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The Solubilities of Esters of 4-Hydroxybenzoic Acid, Determined Separately and Together, in Aqueous Solutions of 2-Hydroxypropyl-β-cyclodextrin

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ABSTRACT

The solubilities of four esters of 4-hydroxybenzoic acid have been investigated singly and together in water and in aqueous solutions of 2-hydroxypropyl-β-cyclodextrin (HPCD). Values obtained for the solubilities of methyl (MP), ethyl (EP), propyl (PP), and butyl (BP) esters in water agreed well with literature values, when determined separately. When measured together the solubilities of the methyl and butyl esters increased slightly, that of the ethyl ester decreased slightly, and the solubility of the propyl ester decreased by approximately 50%. Association constants for complexes of esters and HPCD determined separately in water agreed well with literature values, declining from methyl to ethyl and then increasing through propyl to butyl. When the association constants were determined for the esters together in solutions of HPCD the same pattern was noted, but the large decreases in association constants may indicate some other process in addition to competition between the esters for the HPCD molecules.

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INTRODUCTION

Complexation by cyclodextrins is widely used to increase the solubilities of drug molecules which have limited solubilities in water (1). Most studies have measured solubilities in simple aqueous solutions of the appropriate cyclodextrin. However, in practice, drug solutions are usually more complex than this and other solutes may compete with drug molecules for the cyclodextrin. This competition has been used to calculate the binding constants for cyclodextrin inclusion compounds (2) and in probing the mechanisms of formation of inclusion complexes (3). Competitive effects on the solubility, bioavailability, and pharmacokinetics of drugs in the presence of cyclodextrins have also been studied (4–7).

In this study the solubilities of the methyl (MP), ethyl (EP), propyl (PP), and butyl (BP) esters of 4-hydroxybenzoic acid have been measured singly and together in a range of concentrations of a cyclodextrin (HPCD). These molecules are useful preservatives and are often used together, particularly MP and PP, and as a homologous series may give some data on the competitive effect.

MATERIALS

The esters of 4-hydroxybenzoic acid were purchased from Sigma Chemicals (St. Louis, MO, USA) and were used without further purification (MP, lot 43MO382; EP, lot 62H3Y67; PP, lot 42HD879; BP, lot 62HD265).

HPCD (degree of substitution = 6.5, lot D8031) was a gift from American Maize Products Company (Hammond, IN, USA) and was used without purification.

Water was obtained from a Milli Q Ultrapure Water System (Milli Q Australia, conductivity < 0.05 μS.

All other chemicals were analytical or high-performance chromatographic grades.

METHODS

Solubility Measurements

Solubilities in water, buffer, and solutions of HPCD were determined by adding equimolar excesses of the esters, either singly or in combination, to the appropriate vehicle. Since solubilities were expected to increase in HPCD solutions, which contained 1–10% w/v of the cyclodextrin, increasing equimolar amounts of the esters were added to these vehicles. The suspensions were shaken in an incubator (Incubator-Shaker, Lab-Line, IL, USA) at 25°C ± 0.1 for 5 days, which had previously been shown to afford equilibrium. Samples were filtered through glass wool, diluted appropriately with methanol, and analyzed by a high-performance liquid chromatographic (HPLC) method.

Analysis of Samples

Samples for analysis were injected through a 20 μl loop injector (Rheodyne) onto a Phenomenex Bondclone (10 μ, C18, 300 × 3.9 mm ID) column. Peaks were analyzed by a Waters (Millipore) 486 MS Detector and a Hewlett Packard 3396A Integrator at a wavelength of 256 nm. The mobile phase consisted of 55:45 methanol:0.04 M ammonium acetate in water. A flow rate of 1.5 ml/min was achieved using a Waters 501 pump.

Linearities of response (0–20 μg/ml) and coefficients of variation (5 μg/ml, $n = 6$) were determined for all esters from areas under the curve/concentration data. The effect of HPCD on the chromatograms was determined by comparing solutions with and without the complexing agent. Concentrations of unknown samples were obtained from comparison with freshly prepared standard solutions containing all esters.

RESULTS AND DISCUSSION

A typical HPLC chromatogram (Fig. 1) shows good separation of peaks with no interference. Plots of concentrations versus areas under the curve were linear ($r^2 > 0.99$) for all esters and coefficients of variation were 1.42% (MP), 0.98% (EP), 1.30% (PP), and 1.36% (BP). The presence of HPCD in solutions affected neither areas under the curves nor retention times.

In this study the pH values of the various solutions in water ranged from 4.5 to 5.0, which are well below the pK_a values of the esters, quoted as 8.48 ± 0.02 (8). The esters will be in the undissociated form in the pH range of 4.5–5.0 and their solubilities would essentially be unaffected by slight pH changes, which is confirmed by considering the solubilities obtained singly in water and in phosphate buffer (6.0) (Table 1). Values for solubilities determined singly in water agree with some previous data (9–14) but not with other data (15,16). Although recent work (14) has precluded the presence of solvates or polymorphs for the esters dissolved singly in water, the presence of metastable supersaturated solutions, which are not at the equilibrium state, cannot be precluded (17,18), and these may affect apparent solubility values.

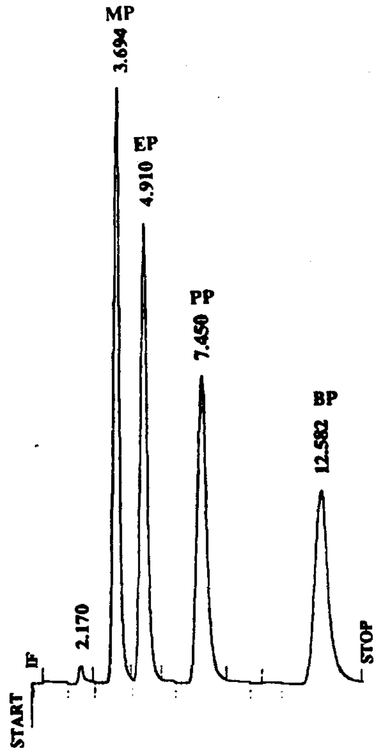

Figure 1. Representative chromatogram of esters determined together (MP–methyl ester; EP–ethyl ester; PP–propyl ester; BP–butyl ester).

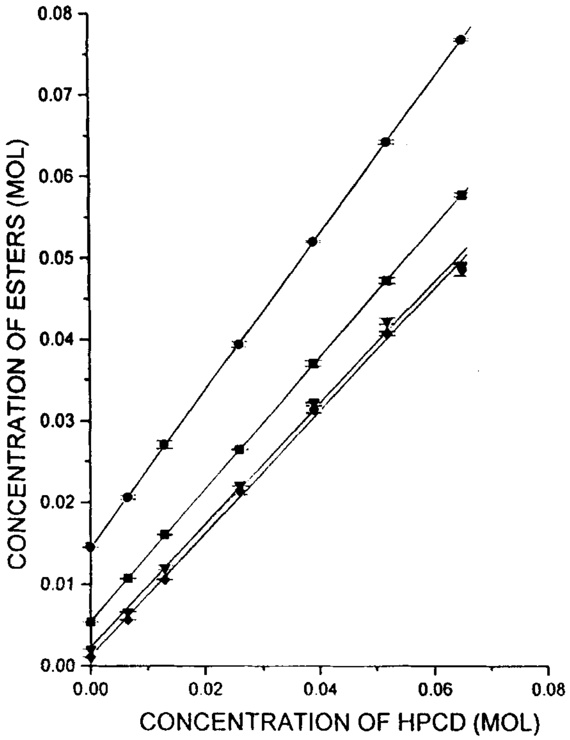

Figure 2. Solubilities of 4-hydroxybenzoic acid esters in HPCD solutions, measured separately (●, MP; ■, EP; ▼, PP, ◆, BP) ± 1 *SD*, *n* = 3.

Figure 2 shows plots of solubilities of the esters, measured singly, versus concentrations of HPCD (molar). The apparent mean molecular weight of HPCD was calculated from the theoretical structure, giving a value of 1512. These plots are linear ($r^2 > 0.99$) in all cases, indicating the probability of formation of 1:1 complexes between the esters and HPCD.

The binding constants ($K_{1:1}$) can be calculated from Eq. (1):

Table 1

Solubilities in Water, Slopes of Solubilities of Esters Versus Concentrations of HPCD, and Apparent Association Constants $K_{1:1}$ for Esters with HPCD in Aqueous Solution

	Solubilities in water; mol ± 1 *SD* (*n* = 3)		Slopes and $K_{1:1}$ values			
			Determined Singly		Determined in Combination	
Esters	Determined singly[a]	Determined in Combination	Slope	$K_{1:1}$	Slope	$K_{1:1}$
Methyl	$1.446 \times 10^{-2} \pm 0.009$ $(1.480 \times 10^{-2} \pm 0.006)$	$1.579 \times 10^{-2} \pm 0.016$	0.941	1099	0.578	87
Ethyl	$0.5381 \times 10^{-2} \pm 0.002$ $(0.515 \times 10^{-2} \pm 0.008)$	$0.481 \times 10^{-2} \pm 0.002$	0.790	699	0.246	68
Propyl	$0.202 \times 10^{-2} \pm 0.003$ $(0.200 \times 10^{-2} \pm 0.004)$	$0.112 \times 10^{-2} \pm 0.004$	0.730	1338	0.093	92
Butyl	$0.110 \times 10^{-2} \pm 0.001$ $(0.114 \times 10^{-2} \pm 0.004)$	$0.116 \times 10^{-2} \pm 0.002$	0.733	2495	0.170	177

[a] Solubilities in buffer in parentheses.

$$K_{1:1} = \frac{\text{slope}}{S_0(1 - \text{slope})} \qquad (1)$$

where S_0 is the solubility of the solute in water. Slopes and $K_{1:1}$ values are shown in Table 1.

The values for EP, PP, and BP agree reasonably well with literature values (10,19,20) while values for MP and PP agree with values calculated from literature data (1,11). However, the value for MP is very much higher than a value of 140 quoted in the literature [19]. The slope of the plot for the solubilities of MP versus HPCD concentration tends towards unity; consequently any slight measured error in this value alters $K_{1:1}$ dramatically. A slope greater than unity indicates that a 1:1 complex is not being formed (1). The $K_{1:1}$ values increase regularly from EP to PP to BP, as would be expected from hydrophobicity considerations.

Also shown in Table 1 are the solubilities of the esters when determined together. The solubility of MP increases slightly (~9%), that for BP increases by a lesser amount, and the value for EP declines slightly. However, that of PP has declined massively (~50%). It has been noted previously (21) that the presence of PP increases the solubility of MP by 6% due to the formation of a dipole-dipole interaction between MP and PP. However, this would be expected to increase the solubility of PP also, which is not so here. Competition by the esters for water molecules is precluded although it is interesting to note that the sum of the molar solubilities of the esters when measured singly in water is within 1% of the sum of total solubilities of the esters when measured together.

Solubilities of the esters when measured together in the presence of HPCD are shown in Fig. 3, and slopes and $K_{1:1}$ values are given in Table 1. It is noticeable that the solubility line for BP crosses that of PP, which is probably a reflection of the much reduced solubility of PP in water in the presence of the other esters. Slopes and $K_{1:1}$ values, which may only be considered to be apparent under the circumstances, are much reduced for all the esters. Again, with the exception of MP the $K_{1:1}$ values increase as hydrophobicities of the esters increase, indicating a greater ability to compete for the hydrophobic core of the HPCD molecules. Since previous studies on competitive complexation with cyclodextrins have concentrated on changes in the affinity of a single molecule it is not possible to say whether the vastly reduced apparent $K_{1:1}$ values for all esters would be observed generally, or whether specific interactions between the esters are the cause of the decreases.

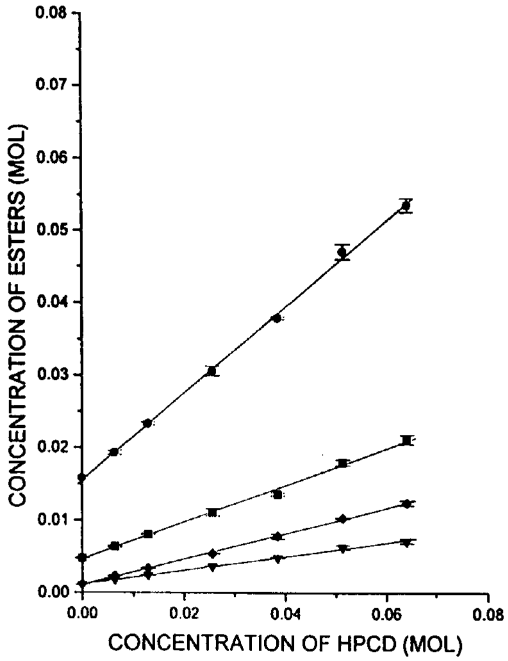

Figure 3. Solubilities of 4-hydroxybenzoic acid esters measured together in HPCD solutions (●, MP; ■, EP; ▼, PP; ◆, BP) ± 1 *SD*, *n* = 3.

If the latter is the case, then causes may include specific interactions in solution between the esters, more complex and less soluble associations between the esters and HPCD, or changes in the state of the solid esters which will not affect the solubility but may produce nonequilibrium metastable solutions.

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