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INCLUSION COMPLEXES OF TOLNAFTATE WITH ß-CYCLODEXTRIN AND HYDROXYPROPYL ß-CYCLODEXTRIN

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ABSTRACT

Tolnaftate, an antifungal agent, was found to form inclusion complexes with both ß-cyclodextrin (ß-CD) and hydroxypropyl ß-cyclodextrins (HPBCDs) with two different degrees of substitution [HPBCD(A)-8% and HPBCD(B)-3%]. Complex formation in the solution state was studied using phase solubility and spectral shift methods. Solid complexes were prepared by the coprecipitation method. Solubilities and dissolution rates were determined for each solid complex, its corresponding physical mixture, and free drug. The increase in solubility of tolnaftate with added HPBCD was found to be significantly greater than with added ß-CD. For both HPBCD(A) and HPBCD(B), over the concentration range 0-0.05 M, 1:1 complexes with stability constants of 1460 ± 139 M^{-1} and 1860 ± 165 M^{-1} were observed, respectively. Over the ß-CD concentration range 0-0.02 M, a 1:1 complex with a stability constant of 1190 ± 105 M^{-1} was observed. At higher HPBCD concentrations, the increase in solubility was observed to show a positive deviation from linearity (type A_P phase diagram). Using the spectral method, in a 25% v/v methanol in water system, the stability constants were determined to be 1020 ± 150 M^{-1}, 1110 ± 120 M^{-1} and 1100 ± 260 M^{-1} for HPBCD(A), HPBCD(B) and ß-CD, respectively. The solid complexes prepared showed improved dissolution over physical mixtures and free drug.

TABLE 1

Basic Properties of Cyclodextrins[2]

Cyclodextrin	No. of glucose units	Molecular Weight	Solubility in Water (g/100 ml) @ 25C	Cavity Dimensions(Å)	
				Depth	i.d[a]
Alpha-CD	6	972	14.50	7.9	5.0
Beta-CD	7	1135	1.85	8.0	6.0
Gamma-CD	8	1297	23.20	8.0	7.5
HPBCD	7	1550[b]	>50.0	8.0	6.0

[a]i.d = internal diameter
[b]1550 = average molecular weight

INTRODUCTION

Complexation plays an important role in altering the physical, chemical and biological properties of many important drugs. The discovery of cyclodextrins (CDs) as inclusion complexing agents led to a solution of a number of problems encountered by the pharmaceutical companies during the formulation phase of product development. The primary reasons for the great pharmaceutical interest in the CDs [1] compared to the other inclusion compounds are: (i) size of channel diameter; (ii) good stability; and (iii) absence of toxicological side effects. Some basic properties of the cyclodextrins are listed in Table 1.

One of the important characteristics of the CDs is their ability to form inclusion complexes with various compounds (guests) in which the guest compounds are included in the CD's cavity (hosts). Guest compounds range from polar reagents such as acids, amines, small ions such as ClO_4^-, SCN^-, and halogen ions to highly apolar aliphatic and aromatic hydrocarbons and even rare gases[2]. The effect of CD complexation varies depending upon the specific CD and the specific drug moiety employed. For example, aspirin has increased stability with CD complexation[3], indomethacin has enhanced bioavailability and reduced side effects following

complexation with ß-CDs[4], and Lach and Chin[5] reported the lower rates of hydrolysis of benzocaine when complexed with CDs. In vitro dissolution tests of a number of drugs have indicated that the drug release is faster from CD complexes as compared to physical mixtures of the corresponding CD and drug. Pure drug generally exhibits the slowest release rates of these three systems[6].

The objective of the present investigation was to increase the aqueous solubility of a water insoluble drug using CDs. Tolnaftate was used as a model drug. ß-CD and HPBCDs with two different degrees of substitution were employed in the preparation of inclusion complexes. Complex formation was studied using both phase solubility and spectral methods. Solid complexes were prepared by the coprecipitation method. The stoichiometric ratio and stability constants were determined using the solubility and spectroscopic data. To evaluate the enhancement in the dissolution rate of tolnaftate-CD complexes, in vitro dissolution tests were performed using the USP paddle method.

MATERIALS AND METHODS

Materials

The materials used were tolnaftate (Schering-Plough, Memphis, TN), ß-CD and HPBCDs (Pharmatec, Alachua, FL), methanol, acetone (Fisher-Scientific, Fair Lawn, NJ). Distilled water was used in all experiments.

Procedures

The experiments were designed to compare the increase in the aqueous solubility of tolnaftate using ß-CD and HPBCDs. Three types of studies were conducted: (i) phase solubility studies; (ii) spectral studies; and (iii) dissolution studies.

Phase Solubility Studies: Solubility measurements were conducted according to the method of Higuchi and Lach[7]. Excess amounts of tolnaftate (80-100 mg) were added to aqueous solutions (100 ml) containing various concentrations of ß-CD or HPBCDs. Two different HPBCDs were used for all the studies, HPBCD(A) and HPBCD(B), having degrees of substitution (DS) of 8.0% and 3.0% respectively. Amber colored containers with air-tight lids were placed in a mechanical shaker-constant temperature water bath (Precision Scientific, Chicago, IL) at 30 ± 0.2°C. After equilibrium was attained (approximately 7 days), an aliquot was filtered and analyzed for tolnaftate content by UV spectrophotometry (Perkin-Elmer, Norwalk, CT).

Spectroscopic Studies: Complex formations of tolnaftate with ß-CD, HPBCD(A) and HPBCD(B) were studied using the spectral shift method[8] in a 25% v/v methanol

in water system. To 10 ml of tolnaftate solution (concentration = 2.26 x 10^{-6}M) in 20 ml glass vials, different quantities of ß-CD were added (0 to 200 mg). The vials were gently warmed to solubilize the ß-CD and were then allowed to cool to room temperature. Similarly, different quantities of the HPBCDs were added and gently shaken at room temperature. The changes in absorbance of the substrate (tolnaftate) resulting from the addition of various concentrations of ligand (cyclodextrin) were measured at 258 nm and the data were analyzed to evaluate the stoichiometry and stability constants of the resultant complexes.

Preparation of Solid Complexes: The coprecipitation method was utilized to prepare solid complexes. ß-CD (11.5 g) was dissolved in 100 ml of distilled water at 70°C. Tolnaftate (300 mg) was dissolved in a sufficient volume of acetone (10 ml) which was added dropwise with constant stirring and maintained at 70°C for 1 hour, then gradually cooled to room temperature over a period of 6 hours. The stirring was continued 7 days and the precipitate obtained was filtered by vacuum filtration, washed repeatedly with acetone and dried at 60°C for 4 hours.

The HPBCD(A) solution was prepared by mixing 10.0 g in 20 ml of distilled water. Tolnaftate (300 mg) was dissolved in sufficient volume of acetone and added dropwise to the HPBCD(A) solution under constant stirring. The mixture was stirred constantly for 7 days at room temperature. The unincorporated drug was removed by vacuum filtration and the filtrate was evaporated using Rotavap (Rinco Instruments, Greenville, IL), collecting the dried tolnaftate-HPBCD(A) complex. The procedure for the preparation of the tolnaftate-HPBCD(B) complex was the same as with HPBCD(A).

Dissolution Studies: Dissolution studies were conducted for tolnaftate, physical mixtures of the drug and CDs, and inclusion complexes using the USPXXII paddle method (Model 2000, Distek, Somerset, NJ), using the rotation speed of the paddles of 50 ± 2 rpm. The dissolution medium was distilled water (400 ml) and the bath temperature was maintained at 37 ± 0.2°C. Samples of 5 ml were collected at 1, 5, 10, 15, 30, 45, 60, 90, 120, and 180 minutes. The samples were filtered and the filtrates were analyzed spectrophotometrically for tolnaftate content.

RESULTS AND DISCUSSION

Phase Solubility Studies

Phase solubility diagrams (Figure 1) of tolnaftate with ß-CD, HPBCD(A) and HPBCD(B) illustrate the solubility enhancement capabilities of the cyclodextrins.

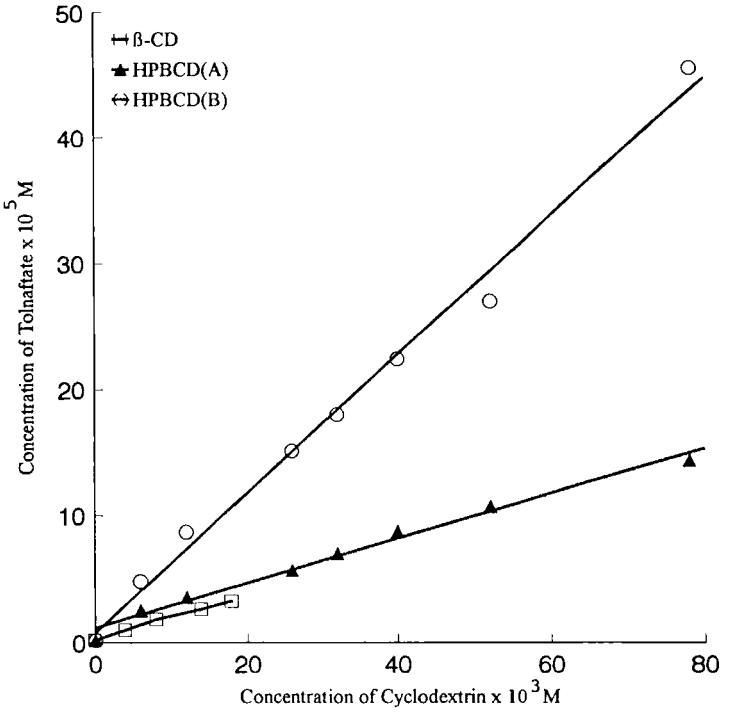

Figure 1. Phase Solubility Diagrams of Tolnaftate with three CDs

The ß-CD system can be classified as a solubility curve of type A_L[8], i.e., the solubility of tolnaftate increased linearly with the increasing concentrations of ß-CD. There was a 30 fold increase in solubility of tolnaftate in 0.02 M ß-CD. The increase in solubility at the higher concentrations of HPBCD than those illustrated in Figure 1 showed a positive deviation from linearity. These types of solubility curves are generally classified as A_P indicating the formation higher order complexes at the higher concentrations of ligand[8].

The linear relationship between tolnaftate and ß-CD indicate 1:1 stoichiometry. Similarly, the linear relationships, at the lower concentrations, between tolnaftate and the HPBCDs indicate 1:1 stoichiometry of the complex formed. The stability constants for ß-CD, HPBCD(A), and HPBCD(B) obtained were 1190 ± 105 M^{-1}, 1460 ± 139 M^{-1}, and 1860 ± 165 M^{-1} respectively. All stability constants were calculated from the equation:

$$K_{1:1} = \frac{\text{Slope}}{S_o\ (1\text{-Slope})}$$

$K_{1:1}$ = Stability Constant
S_o= Solubility of tolnaftate in water.

The results of preliminary saturation solubility test for tolnaftate indicated that it was very slightly soluble (50 mcg/100 ml) in water. The results of phase solubility studies indicated that the aqueous solubility of tolnaftate was greatly enhanced in the presence of cyclodextrins. The solubilizing effect of cyclodextrin derivatives is in the following order, HPBCD(B) > HPBCD(A) > ß-CD. The enhancement in solubility was 180 fold for HPBCD(B), 120 fold for HPBCD(A) and 30 fold for ß-CD system.

Spectral Shift Studies

The method utilizes varying concentrations of CDs with a constant concentration of tolnaftate, in contrast to the phase solubility studies where the CD concentrations were held constant and the tolnaftate concentration was varied. The presence of 25% v/v methanol was found to be essential to keep the tolnaftate in solution. As a result of varying the concentrations of the CDs, differing amounts of tolnaftate were complexed. Small changes in the total absorbance were measured due to the change in the absorptivity of tolnaftate in its complexed form as compared to its free form. The Benesi-Hildebrand plots (Figure 2) indicated linear relationships for tolnaftate with all the three CDs. The stability constants calculated from the ratio of intercept/slope of these plots were 1100 ± 260 M^{-1}, 1029 ± 150 M^{-1}, and 1110 ± 120 M^{-1} for ß-CD, HPBCD(A) and HPBCD(B) respectively. These results confirmed the 1:1 stoichiometry of the complexes as seen in the phase solubility studies. The lower values of stability constants calculated from spectral studies plots, as compared to those obtained from the phase solubility studies, may be due to some weakening of complexation forces in 25% v/v methanol.

Statistical analyses for the determinations of differences in the stability constants for the different CD systems are summarized in Table 2. A two-tailed t-test was performed to examine the differences between each of the following pairs of systems: (i) ß-CD and HPBCD(A); (ii) ß-CD and HPBCD(B); and (iii) HPBCD(A) and HPBCD(B). The stability constants obtained from solubility studies for all the three CDs were significantly different from one another ($p<0.05$). In contrast, no significant differences were observed

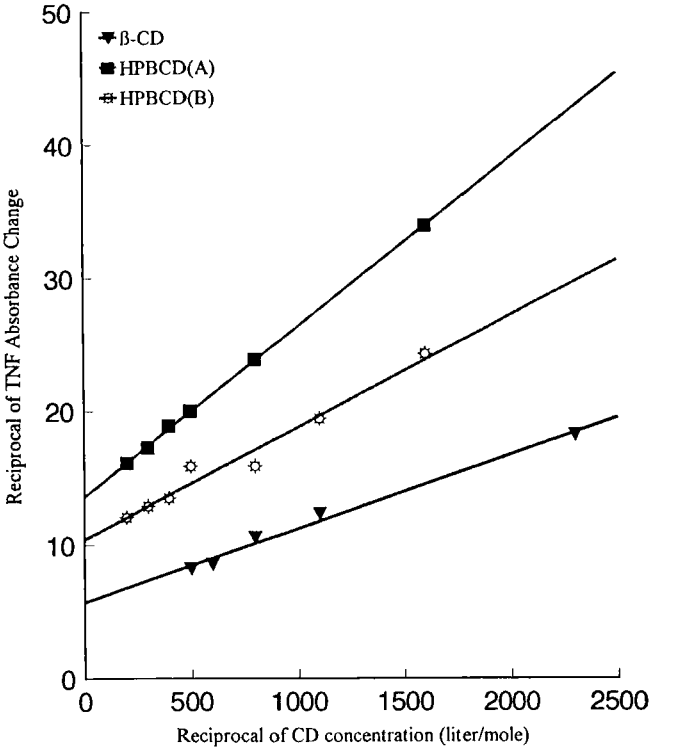

Figure 2. Benesi-Hildebrand Plots for the Three CD Systems

between the values of the stability constants obtained from the spectral shift studies.

Dissolution Studies

Dissolution profiles of tolnaftate (alone), physical mixtures of tolnaftate with the three CDs, and their respective complexes are illustrated in Figure 3. The HPBCD(B) complex appears to exhibit the fastest dissolution rate followed by HPBCD(A) and ß-CD complexes. The physical mixtures and tolnaftate alone have the lower dissolution profiles. The physical mixtures showed a slight improvement over the free drug but had a much slower initial rate and lower apparent solubility as compared to their respective complexes. The dramatic differences in the dissolution profiles of tolnaftate from different systems highlights the solubility improvement brought about by complexation.

TABLE 2

Comparison of Stability Constants

System	$K_{1:1}$ Mean Values with Standard Error	
	Solubility (M^{-1})	Spectral (M^{-1})
TNF[a]-ß-CD	1190 ± 105	1100 ± 260
TNF-HPBCD(A)	1460 ± 139	1020 ± 150
TNF-HPBCD(B)	1860 ± 165	1110 ± 120

[a]TNF - Tolnaftate

Calculated t-values* for indicated comparisons

	Solubility	Spectral
ß-CD vs. HPBCD(A)	4.154** (2.18)	0.618 (2.23)
ß-CD vs. HPBCD(B)	9.300** (2.18)	0.100 (2.23)
HPBCD(A) vs. HPBCD(B)	5.24** (2.36)	1.23 (2.16)

* Critical t-values from tables in parentheses.
** Indicates significant difference.

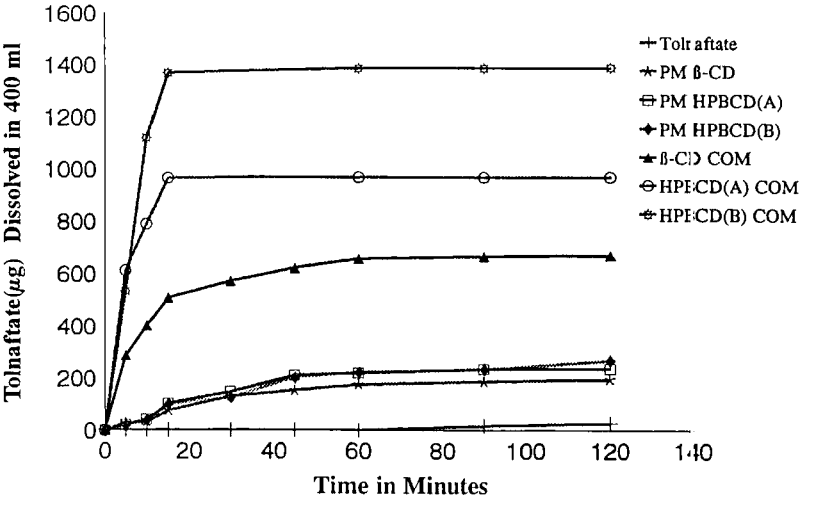

Figure 3. Dissolution Profiles of Test Compounds

CONCLUSIONS

The HPBCDs improved the aqueous water solubility of tolnaftate significantly. Stability constants obtained from solubility studies and spectral shift methods were all of the same order of magnitude. Rapid dissolution was obtained from inclusion complexes. Cyclodextrins and their derivatives are useful in enhancing the solubility and dissolution rates of water insoluble drugs such as tolnaftate. The solubility study approach appeared to be more sensitive than spectral shift studies to alterations in the CD molecule. However, in the present case, the lack of sensitivity may have simply been due to a reduction in the magnitude of the interaction as a result of having the methanol present as a cosolvent while conducting the spectral shift studies.

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