L., Interaction of β-cyclodextrin with steroid compounds in aqueous solutions. *Pharmazie*, 44 (1989), 623–626.

Lindler, K. and Saenger, W., Crystal structure of the γ-cyclodextrin-*n*-propanol inclusion complex: correlation of α-, β-, γ-cyclodextrin geometries. *Biochem. Biophys. Res. Commun.*, 92 (1980) 933–938.

Loftsson, T., Brewster, M.E., Derendorf, H. and Bodor, N., 2-Hydroxypropyl-β-cyclodextrin: Properties and usage in pharmaceutical formulations. *Pharm. Ztg. Wiss.*, 136 (1991) 5–10.

Martin, A., Wu, P.L., Adjei, A., Lindstrom, R.E. and Elworthy, P.H., Extended Hildebrand solubility approach and the log linear solubility equation. *J. Pharm. Sci.*, 71 (1982) 849–856.

Matsui, Y. and Mochida K., Binding forces contributing to the association of cyclodextrin with alcohols in aqueous solution. *Bull. Chem. Soc. Jap.*, 52 (1979) 2808–2814.

Orstan, A. and Ross, A., Investigation of the β-cyclodextrin-indole inclusion complex by absorption and fluorescence spectroscopies. *J. Phys. Chem.*, 91 (1987) 2739–2745.

Pitha, J., In situ modification of polynucleotide films for infrared studies. *Biochim. Biophys. Acta*, 232 (1971) 607–610.

Pitha, J. and Pitha J., Amorphous water-soluble derivatives of cyclodextrins: nontoxic dissolution enhancing excipients. *J. Pharm. Sci.*, 74 (1985) 987–990.

Pitha, J., Irie, T., Sklar, P.B. and Nye, J.S., Drug solubilizers to aid pharmacologists: amorphous cyclodextrin derivatives. *Life Sci.*, 43 (1988) 493–502.

Pitha, J., Hoshino, T., Torres, J.L. and Irie, T., Preparation of drug: hydroxypropylcyclodextrin complexes by a method using ethanol or aqueous ammonium hydroxide as co-solubilizers. *Int. J. Pharm.*, 80 (1992) 253–258.

Saenger, W., Cyclodextrin inclusion compounds in research and industry. *Angew. Chem. Int. Ed. Engl.*, 19 (1980) 344–362.

Suzuki, M., Ueda, S. and Kusai, A., Application of freezing point depression to drug interaction studies. I. Interaction between cyclodextrins and alcohols. *Chem. Pharm. Bull.*, 36 (1988) 720–725.

Szejtli, J., *Cyclodextrin Technology*, Kluwer, Dordrecht, The Netherlands, 1988.

Tokuoka, R., Abe, M., Fujiwara, T., Tomita, K. and Saenger, W., Crystal structure of a β-cyclodextrin · ethanol · octahydrage. *Chem. Lett.*, (1980) 491–494.

Uekama, K., Hirayama, F. and Irie, T., Modifications of drug release by cyclodextrin derivatives. In Duchene, D. (Ed.), *New Trends in Cyclodextrins and Derivatives*, Editions de Sante, Paris, 1991, pp. 409–446.

Yalkowsky, S.H., Flynn, G.L. and Amidon, G.L., Solubility of non-electrolytes in polar solvents. *J. Pharm. Sci.*, 61 (1972) 983–984.