Pharmaceutical Research, Vol. 7, No. 6, 1990

Report

Pharmaceutical Usefulness of Hydroxypropylcyclodextrins: "E Pluribus Unum" Is an Essential Feature

C. Trinadha Rao,[1] Henry M. Fales,[2] and Josef Pitha[1,3]

Received October 24, 1989; accepted January 4, 1990

A series of hydroxypropyl-β-cyclodextrins was prepared by a method that leads to a preferential substitution on the secondary hydroxyls, mainly O-2, of β-cyclodextrin with (*S*)-2-hydroxypropyl groups. The series consisted of mixtures of compounds with average degrees of substitution of 8, 3, and 1.6 and of a specially isolated monosubstituted compound; thus, the number of components progressively decreased in this series. The crystallinity in the series increased progressively, the first member being fully amorphous and the last one fully crystalline. All members of the series formed clear aqueous solutions at concentrations of >50.0, 2.0, 0.6, and 0.3%, respectively. Therefore, pharmaceutically useful hydroxypropylcyclodextrin preparations are those containing a large number of chemically individual compounds—a feature resulting in an amorphous state and high water solubility.

KEY WORDS: hydroxypropylcyclodextrin; drug solubilizers; amorphous character; cyclodextrin derivative.

INTRODUCTION

Poor solubility of some drugs in water may be overcome with the use of cyclodextrins which form guest-host complexes with suitable lipophiles (1–3). When these complexes of improved water solubility are administered per os or parenterally, the lipophilic guest is exchanged for an endogenous lipid and thus, the free drug may enter the tissue. Cyclodextrins themselves and their complexes with drugs are crystalline; thus, there are limits to their solubility (1–4). However, cyclodextrins can be transformed by nonspecific substitution into multicomponent, amorphous mixtures (see Fig. 1) which are very soluble and lack toxicity (5–11). Hydroxypropyl-β-cyclodextrin (12), represents one of the pharmaceutically most useful derivatives (6,7). Studies on the optimal average degree of substitution in hydroxypropylcyclodextrins for drug solubilization generally point to lower degrees of substitution, typically with an average of two to five substituents per β-cyclodextrin molecule (11,13,14). In this work the number of components in mixtures of hydroxypropyl-β-cyclodextrins was compared to their solubility and crystallinity. The results show that unless the preparation (i.e., "unum" in the title) is a union of many components ("e pluribus"), its pharmaceutical usefulness may be limited.

[1] NIA/GRC, National Institutes of Health, 4940 Eastern Avenue, Baltimore, Maryland 21224.

[2] NHLBI, National Institutes of Health, 9000 Rockville Pike, Bethesda, Maryland 20892.

[3] To whom correspondence should be addressed at National Institute on Aging, Gerontology Research Center, 4940 Eastern Avenue, Baltimore, Maryland 21224.

MATERIALS AND METHODS

β-Cyclodextrin was provided by U.R. Industries, Inc., New Jersey, and *S*-(−)-propylene oxide was purchased from Aldrich Chemical Co., Inc., Wisconsin.

Plasma desorption spectra (positive ion, Cf-252 used for ionization) were measured on a spectrometer constructed by Dr. R. D. Macfarlane for NHLBI and subsequently modified by one of the authors (H. M. Fales) and L. K. Pannell. The average degrees of substitution in (*S*)-2-hydroxypropyl-β-cyclodextrin are given per β-cyclodextrin molecule and were calculated from the mass spectra according to the formula, average degree of substitution = Σ(peak height × degree of substitution)/Σ(peak height). The average degrees of substitution were also deduced from ^{1}H-NMR spectra (recorded on a Varian XL-200 instrument) from the ratio of the integrals of the methyl protons to that of anomeric protons. Thin-layer chromatography was performed on a precoated silica gel plate ($60F_{254}$, Merck Co.) using 1-propanol–water–ethyl acetate–ammonium hydroxide (6:3:1:1) as solvent for developing. The components were revealed by heating the plate stained with Vaughn's reagent (a solution of ceric sulfate, 1 g, and ammonium molybdate, 24 g, in 10% sulfuric acid, 500 ml).

The powder X-ray diffraction patterns were recorded by Oneida Research Services, Inc., New York, on a Siemens D500 automated diffractometer with a graphite monochromator. Preparations 2–4 were dried out of water solution at room temperature and ambient room humidity. Preparation 1 was dried out of water under reduced pressure and then left for several days to equilibrate at ambient room humidity.

Preparation 1. β-Cyclodextrin (2.3 g, corresponding to 2.0 g anhydrous compound, 1.76 mmol) was dissolved in

0724-8741/90/0600-0612$06.00/0

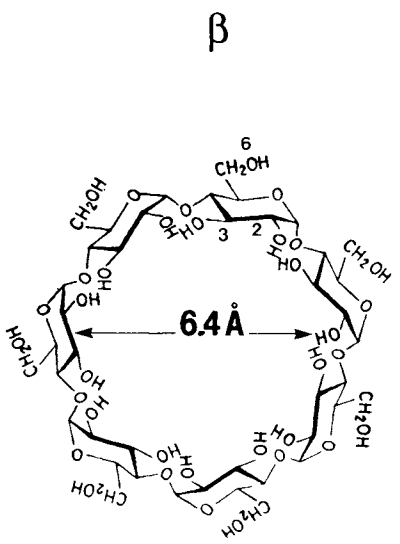

Fig. 1. Structure of β-cyclodextrin. The hydroxy groups are located on the edge of the toroid; thus, the substitution on them leaves the cavity more or less undisturbed. In the reaction with racemic propylene oxide any OH group can be converted into $-O-CH_2-CHOH-CH_3$ or $-O-CH(CH_3)-CH_2OH$ groups, the latter conversion occurring less frequently; again both of these groups may have (*R*) or (*S*) chirality. The substitutions may occur on 6–O, 3–O, or 2–O, on the same or on different glucose units, or on a previously introduced group; thus, the number of compounds possibly present in the reaction product is very high. The preparative method used here strongly increased the relative reactivity of 2–O and introduced only substituents of uniform chirality; thus, the number of compounds present in mixtures 1–3 was strongly reduced compared to those routinely made using non-selective conditions.

aqueous 1.5% (w/w) NaOH (9 ml) by heating at 60°C for 10 min. The clear solution was cooled in an ice bath and *S*-(−)-propylene oxide (2 g, 34.5 mmol) was introduced during 15 min. The reaction mixture was stirred in the ice bath for 5 hr, then at room temperature for 36 hr, neutralized carefully while cooling in ice bath to pH 7.0–7.5 with 1 *M* HCl, and dialyzed for 5 hr at room temperature against distilled water. The retained solution was evaporated *in vacuo* at a temperature less than 50°C to give a thick syrup which, upon coevaporation with ethanol (2 × 25 ml), again *in vacuo*, gave preparation 1 as a white foam (2.8 g).

Preparations 2 and 3. Preparation 2 was made in a manner similar to that used for preparation 1 using β-cyclodextrin (13.3 g, corresponding to 11.52 g anhydrous compound) in 1.5% (w/w) NaOH (54 ml) and *S*-(−)-propylene oxide (8.3 g, 143.1 mmol). The reaction mixture in this case was stirred at ice bath temperature for 12 hr and then at room temperature for 4 hr. Evaporation of the suspension obtained after neutralization and dialysis gave preparation 2 (14.23 g).

Preparation 2 (7 g) was stirred in distilled water (50 ml) for 5 hr and filtered. The residue was dried to obtain preparation 3 (1.20 g).

Preparation 4. The individual compound 2-*O*-[(*S*)-2′-hydroxypropyl]-β-cyclodextrin was prepared by a procedure in which precipitation and crystallization from water were used for separation of the desired compound from the excess of β-cyclodextrin present in the reaction mixture (15).

RESULTS

Since one of the purposes of this work was to find whether any crystallinity may ever occur in mixtures of hydroxypropylcyclodextrins, preparative conditions for the latter were specifically chosen to favor that process. A very low concentration of alkali hydroxide in reaction mixtures of propylene oxide-cyclodextrin and the use of optically pure propylene oxide limit the number of components in mixtures (15); therefore alkylations with (*S*)-propylene oxide catalyzed by 1.5% sodium hydroxide were used. Furthermore, β-cyclodextrin (Fig. 1) was chosen since that compound and its derivatives invariably have a better tendency to crystallize than the corresponding α- or γ-cyclodextrins. Altogether four preparations were studied. Preparation 1 was a mixture with high substitution and was obtained without any fractionation. Preparation 2 was obtained in a similar way except less (*S*)-propylene oxide was used; preparation 3 was a less soluble fraction of preparation 2. Preparation 4 is 2-*O*-[(*S*)-2′-hydroxypropyl]-β-cyclodextrin, a chemically distinct compound. Average degrees of substitution were measured by NMR spectroscopy; results are given in Table I. Furthermore, detailed distribution was determined by ^{252}Cf plasma desorption mass spectrometry; results are collected in Fig. 2. (left panels). Also, these data were used to calculate the average degrees of substitution given in Table I. The differences between values found by these two methods (Table I) are considerable but within the limits expected on the basis of fundamentally different approaches and different sensitivities to impurities. The composition of mixtures was also evaluated by thin-layer chromatography (Fig. 3). The system used enabled reliable detection of β-cyclodextrin in mixtures and separated well β-cyclodextrins substituted up to six times with 2-hydroxypropyl groups.

Table I lists the maximal concentrations at which preparations 1–4 dissolved fully in water. For the chemically unique preparation 4; this concentration (coinciding in this particular case with its water solubility) is diminutive (0.3%), whereas for mixture 1 the value is above 50%.

Powder X-ray diffractions of preparations 1–4 are shown in the right-hand panels in Fig. 2; the corresponding left and right panels present data (mass spectrum and X-ray diffraction) on the same preparation. The differences again are quite dramatic. Whereas preparation 1 clearly has an amorphous pattern, the crystallinity, as indicated by sharp

Table I. Average Degree of Substitution and Water Solubility of Preparations 1–4

Preparation No.	Average degree of substitution		Maximal concentration (%, w/w) at which full dissolution in water occurred[a]
	^{252}Cf pd mass spectrometry	NMR	
1	8.5	7.6	>50.00
2	3.4	2.7	2.1
3	1.7	1.5	0.65
4	1.0	1.0	0.31

[a] Measured at 20–22°C. For preparation 4, which is a chemical individuum, this parameter corresponds to solubility.

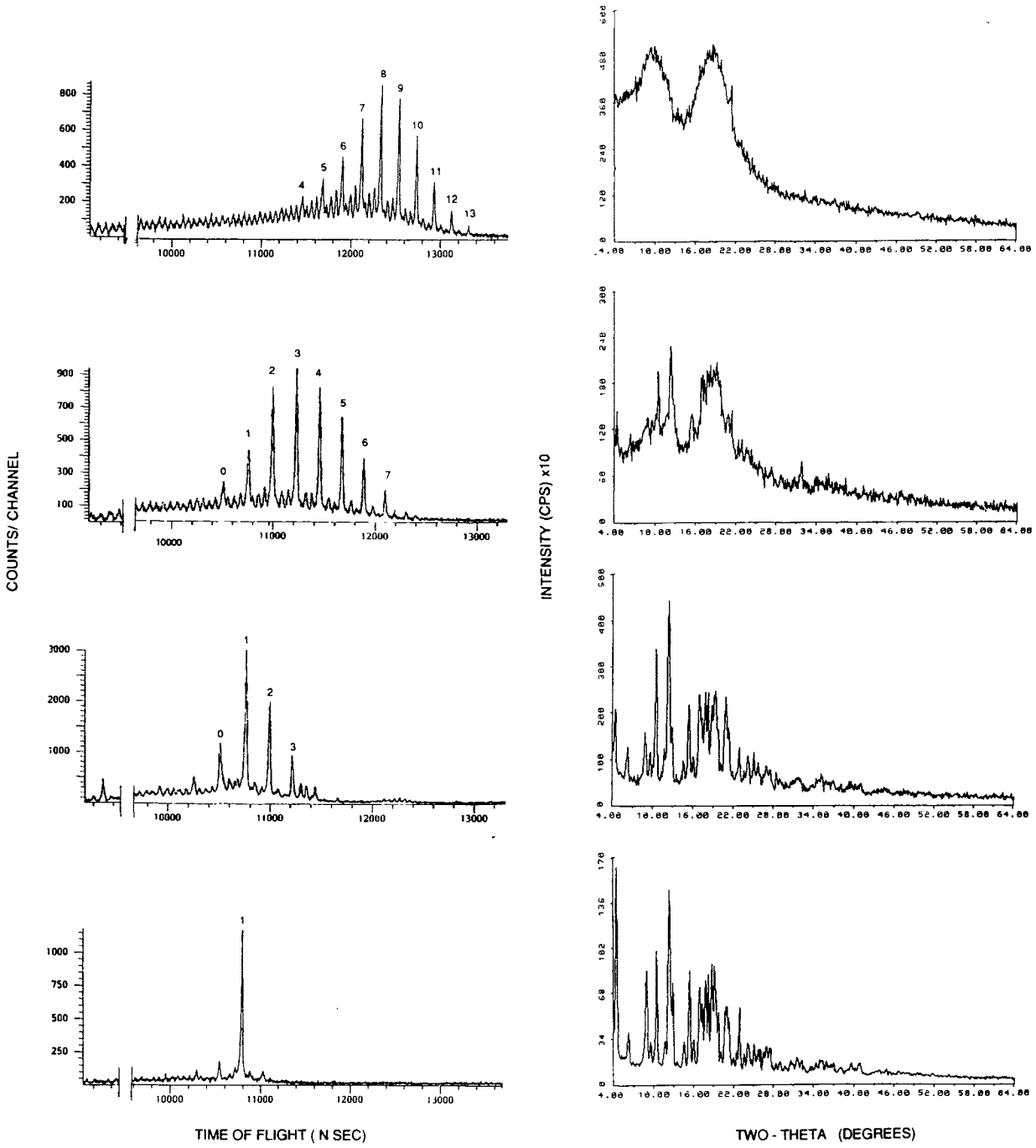

Fig. 2. Mass spectrum (^{252}Cf plasma desorption) and X-ray powder diffraction patterns: top row, preparation 1; second row, preparation 2; third row, preparation 3; fourth row, preparation 4. The number above the peak in mass spectra denotes the number of substituents on the cyclodextrin ring.

signals, increases for preparations 2 and 3 and the individual compound 4 has a pattern of crystallinity comparable to that of β-cyclodextrin (pattern not shown).

DISCUSSION

The purpose of improving cyclodextrin solubility without affecting its complexation potency was realized by chemical conversion into mixtures of hydroxypropylcyclodextrins (5–7). The hypothesis that success of the design was based on a great number of components in these mixtures has presently been documented. Conversion of β-cyclodextrin to an individual hydroxypropyl-β-cyclodextrin (preparation 4) decreased its solubility from 1.8 to 0.3%, respectively, and probably also its usefulness in pharmaceutics. Increasing the degree of substitution to 1.7 and 3.4 enhanced solubility to 1.5 and 2.1%, respectively (Table I); however, when the average degree of substitution was in-

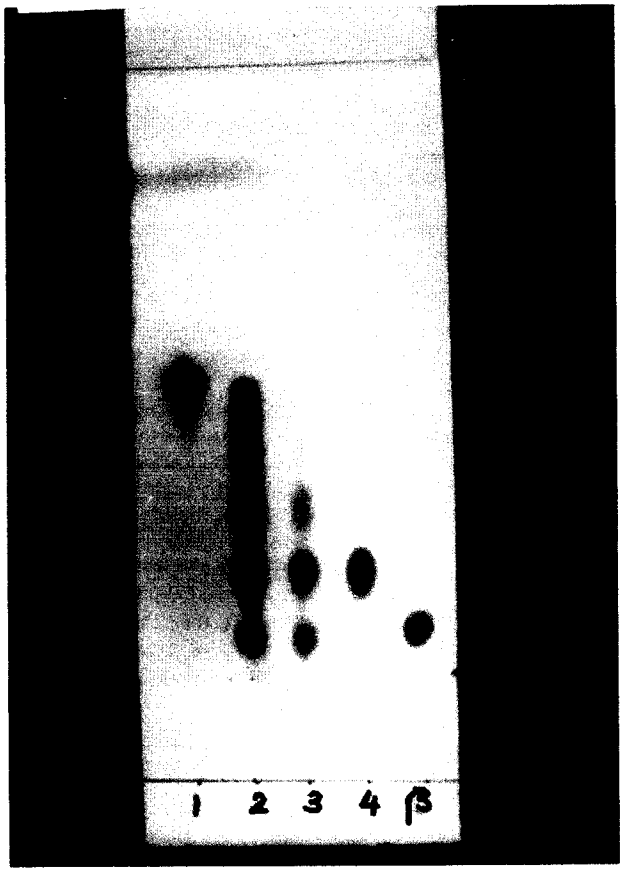

Fig. 3. Thin-layer chromatography of preparations 1–4 (denoted by respective numbers) and that of β-cyclodextrin (denoted by β).

creased to 8.5, the solubility improved dramatically to more than 50%. Since similar reaction conditions were used to produce all preparations, their substitution patterns had to be similar (15). On the other hand, an increase in the average degree of substitution had to be reflected in an increase in the number of components in the mixture since the number of combinations of substituent distribution is a function of the number of substituents present (Fig. 1).

The regiospecificity of the reaction of propylene oxide under alkaline conditions routinely used (5–17% sodium hydroxide) with β-cyclodextrin is low but can be improved when the concentration of sodium hydroxide is either decreased or increased, thereby favoring substitution on O–2 and O–6, respectively (15). Since the 2-hydroxypropyl substituent contains a chiral carbon, and cyclodextrins are also chiral, every specific substitution by racemic propylene oxide leads to the formation of a pair of diastereomers, i.e., compounds differing in their chemical and physical properties. The preparations evaluated here differ from those routinely used since very low sodium hydroxide concentration (1.5%) and (*S*)-propylene oxide were used, conditions which limit the number of components of mixtures.

ACKNOWLEDGMENTS

The authors express their appreciation to Mr. E. Sokolski for recording the spectra and to Mrs. D. Lamartin for typing and improving the manuscript.

REFERENCES

1. J. Pitha, L. Szente, and J. Szejtli. In S. D. Bruck (ed.), *Controlled Drug Delivery, Vol. I,* CRC Press, Boca Raton, FL, 1983, pp. 125–148.
2. K. Uekama and M. Otagiri. *Crit. Rev. Ther. Drug Carrier Syst.* 3:1–40 (1987).
3. D. Duchene, F. Glomot, and C. Vaution. In D. Duchene (ed.), *Cyclodextrins and Their Industrial Uses,* Editions de Sante, Paris, 1987, pp. 211–259.
4. M. J. Jozwiakowski and K. A. Connors. *Carbohydr. Res.* 143:51–59 (1985).
5. J. Pitha for U.S. Government, U.S. Patent 4,371,673 (1983).
6. J. Pitha for U.S. Government, U.S. Patent 4,596,795 (1986).
7. J. Pitha and J. Pitha. *J. Pharm. Sci.* 74:987–990 (1985).
8. J. Pitha, S. M. Harman, and M. E. Michel. *J. Pharm. Sci.* 75:165–167 (1986).
9. B. W. Müller and U. Brauns. *Int. J. Pharm.* 26:77–88 (1985).
10. B. W. Müller and U. Brauns. *Pharm. Res.* 2:309–310 (1985).
11. B. W. Müller and U. Brauns. *J. Pharm. Sci.* 75:571–572 (1986).
12. R. E. Gramera and R. J. Caimi. U.S. Patent 3,459,731 (1969).
13. J. Pitha, J. Milecki, H. Fales, L. Pannell, and K. Uekama. *Int. J. Pharm.* 29:73–82 (1986).
14. L. Szente and J. Szejtli. In J. Szejtli and O. Huber (eds.), *Proceedings of the 4th International Symposium on Cyclodextrins,* D. Reidel, Dordrecht, Holland (in press).
15. J. Pitha, C. T. Rao, B. Lindberg, and P. Seffers. *Carbohydr. Res.* (in press).