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A Study of the Complexation Between Danazol and Hydrophilic Cyclodextrin Derivatives

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ABSTRACT

Complexation between danazol, a steroid used for endometriosis, and both hydroxypropyl-β-cyclodextrin (HPCD) and sulfobutyl ether-β-cyclodextrin (SBE) was studied in solution and solid state. Complexation was evaluated in solution using solubility studies and proton magnetic resonance (^{1}H NMR) spectroscopy, and in the solid state using x-ray diffraction, Fourier-transform infrared spectroscopy (FTIR), and dissolution studies. Solubility studies suggested the existence of a 1:1 complex between danazol and either HPCD or SBE. ^{1}H NMR showed that complexation occurs by inclusion of the isoxazole ring of danazol into the cyclodextrin cavity in both cases. Powder x-ray diffraction indicated that danazol existed in a crystalline noncomplexed form at low danazol-to-cyclodextrin ratios in the coprecipitates prepared by solvent evaporation method, while at higher ratios danazol existed in an amorphous complexed form. This ratio was 1:10 w/w for HPCD and 1:20 for SBE; the higher ratio in the case of SBE is attributed to early precipitation of danazol from the solvent used for preparation. FTIR studies showed that the complexation was accompanied by a shift of the O-H stretching of danazol hydroxyl group to a higher frequency, which is attributed to the disruption of the intermolecular hydrogen bonding. The dissolution rate of danazol from HPCD coprecipitates was higher than crystalline danazol in aqueous-isopropanolic medium, while SBE coprecipitates showed reduced dissolution rates due to the low solubility of SBE in isopropanol. However, SBE coprecipitates showed higher dissolution rates in water than in the isopropanolic medium.

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INTRODUCTION

Danazol (17-α-pregna-2,4-dien-20-yno[2,3-*d*] isoxazol-17-ol) is a synthetic steroid that has multiple biological effects and consequently has found diverse clinical applications. The compound is used in the treatment of endometriosis and/or infertility, fibrocystic breast disease, and hereditary angioedema (1). It has very high potential for the treatment of acute myeloid leukemia and tropical spastic paraperesis—a progressive spastic disorder associated with T-lymphotropic virus (2,3). Among other indications, danazol has also been investigated for the use in premenopausal abnormal bleeding and metastatic breast cancer (4). Danazol is poorly soluble in water (5) and was shown to have dissolution rate limited oral bioavailability. Consequently steady-state plasma concentrations did not increase proportionally with the increasing dose (6). Recently, a soluble danazol complex developed using hydroxypropyl-β-cyclodextrin has been reported to significantly increase oral bioavailability of danazol in rats (7).

Newly developed sulfoalkyl ether derivatives of cyclodextrins have been reported to possess some advantages over parent cyclodextrins and other cyclodextrin derivatives (8). One of these derivatives, sodium salt of sulfobutyl ether-β-cyclodextrin (SBE) was found to be less hemolytic to human erythrocytes than β-cyclodextrin and hydroxypropyl-β-cyclodextrin (HPCD) (9). SBE is a highly hydrophilic amorphous compound that forms highly water-soluble complexes with many drugs, and such complexes were found to be more soluble than the corresponding HPCD complexes.

The purpose of this work was to study complexation between danazol and SBE using techniques used previously for the study of danazol–HPCD complex (e.g., x-ray diffraction, solubility studies, dissolution rate) and to compare these results with those of a danazol–HPCD complex. In addition, complexation between danazol and either HPCD or SBE was also studied with other techniques such as proton nuclear magnetic resonance (^{1}H NMR) and Fourier-transform infrared spectroscopy (FTIR).

MATERIALS AND METHODS

Materials

Danazol was purchased from Miat Chemical Company (Italy). Hydroxypropyl-β-cyclodextrin was donated by Roquette Corporation (Gurnee, IL). Sulfobutyl ether-β-cyclodextrin sodium salt was donated by CyDex L.C. (Overland Park, KS). Isopropanol and methanol were of analytical grade.

Solubility of Danazol in SBE Solutions

Solubility of danazol in SBE solutions of varying concentrations was determined at 22°C and 37°C by adding 10 ml of aqueous SBE solution to the excess amount of danazol in a screw-capped bottle. The bottles were shaken in a thermostatically controlled water bath shaker until equilibrium was attained as demonstrated by a constant danzol content of three successive samples at 6, 12, and 24 hr. Samples of 0.5 ml were filtered and analyzed for danazol content using the high-performance liquid chromatographic (HPLC) method reported previously (5,7).

Preparation of Danazol–SBE Coprecipitates

Coprecipitates of danazol with SBE were prepared at different w/w ratios of 1:3, 1:5, 1:10, and 1:20 by mixing 1% w/v methanolic solution of danazol with methanolic solutions of SBE having different concentrations. The resulting solution was stirred at an ambient temperature until complete evaporation of the solvent occurred. The resulting coprecipitates were kept in a desiccator for at least 48 hr and then ground in a glass mortar for size reduction.

X-ray Diffraction of Danazol–SBE Coprecipitates

X-ray diffraction patterns of danazol–SBE coprecipitates were determined between $2\theta = 10°$ to 35° using a Phillips PW 3710 scanner/PW 1830 generator with a CuKα anode at 40 kV and 40 mA.

Solubility of Danazol–SBE Coprecipitates

Solubility of danazol from coprecipitates in water was determined by adding 10 ml of water to an accurately weighed amount of the coprecipitate containing 50 mg of danazol in a screw-capped bottle. The bottles were shaken at a constant temperature until equilibrium was attained. Samples of 0.5 ml were filtered and analyzed for danazol content.

Dissolution of Danazol–SBE Coprecipitates

An accurately weighed amount of the powdered danazol–SBE coprecipitate having mean particle size of 110 μm, and containing 30 mg of danazol was subjected

to dissolution study using USP type 1 dissolution tester at 37°C. The powder was placed in baskets having screen size of 35 μm (VanKel Industries, Edison, NJ); the dissolution medium consisted of 900 ml of 30% isopropanol in water and stirring speed was 80 rpm. Samples were taken at different time intervals and analyzed for danazol content using HPLC. Dissolution profiles of danazol–SBE coprecipitates were also obtained employing dissolution medium composed of 100 ml distilled water on a USP dissolution apparatus type 1, and using the same conditions described above.

Nuclear Magnetic Resonance (^{1}H NMR) Studies

^{1}H NMR spectra were acquired at 300 MHz on a Bruker WH-300 spectrometer. Data were accumulated by 16 K size with 0.5 sec delay time and 70° tip angle. All spectra were obtained using a solvent composed of 30% deuterated methanol (CD_3OD) in D_2O, since it was not possible to dissolve enough danazol in D_2O alone to obtain its spectrum. Even with the use of CD_3OD, a maximum concentration of only 10 μg/ml was achieved. Spectra were also obtained for solutions containing 10 μg/ml of danazol together with 400 μg/ml of either HPCD or SBE in the same solvent. Chemical shifts were calculated using the methanol peak at 3.12 ppm as a reference.

Fourier-Transform Infrared Studies

Infrared spectra were obtained using the Perkin-Elmer model 1605 FTIR instrument. Sixteen scans were obtained at a resolution of 2 cm^{-1}, averaged, and then smoothed by deconvolution. Potassium bromide disks containing the sample at a concentration of 5% were used to obtain spectra of danazol, danazol–HPCD coprecipitates, danazol–SBE coprecipitates, and corresponding physical mixtures prepared at the same ratios. The danazol spectrum was obtained using the spectrum of potassium bromide as background, while spectra of the coprecipitates and physical mixtures were obtained using either the HPCD or the SBE spectrum as background.

RESULTS AND DISCUSSION

Solubility of Danazol in SBE Solutions

Solubility of danazol in water is very low and was previously reported to be 0.61 and 0.32 mg/liter at 37°C and 22°C, respectively (7). Solubility diagrams of danazol in aqueous SBE solutions at 22°C and 37°C were linear, similar to those obtained previously for HPCD solutions (Fig. 1). The linear A_L-type of these curves also suggests 1:1 complexation between danazol and SBE (10). The stability constant (K) of the 1:1 complex was calculated according to the equation (11):

$$[S_t] = [S_0] + \frac{K_{1:1}[S_0][L_t]}{1 + K_{1:1}[S_0]} \quad (1)$$

where $K_{1:1}$ is the stability constant of the 1:1 complex, $[S_0]$ is the intrinsic solubility of danazol in the pure solvent, $[S_t]$ is the solubility of danazol in SBE solution, and $[L_t]$ is the total concentration of ligand (SBE). The value of $K_{1:1}$ was 132.6 × 10^3 M^{-1} and 75.9 × 10^3 M^{-1} at 22°C and 37°C, respectively. This is higher than the corresponding values for the danazol–HPCD complex at the same temperatures, the values of which were 76.6 × 10^3 M^{-1} and 61.9 × 10^3 M^{-1} at 22°C and 37°C, respectively. The decrease in stability of the danazol–SBE complex by temperature is greater than that of the danazol–HPCD complex, and this is reflected in higher enthalpy of complexation ($\Delta H°$) for the SBE complex. $\Delta H°$ was calculated using the van't Hoff equation [Eq. (2)] for the SBE complex:

$$\ln \frac{k_2}{k_1} = \frac{\Delta H°[T_2 - T_1]}{RT_2T_1} \quad (2)$$

The $\Delta H°$ was calculated to be -28.3 kJ/mol as compared to a value of -9.9 kJ/mol determined for the

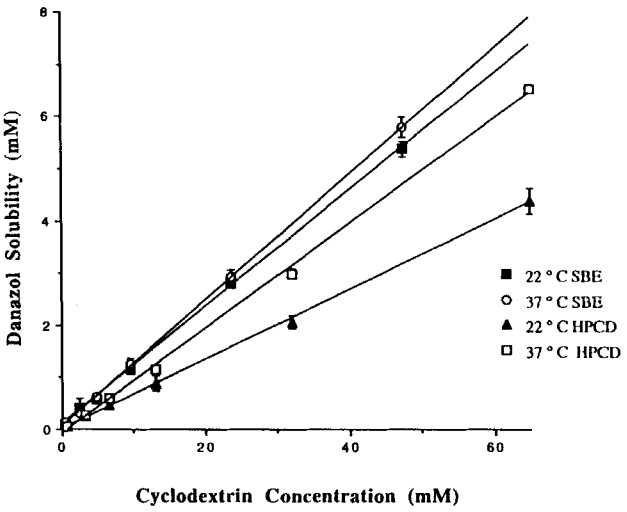


Figure 1. Solubility of danazol (mean ± SD) in aqueous solutions of SBE and HPCD.

HPCD complex (7). While complexation was exothermic in both cases, the value of $\Delta H°$ was higher for SBE, which explains why the value of its stability constant is more affected by change in temperature than in the case of HPCD.

X-ray Diffraction of Danazol–SBE Coprecipitates

X-ray diffraction of SBE coprecipitates (prepared by coprecipitation from methanolic solution) showed distinct crystalline peaks of danazol in the 1:10 coprecipitate. The crystalline peaks were greatly reduced in the diffractogram of the 1:20 coprecipitate, indicating the amorphous nature of danazol in this coprecipitate (Fig. 2). In contrast, danazol–HPCD coprecipitate 1:10 prepared by a similar method was found to be amorphous, suggesting that danazol exists in a complexed form in this coprecipitate at a lower danazol–cyclodextrin ratio than SBE. It was shown previously that the stability of the danazol–HPCD complex is markedly decreased in organic solvents (7), an observation that is in agreement with that of Pitha and Hoshino, who concluded that the complex forms only after the evaporation of the organic solvent (12). Absence of complexation in the 1:10 SBE coprecipitate could be due to limited solubility of danazol in methanol and consequent early precipitation of danazol before complete evaporation of the solvent, while SBE was still in solution. This was demonstrated by stopping the evaporation process before complete evaporation of the solvent and analyzing for drug content in the early precipitate. This precipitate was found to be very rich in danazol. In the case of the 1:20 coprecipitate, this early precipitation was not observed, probably due to higher viscosity of the more concentrated SBE solution, which retarded nucleation of danazol crystals.

Solubility of Danazol–SBE Coprecipitates

Solubility of danazol from its coprecipitates (equilibrium concentration of danazol in presence of excess undissolved coprecipitate) was superimposable with its solubility from the corresponding physical mixture up to a 1:10 ratio. The amorphous form of danazol obtained in the 1:20 coprecipitate was highly soluble in water at 22°C when compared with the corresponding physical mixture [Fig. 3(a)]. In the case of the 1:20 ratio only, the coprecipitate dissolved completely, resulting in a high concentration of danazol in solution. Unlike the danazol–HPCD complex (1:10 coprecipitate), there was no decline in concentration with time [Fig. 3(b)]. This may be due to the higher viscosity of the solution in this case, which is due to the high content of SBE and which retards the crystallization of danazol.

Dissolution Studies

Dissolution rates of danazol from different SBE coprecipitates in 30% isopropanol were found to be lower than that of crystalline danazol and decreased with increasing SBE content in the coprecipitate [Fig. 4(a)]. Aqueous-isopropanolic solution is the pharmacopeial medium of dissolution for danazol. Isopropanol is added

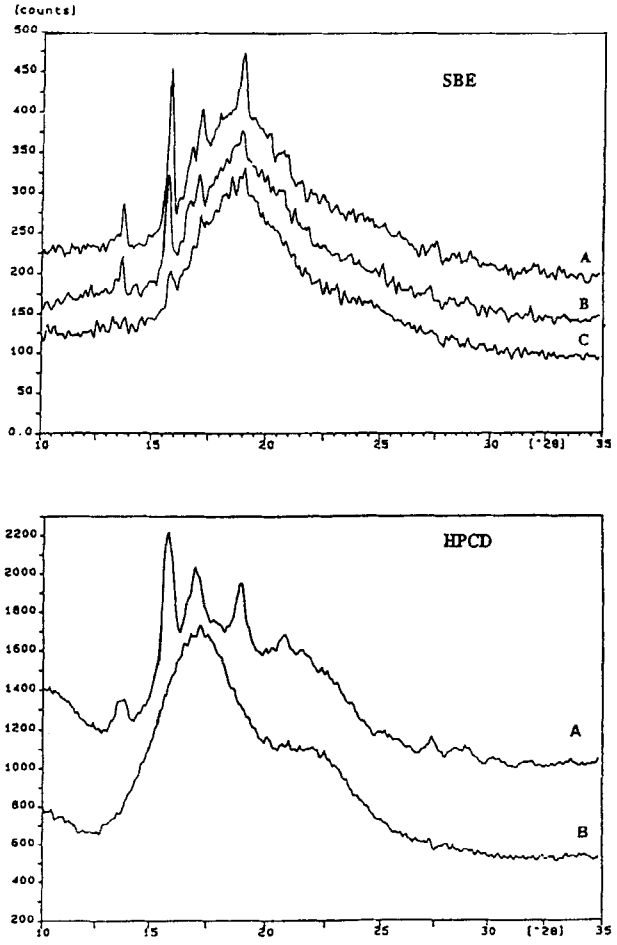


Figure 2. (Top) X-ray diffraction patterns of danazol–SBE 1:10 coprecipitate, A; danazol–SBE 1:20 physical mixture, B; and 1:20 coprecipitate, C. (Bottom) X-ray diffraction patterns of danazol–HPCD physical mixture 1:10, A; and danazol–HPCD coprecipitate 1:10, B.

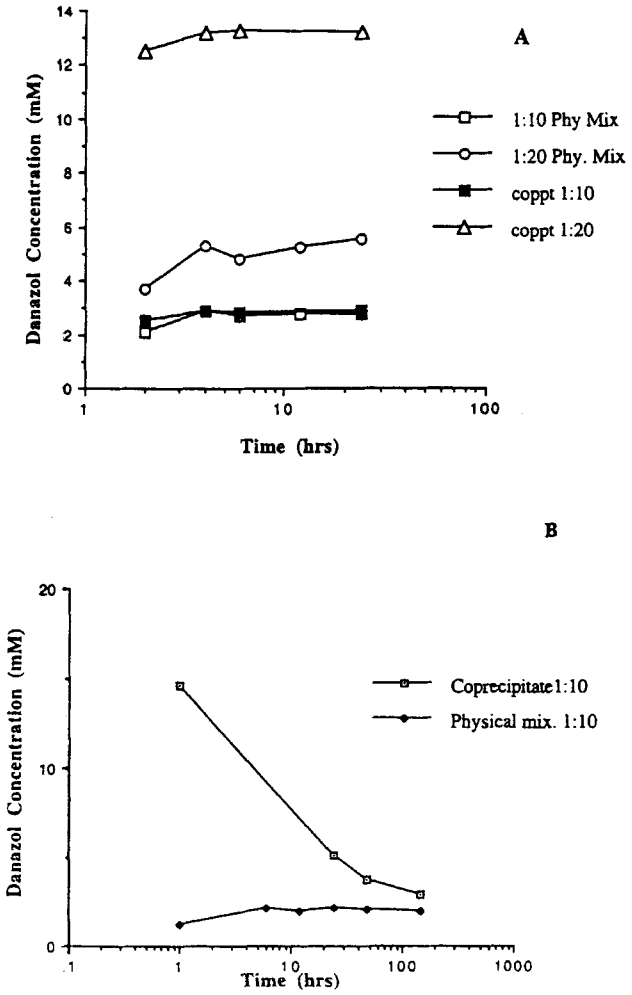


Figure 3. Solubility of danazol from its coprecipitates and physical mixtures with SBE, A; and HPCD, B, as a function of time.

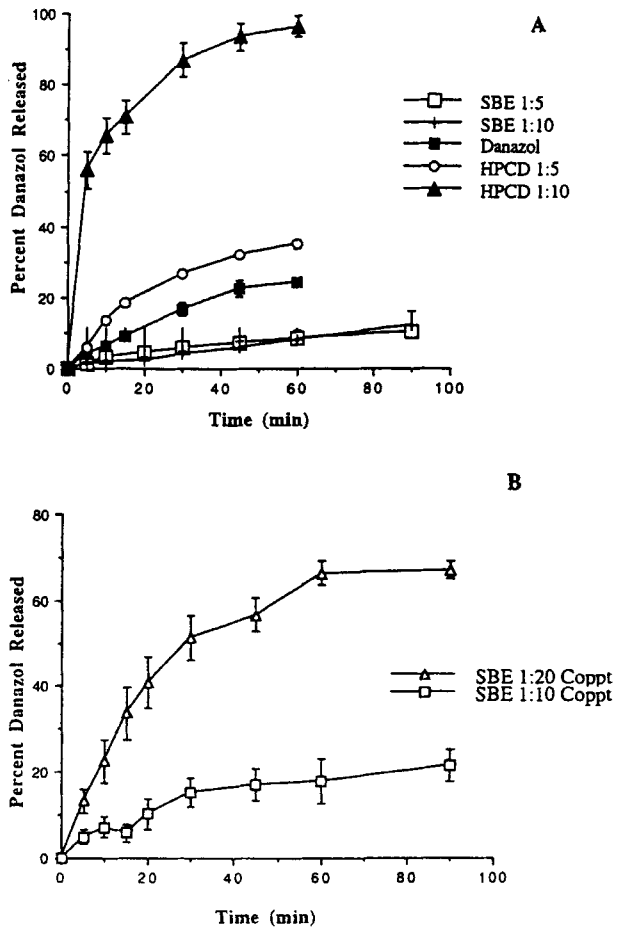
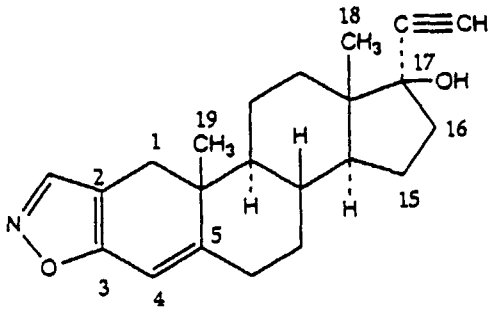


Figure 4. Dissolution profiles of crystalline danazol and its coprecipitates (mean ± *SEM*) in 30% isopropanol in water (HPCD and SBE), A; and water alone (SBE), B.

to increase the solubility of danazol in dissolution medium, thus maintaining sink condition. However, SBE has only limited solubility in isopropanol, and the decrease of dissolution rate is attributed to the nature of dissolution medium, which might not be predictive of the dissolution rate in the aqueous medium of the GI tract. The dissolution rates of the different coprecipitates were therefore tested in distilled water, although it was not possible to maintain sink condition in this medium. The dissolution rate of danazol from the SBE coprecipitate was found to be higher in water than it was in aqueous-isopropanolic medium. The 1:20 danazol–SBE coprecipitate exhibited a much higher dissolution rate than the 1:10 coprecipitate, which is due not only to the higher content of SBE but also to the amorphous nature of danazol in the former coprecipitate [Fig. 4(b)].

Nuclear Magnetic Resonance (^{1}H NMR) Studies

Although solubility studies indicated the existence of complexation between danazol and HPCD or SBE in solution, the exact mechanism of complexation could not be deducted from these studies. A deeper insight into the complexation mechanism was obtained from ^{1}H NMR. Cyclodextrins complexes are known to be of the inclusion type, and a molecular modeling study was done using ALCHEMY molecular modeling software (Tripos Associates, St. Louis, MO) to predict how danazol



Danazol

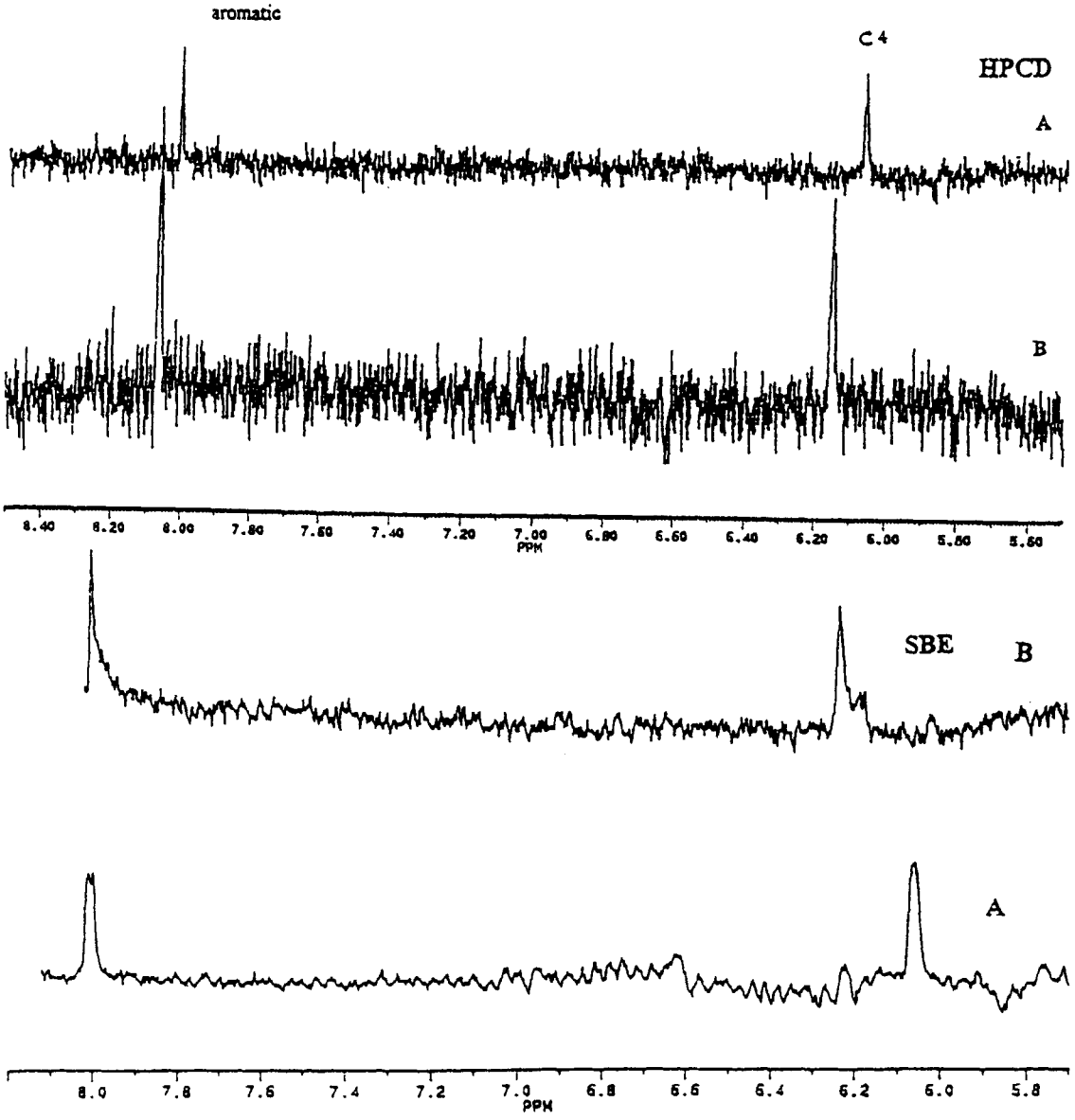


Figure 5. (Top: HPCD) Proton NMR of aromatic region of danazol 10 μg/ml using a solvent composed of 30% CD_3OD in D_2O, A; and danazol 10 μg/ml in the presence of 400 μg/ml HPCD in the same solvent, B. (Bottom: SBE) Proton NMR of aromatic region of danazol 10 μg/ml using a solvent composed of 30% CD_3OD in D_2O, A; and danazol 10 μg/ml in the presence of 400 μg/ml SBE in the same solvent, B.

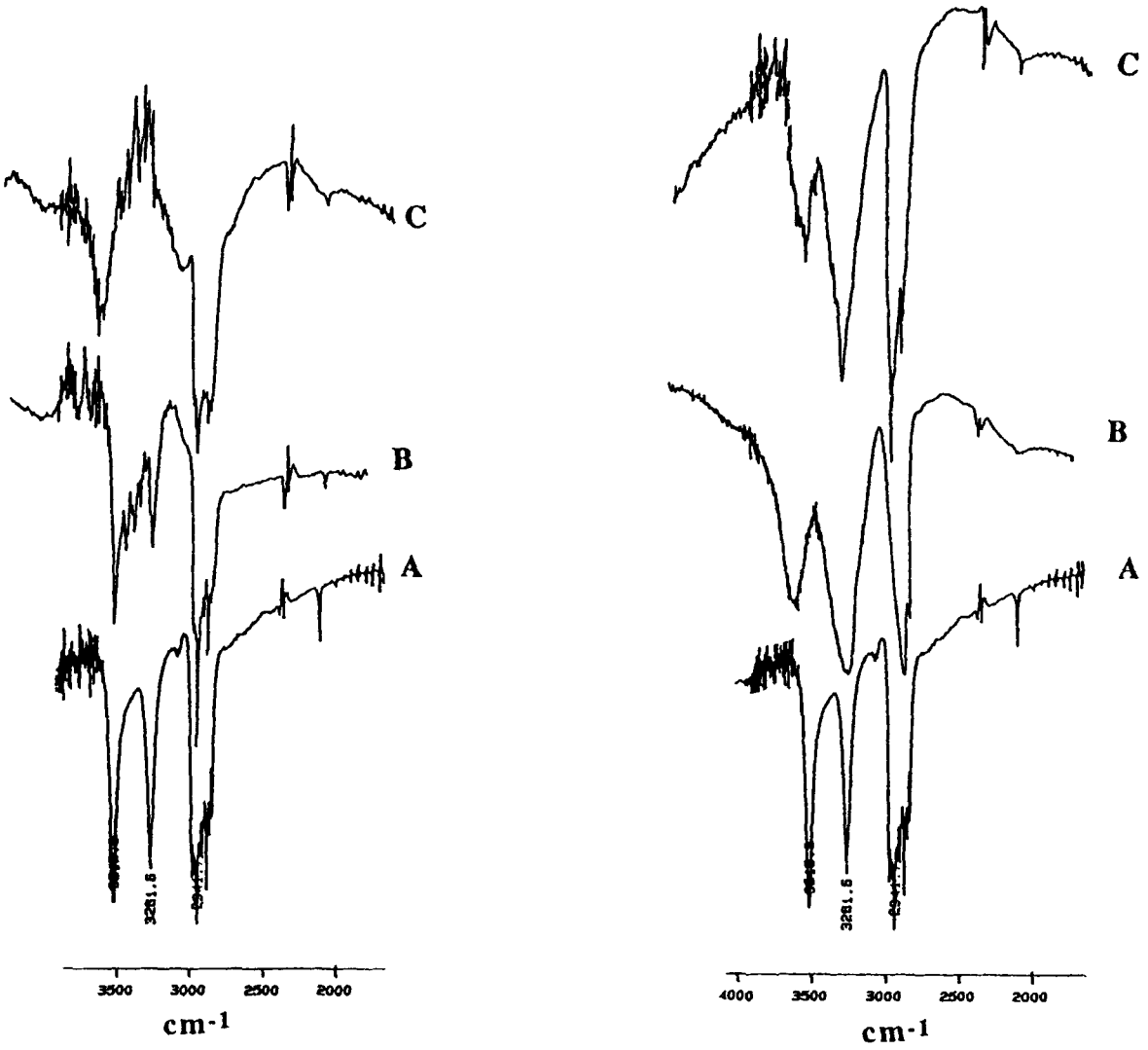


Figure 6. (Left) FTIR Spectra of danazol, A; danazol-HPCD 1:10 physical mixture, B; and coprecipitate, C. (Right) FTIR spectra of danazol, A; and danazol-SBE coprecipitates 1:20, B; and 1:10, C.

would fit into the cyclodextrin cavity. This study suggested that danazol will fit into the cavity with its axis parallel to, and not perpendicular to, the axis of the cavity. Also, the isoxazole end of the molecule will be the part that fits into the cavity, since the acetylenic group at the opposite end protrudes out of the plane of the cyclopentane ring, making this side of the molecule too large to fit into the cavity. The aromatic proton on the isoxazole ring has a chemical shift, δ, of 8.006 ppm (parts per million), while the olefinic proton at the 4 position has δ of 6.062 in the spectrum of danazol in 30% CD_3OD in D_2O. The formation of an inclusion complex with HPCD resulted in a significant downfield shift of the peaks of these protons, indicating that the latter protons are surrounded by the hydrophobic cavity of cyclodextrin in this case (Fig. 5). However, in the case of SBE, a downfield shift was only observed for the olefinic proton, while the aromatic proton did not exhibit such a shift. This is attributed to charged sulfate groups, which make that part of the cyclodextrin cavity more polar. For both cyclodextrins, complexation was accompanied by downfield shift of the peak of C-19 protons. However, C-18 protons did not show any change in chemical shift, suggesting that the cyclopentane end of the molecule does not fit into the cyclodextrin cavity.

Fourier-Transform Infrared Studies

Infrared spectrum of danazol showed a peak at 3518 cm^{-1}, which corresponds to O–H stretching of the hydroxyl group at the 17 position. This peak was shifted to a higher frequency in the danazol HPCD complex (1:10 coprecipitate), whereas in the 1:10 physical mixture there was no such shift (Fig. 6). Also, this peak shifted to a higher frequency in the SBE complex (1:20 coprecipitate) but not in the 1:10 coprecipitate, in which danazol was found by x-ray diffraction to exist in crystalline noncomplexed form. The peak of O–H stretching, therefore, appears to shift only when danazol is complexed. Danazol molecules are closely and uniformly packed in the crystalline lattice, which allows for intermolecular hydrogen bonding between hydroxyl groups. In the complex, danazol molecules have less chance for hydrogen bonding since the crystalline structure is lost.

CONCLUSIONS

Both SBE and HPCD were found to complex with danazol in aqueous solution, resulting in pronounced enhancement of danazol solubility and dissolution rate. The values of stability constants were comparable for the two cyclodextrins, and complexation was exothermic in both cases. Complexation occurred by inclusion of the isoxazole side of danazol in either the SBE or HPCD cavity. However, a higher danazol:cyclodextrin ratio was needed to form the complex in the solid state in the case of SBE than HPCD. In both cases, solubility of the complex was higher than the corresponding physical mixture. The complexation characteristics of both cyclodextrins with danazol were closely similar.

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