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INTERACTIONS BETWEEN PRESERVATIVES AND 2-HYDROXYPROPYL-β-CYCLODEXTRIN

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ABSTRACT

The interactions between several commonly used preservatives, i.e. benzalkonium chloride, chlorhexidine gluconate, chlorobutanol, methylparaben and propylparaben, and 2-hydroxypropyl-β-cyclodextrin were investigated. The interactions were shown to be twofold. Firstly, the preservative molecules can displace the drug molecules from the cyclodextrin cavity, thus, reducing the solubilizing effects of the cyclodextrin. Secondly, the antimicrobial activity of the preservatives were reduced by formation of preservative-cyclodextrin inclusion complexes. The magnitude of the interactions were dependent on the degree of complexation.

INTRODUCTION

2-Hydroxypropyl-β-cyclodextrin (HPβCD) is a cyclic oligosaccharide which is formed by treating β-cyclodextrin with propylene oxide. It has a hydrophilic outer surface and a lipophilic cavity in the center. HPβCD is known to form noncovalent inclusion complexes with a wide variety of hydrophobic drug molecules by taking up a whole molecule, or some part of it, into the cavity[1]. Cyclodextrin encapsulation of a drug molecule will affect many of its physicochemical properties, such as its solubility and stability in aqueous solutions[1-4]. The usage of HPβCD and other cyclodextrins in drug formulations are now extensively investigated[2-4].

It is sometimes difficult to select antimicrobial preservatives for complex drug formulations and frequently specific preservative systems have to be tailored to certain formulations[5]. Since only the free preservative molecules are effective they must not be bound to other components of the formulation. For example, in a o/w emulsions the preservative molecules will partition into the oil phase with only small proportion remaining unbound in the aqueous phase where it is really needed[6].

In this present study, the interactions between several preservatives and HPβCD in aqueous solutions were investigated.

MATERIALS AND METHODS

Materials

2-hydroxypropyl-β-cyclodextrin was obtained from Pharmatec (Alachua, FL, U.S.A.), benzalkonium chloride from Sigma Chemical Company (St. Louis, MO, U.S.A.), chlorobutanol from May and Baker Ltd. (England), chlorhexidine gluconate was obtained as 20% (w/v) aqueous solution from Imperial Chemical Industries (England), methylparaben from Norsk

TABLE 1

Conditions of quantitative determination by HPLC

Drug	Mobile phase	Flow rate (ml/min)	Wave length (nm)	Retention time (min)
Chlorobutanol	Acetonitrile, water (4:6)	2.00	205	3.2
Methylparaben	Acetonitrile, water (3:7)	1.50	260	3.3
Propylparaben	Acetonitrile, water (4:6)	2.00	260	3.6
Hydrocortisone	Acetonitrile, tetrahydrofuran water (35:1:64)	1.50	240	2.4
Prednisolone	Acetonitrile, acetic acid, water (30:1:69)	1.50	242	4.0

Medisinaldepot (Norway), propylparaben from Norsk Medisinaldepot, hydrocortisone from Sigma, prednisolone from Sigma, and triamcinolone acetonide from Sigma. All other chemicals were commercially available products of special reagent grade.

Quantitative analysis

The quantitative determinations of chlorobutanol, methylparaben, propylparaben, hydrocortisone and prednisolone were performed on a high-performance liquid chromatographic (HPLC) equipment consisting of a Milton Roy ConstaMetric 3000 solvent delivery system, a Rheodyne 7125 injector, a Beckman Ultrasphere ODS 5 μm (4.6 x 150 mm) column and a Spectra-Physic SP8450 uv/vis detector. For other HPLC conditions see table 1. The quantitative determination of triamcinolone acetonide was done spectrophotometrically (Perkin-Elmer 550SE uv/vis spectrophotometer).

Solubility studies

Phase-solubility experiments were conducted by adding excess amounts of the drug or the preservative to be studied to aqueous solutions containing 0 to 40% (w/v) HPβCD. The suspensions formed were sonicated in an ultrasonic bath for 2 h then allowed to equilibrate at room temperature for 24 to 84 h. After filtration through 0.45 μm polyethylene microfilter the saturated solutions were diluted with 70% aqueous methanol solution and analyzed by HPLC or UV.

The preservative efficiency testing

The following micro-organisms were used in the test: *Escherichia coli* (ATCC 25922), *Staphylococcus aureus* (ATCC 25923), *Pseudomonas aeruginosa* (ATCC 27853), *Candida albicans* and *Aspergillus niger*. Each micro-organism was incubated in a liquid Muller-Hinton broth (Difco, Detroit, MI, U.S.A.) at 37°C for 18 to 24 h and the microbial count reduced to about 10^6 organisms per ml by dilution. Then, 0.10 ml of the inoculum was added to a solution consisting of 0.50 ml of a sterilised cyclodextrin solution and 0.40 ml of Muller-Hinton broth. The HPβCD and preservative concentrations shown are the final concentrations after dilution in the culture medium. Tubes containing the solutions were incubated at 37°C and examined visually after 24 h for microbial growth.

RESULTS AND DISCUSSION

Solubility

Figure 1 shows the effect of HPβCD on the solubility of chlorobutanol, methylparaben and propylparaben in water. Of these three preservatives propylparaben had the lowest solubility in pure water at room temperature, only 0.2 mg/ml, the solubility of methylparaben was determined to be 1.8 mg/ml and that of chlorobutanol 7.5 mg/ml. The solubilities of all three

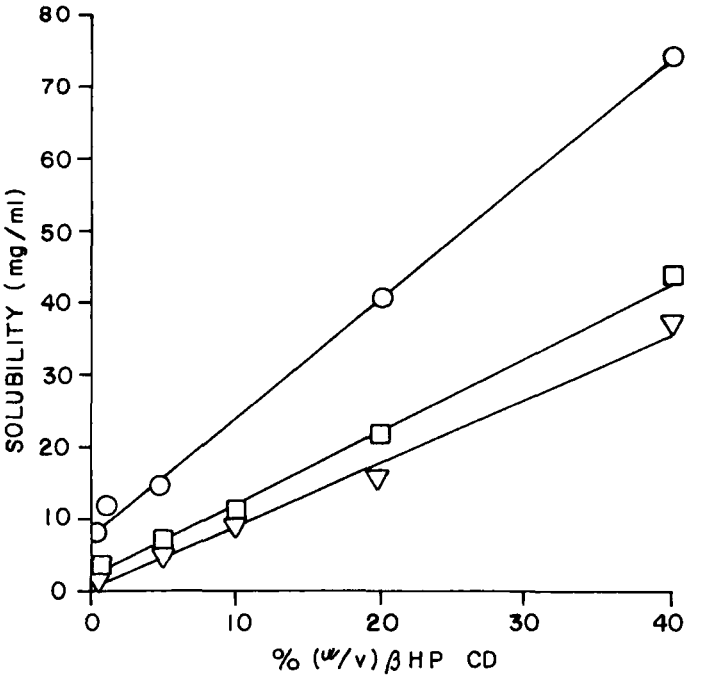

FIGURE 1

Phase-solubility diagrams for various preservatives in aqueous solutions at room temperature: ○, chlorobutanol; □, methylparaben; ▽, propylparaben.

preservatives were increased upon addition of HPβCD, but the enhancement was lowest for chlorobutanol, from 7.5 in water to 74.2 mg/ml in 40% (w/v) aqueous HPβCD solution or about 10-fold increase, and highest for propylparaben, from 0.2 to 36.9 mg/ml or almost 200-fold increase. The other two preservatives tested, i.e. benzalkonium chloride and chlorhexidine gluconate, have very good solubility in water.

HPβCD also increased the aqueous solubility of hydrocortisone, prednisolone and triamcinolone acetonide, but addition of the rather lipophilic water-insoluble chlorobutanol decreased the solubilizing effects of HPβCD (Figure 2). Methylparaben and propylparaben had similar effect as chlorobutanol, but benzalkonium chloride and chlorhexidine gluconate, which are water-soluble, had much less effect on the drug-HPβCD complexation.

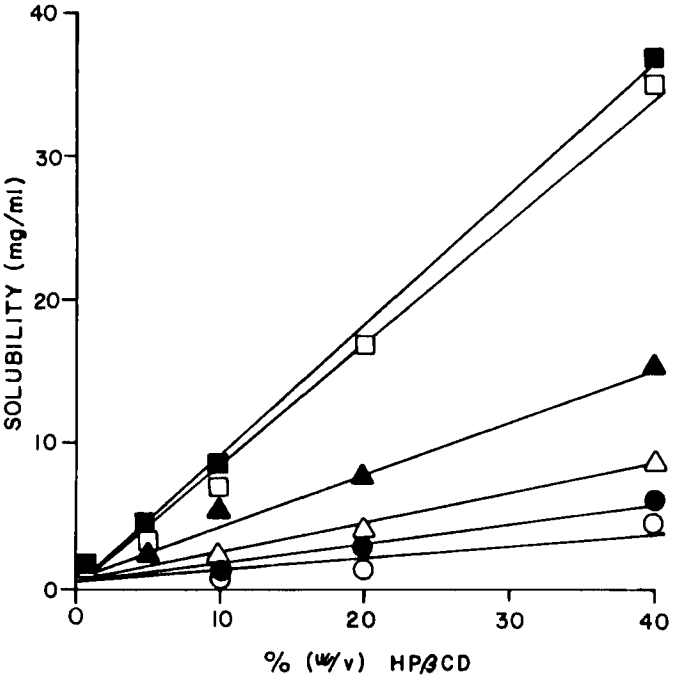

FIGURE 2

The effect of chlorobutanol on the slobilizing effects of HPβCD in aqueous solutions. The open signs when 1.0% chlorobutanol was present (0.5% in the case of hydrocortisone), closed signs when no preservative was present. ■ and □, hydrocortisone; ▲ and △, prednisolone; ● and ○, triamcinolone acetonide.

Antimicrobial effect

HPβCD itself in up to 20% (w/v) aqueous solutions does not possess any antimicrobial activity. In aqueous HPβCD solutions chlorobutanol had some antimicrobial effect but since only the free preservative molecules are effective its effectiveness was greatly reduced due to HPβCD complexation (Table 2). The lipophilic water-insoluble preservatives, methylparaben and propylparaben both of which have much higher degree of HPβCD complexation than chlorobutanol, had little or no effect in aqueous HPβCD solutions.

TABLE 2

Antimicrobial preservative effectiveness in aqueous HPβCD solutions.

Preservative	Estimated antimicrobial activity in aqueous HPβCD solutions.				
Micro-organism	0%[a)]	0.5%	5%	10%	20%
Chlorobutanol					
E. coli	0.25[b)]	0.25	>0.5	>0.5	>0.5
S. aureus	0.5	0.5	>0.5	>0.5	>0.5
Ps. aeruginosa	0.5	0.5	>0.5	>0.5	>0.5
A. niger	0.5	0.5	>0.5	>0.5	>0.5
C. albicans	0.25	0.25	0.5	0.5	0.5
Benzalkonium chloride					
E. coli	0.005	0.01	0.07	0.07	0.07
S. aureus	0.005	0.005	0.005	0.01	0.05
Ps. aeruginosa	0.005	-[c)]	0.07	>0.1	>0.1
A. niger	0.01	0.01	-	-	-
C. albicans	0.005	0.005	0.01	0.01	0.01

a) % (w/v) HPβCD in water.

b) mg preservative/ml test solution which completely prevented microbial growth.
>: The preservative concentration shown did not prevent microbial growth.

c) Not determined.

Benzalkonium chloride was effective on all the micro-organisms tested except for maybe *Ps. aeruginosa* (Table 2). Chlorhexidine gluconate, 0.05 mg/ml solution, prevented the growth of all the micro-organisms tested in all the HPβCD solutions, i.e. from 0 to 20% (w/v) aqueous HPβCD solutions. Thus, it appears that the effectiveness of the preservatives depends on their degree of complexation. Addition of hydrocortisone to the aqueous preservative HPβCD solutions did not enhance to any significant extent the antimicrobial effectiveness of the preservatives.

CONCLUSIONS

The interactions between preservatives and cyclodextrins in aqueous cyclodextrin drug formulation have twofold implications. Firstly, the preservative molecules can displace the drug molecules from the cyclodextrin cavity reducing the solubilizing and stabilizing effect of the cyclodextrins and, secondly, the complexation of the preservatives reduces, or even abolishes, their antimicrobial activity. These interactions depend on the ability of the preservative molecules to form complexes with the cyclodextrin molecules In general, the antimicrobial effectiveness of the lipophilic water-insoluble preservatives are more affected by the presence of cyclodextrins in the drug formulation than the antimicrobial effect of the hydrophilic water-soluble ones.

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