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Preparation of drug : hydroxypropylcyclodextrin complexes by a method using ethanol or aqueous ammonium hydroxide as co-solubilizers

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Summary

The dissolution of lipophilic drugs in aqueous solutions of hydroxypropylcyclodextrins can be accelerated by the addition of co-solubilizers such as ethanol or ammonia. These co-solubilizers can be removed later, together with water, by evaporation or freeze-drying, leaving drug : hydroxypropylcyclodextrin complexes. The co-solubilizer method was used successfully with steroid drugs (5-androstene-3β,17β-diol, 4-androstene-3,17-dione, dehydroepiandrosterone, dexamethasone, 5α-dihydrotestosterone, 6-methylprednisolone and testosterone), peptides (gramicidin S) and a macrocyclic antibiotic (amphotericin B). The complexes prepared in this manner were amorphous and of satisfactory stability and solubility.

Introduction

The complexes of lipophilic drugs with hydroxypropylcyclodextrins are amorphous, and often dissolve readily in water; thus, they are of potential importance in pharmaceutics (Pitha and Pitha, 1985; Pitha et al., 1986; Szejtli, 1988; Brewster et al., 1989; Loftsson et al., 1991; Uekama et al., 1992). We have developed a faster and more convenient method of preparation of complexes which is also suitable for unstable drugs. The improvement was achieved by the addition of a volatile cosolubilizer during the dissolution of components of the complexes which was removed later by evaporation. Some complexes prepared via this new method have already been used in biological study and found to be fully effective (Taylor et al., 1990). The preceding paper describes the physico-chemical aspects of the method (Pitha and Hoshino, 1992), which in this article is shown to be suitable for a number of drugs.

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Materials and Methods

Materials

Hydroxypropyl-β-cyclodextrin and hydroxypropyl-γ-cyclodextrin were purchased from Pharmatec, Inc., Alachua, FL, and had average degrees of substitution between 5 and 6; hydroxypropyl-α-cyclodextrin was prepared in our laboratory by K. Fukunaga and had an average degree of substitution of 4.6. 5-Androstene-3β,17β-diol, 4-androstene-3,17-dione, dehydroepiandrosterone, dexamethasone, 5α-dihydrotestosterone, 6-methylprednisolone, testosterone, gramicidin S, gramicidin D, and amphotericin B were purchased from Sigma Chemical Co., St. Louis, MO. Other chemicals and solvents were of analytical reagent grade.

Preparation of the inclusion complexes

Ethanol as a co-solubilizer. The drug and the hydroxypropylcyclodextrin (1:10 w/w) were dissolved in a minimal volume of either 95 or 75% ethanol, followed by the solution being filtered through a membrane filter (pore size: 0.22 μm). While our previous observations had suggested that a 1:10 ratio was suitable, the choice was arbitrary. The filtrate was evaporated at room temperature either under a vacuum or by blowing a nitrogen stream through it to dryness. The residue was dissolved in distilled water, then the solution was filtered through a membrane filter (pore size: 0.45 μm) and freeze-dried to leave a powdery complex in yields of over 90%.

Aqueous ammonium hydroxide as a co-solubilizer. Amphotericin B was dissolved in an ammonium hydroxide:water (1:2, about 12% ammonia) solution. Various ratios were used successfully including, for example 185 mg of the drug and 150 ml of solvent. This process, when accelerated by sonication, occurred within 5 min. Thereafter, the aqueous solution of hydroxypropyl-β-cyclodextrin (various ratios were used: 1:10, 1:50, 1:100, 1:200, and 1:400 w/w drug:hydroxypropylcyclodextrin) was added. The solutions thus formed were then rapidly filtered through a membrane filter (pore size: 0.45 μm) and the filtrate immediately freeze-dried. The yield of the process was over 80%. The same procedure was employed to prepare amphotericin B:hydroxypropyl-γ-cyclodextrin complexes.

The stability of amphotericin B in the hydroxypropyl-β-cyclodextrin complex (1:10 ratio) was monitored by thin layer chromatography (TLC). The complex was stored in the dark at room temperature. At times, up to 1 month, a sample of the complex was dissolved in dimethylformamide and compared with the original drug. Silica-gel 60 F254 Merck TLC and elution with *n*-propanol : water : ethyl acetate : ammonia (6:3:1:1) were used. The compounds were visualized by heating after the developed plate had briefly been dipped in Vaugh's reagent (ceric sulfate, 1 g; ammonium molybdate, 24 g; sulfuric acid, 10%, up to 500 ml). Evaluation of amphotericin B samples artificially decomposed by alkali or heating indicated that the above method is suited for the study of drug stability.

Powder X-ray diffraction

A Siemens 500 automated powder diffractometer was used. The instrument was set up with Cu radiation ($\lambda = 1.54$ Å) and a graphite monochromator. The measurements were performed by Oneida Research Services, Whitesboro, NY.

Differential scanning calorimetry

A Perkin Elmer model DSC-4 differential scanning calorimeter was employed. Samples were sealed in aluminum pans and measured at a scanning speed of 10°C/min. Measurements were performed at the School of Pharmacy in Santiago de Compostela (Coruna), Spain, by the kind permission of Dr J.L. Vila-Jato.

Solubility studies

The complex (3, 5, 10, 20 and 40 mg) was placed in a test tube and water (97, 95, 90, 80 and 60 μl, respectively) was added. Afterwards, the test tube was sonicated for 3 min and dissolution checked visually.

Measurements of water, ethanol and ammonia content

Water content was determined according to the Karl Fischer method and performed by Gail-

braith Laboratories Inc., Knoxville, TN. Ethanol and ammonia contents were evaluated using alcohol (ethanol) and ammonia diagnostic kits (Sigma Chemical Co., St. Louis, MO). Both kits are based on enzyme reactions and hydroxypropylcyclodextrins did not interfere with quantitation.

Results

In the preparation of complexes of drugs with hydroxypropylcyclodextrins, the addition of ethanol as a co-solubilizer makes the process faster and more convenient than when only water is used in the dissolution step. In contrast to cyclodextrins, the hydroxypropylcyclodextrins dissolve readily even in USP ethyl alcohol (190 proof) (Pitha et al., 1986). Steroids and peptides were used to illustrate the process. From the former class, androstene-3β,17β-diol, 4-androstene-3,17-dione, dehydroepiandrosterone, dexamethasone, 5α-dihydrotestosterone, 6-methylprednisolone, and testosterone, were tested. Steroids were dissolved in ethanol together with 10-times their weight of hydroxypropyl-β-cyclodextrin and the solutions were then evaporated leaving solid residues, sometimes of a glassy appearance. These residues could easily be screened visually for a crystalline phase, the presence of which would indicate a failure. No crystalline phases were observed with any of the above compounds. For additional confirmation of the absence of crystalline phases, X-ray powder diffraction and DSC data were obtained for the combination of testosterone with hydroxypropyl-β-cyclodextrin in a 1:10 weight ratio; the results (not shown) were similar to those presented in the previous paper where a 1:20 weight ratio was used (Pitha and Hoshino, 1992). The dissolution properties of the above solid residues were also in accord with the absence of any crystalline phase. All solid residues dissolved fully in water (final concentration of hydroxypropyl-β-cyclodextrin, about 10% w/w), within 5–15 min. The aqueous solutions thus obtained could be sterilized by ultrafiltration and freeze-dried to yield powders which were amorphous, as judged on the basis of the methods mentioned above. These powders could be tabletted by direct compression. The solubility of freeze-dried preparations was invariably high. All the above complexes of steroids dissolved smoothly in water up to 40% w/w concentration, the solutions being stable for at least 24 h.

The same general method was used successfully with an ethanol-soluble peptide, gramicidin S. The preparation was readily soluble in water (40% w/w) and the absence of a crystalline phase in the preparation was confirmed from the X-ray powder diffraction spectra (results not shown). Gramicidin : hydroxypropyl-α-cyclodextrin was prepared in the same manner. The method failed with gramicidin D. This peptide did not dissolve in ethanol 75%. When ethanol 95% was used, the drug was readily dissolved, however, after evaporation of the solution, the solid residue was not fully soluble in water.

TABLE 1

Amphotericin B: hydroxypropylcyclodextrin preparations made with ammonia as co-solubilizer: physical stability of solutions in water

Hydroxypropyl-cyclodextrin used	Drug: hydroxypropyl-cyclodextrin ratio (w/w)	Time (h) of appearance of detectable precipitate at concentration of the preparation (% w/w)		
		3%	10%	40%
Hydroxypropyl-β-cyclodextrin	1:10	48	1	not dissolved
	1:100	48	48	0.25
	1:200	48	48	0.25
	1:400	48	48	2
Hydroxypropyl-γ-cyclodextrin	1:10	48	0.25	not dissolved
	1:100	48	48	48
	1:200	48	48	48

The use of aqueous ammonia as a co-solubilizer is illustrated on the preparations consisting of amphotericin B and hydroxypropyl-β-cyclodextrin. This antifungal antibiotic has very low solubility and is prone to hydrolysis. Amphotericin B formed complexes with γ-cyclodextrin (Vikmon et al., 1985; Rajagopalan et al., 1986; Chow and Chen, 1989) and hydroxypropyl-γ-cyclodextrin (Anaissie et al., 1988), but attempts to prepare complexes with hydroxypropyl-β-cyclodextrin by the water-only method were not fully successful. Solutions of aqueous ammonia (tested over the range 7.4–18.5%) were used to dissolve the drug, then hydroxypropyl-β-cyclodextrin or hydroxypropyl-γ-cyclodextrin in aqueous solutions were added and the solutions immediately freeze-dried. The powders remaining after freeze-drying were amorphous when examined by X-ray diffraction. The stability of the drug in the complex was reassessed by TLC; 4 weeks of storage at room temperature led to no detectable decomposition.

The solubility of such amphotericin B preparations and the absence of formation of precipitates in these solutions were found to depend on the ratio of drug to hydroxypropylcyclodextrin. Both of these parameters increased when excess hydroxypropylcyclodextrin was used (Table 1). Comparison of the results on preparations of amphotericin B with hydroxypropyl derivatives of β- or γ-cyclodextrins prepared via the ammonia method showed that the latter yielded solutions which were more stable, i.e., precipitates formed only after prolonged standing (Table 1).

The absorption spectra of amphotericin B depend strongly on its association state. In the aggregated state, for example, as observed when deoxycholate is used for solubilization, it was found that among all the absorbing bands of amphotericin B, that which is located at 345 nm

TABLE 2

Residuals of solvents in preparations containing drug and hydroxypropylcyclodextrins

Drug : hydroxypropylcyclodextrin ratio (w/w)	Solvent used in preparation	Drying conditions [a]	Water (% w/w)	Ethanol (% w/w)	Ammonia (% w/w)
Hydroxypropyl-α-cyclodextrin	water	f.d.	7.46		
	ethanol (190 proof)	2 h	8.07	0.25, 0.21	
	ammonia (12%)	2 h	7.62		0.03
Hydroxypropyl-β-cyclodextrin	water	f.d.	7.23		
	ethanol (190 proof)	2 h	7.88	0.31	
	ammonia (12%)	2 h	7.97		0.06
Hydroxypropyl-γ-cyclodextrin	water	f.d.	9.69		
	ethanol (190 proof)	2 h	9.40	0.06	
	ammonia (12%)	2 h	9.50		0.12
Testosterone : hydroxypropyl-β-cyclodextrin					
1 : 20	ethanol (190 proof)	2 h		0.05	
1 : 10	water		5.7		
Testosterone : hydroxypropyl-γ-cyclodextrin					
1 : 20	ethanol (190 proof)	2 h		0.07	
Amphotericin B : hydroxypropyl-γ-cyclodextrin					
1 : 100	ammonia (12%)	f.d.	12.91		0.14

[a] 2 h drying performed in rotatory evaporator at vacuum of water pump and bath temperature 37 °C; f.d. solution was freeze dried; all samples were placed into lightly covered containers and exposed for 2 days to room atmosphere (22 °C, 50% relative humidity) before analyses were performed.

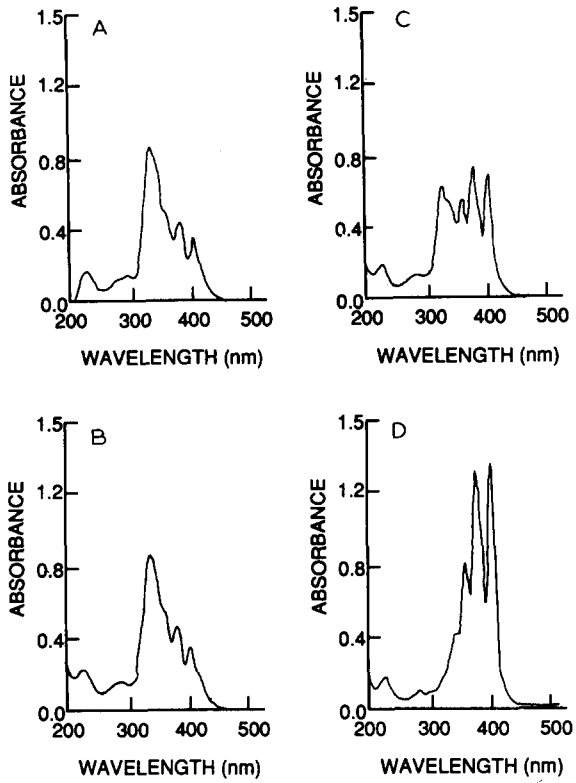

Fig. 1. Absorption spectra of amphotericin B:hydroxypropylcyclodextrin preparations in water (1 cm cuvette; the solutions had the same concentration of drug, approx. 10^{-5} M): (A) amphotericin B : hydroxypropyl-β-cyclodextrin (1 : 100 w/w); (B) amphotericin B : hydroxypropyl-β-cyclodextrin (1:200 w/w); (C) amphotericin B:hydroxypropyl-γ-cyclodextrin (1:100 w/w); (D) amphotericin B:hydroxypropyl-γ-cyclodextrin (1:200 w/w).

is the most prominent (Kajtar et al., 1989). When aggregates of amphotericin B were dissociated by extensive dilution, organic solvent or γ-cyclodextrin, the band at 415 nm became dominant (Kajtar et al., 1989). The spectra of amphotericin B : hydroxypropyl-β-cyclodextrin preparations were found to be of the type observed for self-aggregation of the drug (Fig. 1). The spectra of amphotericin B : hydroxypropyl-γ-cyclodextrin complexes at high excesses of solubilizer were clearly of the fully dissociated type (Fig. 1). When the relative amount of hydroxypropyl-γ-cyclodextrin was decreased (Fig. 1), the spectra were of the intermediate type.

These spectral data in conjunction with stability data in Table 1 suggest that the use of a co-solvent in this instance drove complex formation between amphotericin B and hydroxypropylcyclodextrins beyond the degree attainable in aqueous solutions. When preparations containing these complexes were dissolved in water, the aggregation and precipitation of amphotericin B eventually occurred.

Amorphous preparations invariably retain some of the solvents to which they have been exposed. Previously, water contained in hydroxypropylcyclodextrin samples equilibrated at specified relative humidities was measured and values between 2 and 30% were obtained (Pitha et al., 1986). In the present study, the water, ethanol and ammonia content of preparations made as above and exposed to room humidity (50% relative humidity, 22°C, 48 h) was measured. The results (Table 2) demonstrate that water was present in these preparations at higher concentrations than any of the co-solvents used in their making.

Discussion

Hydroxypropylcyclodextrins, unlike cyclodextrins, are soluble not only in water but also in some water-miscible organic solvents. When a guest compound is added to a solution of hydroxypropylcyclodextrin host in 190 proof ethanol, only dissolution takes place and formation of inclusion complexes does not occur; nevertheless, upon evaporation, an inclusion complex may be obtained (Pitha and Hoshino, 1992). The existence of inclusion complexes in such residues after evaporation of ethanol was proven by measurements of CD spectra for the methyl orange-hydroxypropyl-β-cyclodextrin combination and suggested on the basis of the dissolution properties and of the existence of a stabilized amorphous state for the combination testosterone : hydroxypropyl-β-cyclodextrin (Pitha et al., 1992). The present results show that six other steroids and a peptide antibiotic behave similarly to testosterone. Aqueous ethanol seems thus to be

widely applicable in the co-solvent method of preparation of inclusion complexes.

Another co-solvent, aqueous ammonia, was used for the amphotericin-B-hydroxypropylcyclodextrin combination when the ethanol method failed. Ammonia forms complexes with cyclodextrins that show good stability (Hirsch et al., 1987), but again it is more volatile and can be used at lower concentrations than ethanol.

The co-solubilizer method can obviously be advantageous in the preparation of a number of drug : hydroxypropylcyclodextrin complexes. Nevertheless, when using this method, it is essential to examine the stability of the aqueous solutions of such complexes. Using the co-solubilizer method, the complex is formed via a reversible reaction, and thus is stable, under the conditions during the final stages of evaporation of co-solvent. Such conditions are quite different from those during and after the dissolution of pharmaceutical preparations in aqueous solutions and precipitation of the drug may then occur. Nevertheless, with the exception of amphotericin B, no such complication was observed with any of the eight other drugs investigated. Even when such a complication does occur and solutions of complex are not stable under the conditions envisaged for their pharmaceutical use, some of the beneficial effects of hydroxypropylcyclodextrins may still be salvaged. Hydroxypropylcyclodextrins in solutions slow down the crystallization of drugs. Previously, results on the solubilization of pancratistatin suggested that crystallization may be delayed by an increase in the concentration of hydroxypropylcyclodextrins or a decrease in the concentration of the drug (Torres et al., 1991). Balancing these factors thus may make it possible, at least in some cases, to prepare a suitable formulation even then.

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