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Water-soluble β-cyclodextrins in paediatric oral solutions of spironolactone: solubilization and stability of spironolactone in solutions of β-cyclodextrin derivatives

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Abstract

Water-soluble β-cyclodextrins, hydroxypropyl-β-cyclodextrin (HPβCD), dimethyl-β-cyclodextrin (DMβCD) and sulphobutyl ether β-cyclodextrin (SBE7), were evaluated as potential solubilizers of spironolactone (SP) in paediatric enteral formulations. ^{1}H-NMR was used to verify the formation of inclusion complexes and to detect possible degradation of spironolactone through deacetylation. The effect of temperature on spironolactone stability was studied in solutions of HPβCD and SBE7 to estimate shelf-lives of possible liquid preparations. HPβCD, DMβCD and SBE7 formed true inclusion complexes with spironolactone with a stoichiometry of 1:2 (SP:βCD). No degradation of spironolactone could be detected in the presence of DMβCD, whereas spironolactone degraded through deacetylation according to pseudo-first order kinetics in the presence of HPβCD and SBE7. Spironolactone degradation was slower in solutions of SBE7 than in HPβCD, with the slowest degradation at 6°C in SBE7 solution. Estimated shelf-lives ($t_{90\%}$) for solutions containing 3 mg/ml of spironolactone were, even at 6°C, below 2 h in the presence of HPβCD. The $t_{90\%}$-values in the presence of SBE7 were 4.1, 8.3 and 24.5 h at 22, 13 and 6°C, respectively. According to these results, SBE7 could be considered for the solubilization of spironolactone in paediatric enteral solutions, if the solution were to be prepared and stored at 6°C or below. © 1997 Elsevier Science B.V.

Keywords: Spironolactone; Paediatric; Water-soluble-β-cyclodextrins; Inclusion complex; Solubilization; Stability; Temperature dependence

1. Introduction

Spironolactone (SP) is a potassium sparing diuretic used to reduce lung congestion and thereby

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to improve lung function in low birthweight infants (Atkinson et al., 1988). At the Children's Hospital, Helsinki University Central Hospital, the present dosage regime for neonates is 3.3 mg/kg per day of spironolactone administered in one dose. Lacking liquid preparations, powder papers prepared from commercial tablets (Aldactone) are used, often causing obstruction of nasogastric tubes and loss of a variable part of the dose. A liquid preparation should preferably contain 3–5 mg/ml of spironolactone to minimize the extra water-load to the kidneys. Due to the poor water-solubility of spironolactone, 30 μg/ml, liquid preparations described in the literature contain either an abundance of organic co-solvents (Pramar et al., 1992) or heavy syrups as suspending agents (Committee on Extemporaneous Formulations, 1987; Mathur and Wickman, 1989). These organic excipients, as well as the high-osmolality syrups, are potentially toxic to paediatric patients (Leff and Roberts, 1987). The use of water-soluble derivatives of β-cyclodextrin offers an alternative for the solubilization of spironolactone.

Cyclodextrins are cyclic oligosaccharides that improve the solubility of lipophilic drugs by molecular encapsulation, i.e. by inclusion of the drug into the hydrophobic cavity of the cyclodextrin (Szejtli, 1988). β-Cyclodextrin (βCD) has been used to increase the solubility of spironolactone and thereby its oral bioavailability from solid dosage forms administered to dogs, rats and humans (Seo et al., 1983; Debruères et al., 1985; Vila-Jato et al., 1986; Yusuff et al., 1991). The low solubility of βCD as well as the drug-βCD complexes hampers the use of βCD for the formulation of solutions. Solubility studies with spironolactone and βCD show that concentrations above 1 mg/ml of spironolactone in solution cannot be produced (Seo et al., 1983; Debruères et al., 1985; Yusuff and York, 1991). Water-soluble derivatives of βCD have been developed to overcome the low solubility and/or decrease the parenteral toxicity of the parent cyclodextrin without loss of complexing ability. According to the literature, a spironolactone concentration of 42 mg/ml can be achieved in a 40% solution of hydroxypropyl-β-cyclodextrin (HPβCD) (Pitha et al., 1986), whereas a solubility of 3.6 mg/ml has been reported in a 1.5% solution of dimethyl-β-cyclodextrin (DMβCD) (Uekama, 1985). Evidence on the deacetylation of spironolactone upon complexation with βCD (Szejtli, 1988; Wouessidjewe et al., 1989) further supports the use of modified βCDs.

Effects of HPβCD, DMβCD and sulphobutyl ether β-cyclodextrin (SBE7) on intestinal epithelial integrity have previously been investigated, to establish their safe use as solubilizers of spironolactone in paediatric enteral solutions (Tötterman et al., 1997). According to this study, HPβCD and SBE7 appeared to be safe additives with respect to their local effects in the intestines. In the present study, the effects of HPβCD, DMβCD and SBE7 on spironolactone solubility and stability were evaluated. ^{1}H-NMR was used to verify the formation of inclusion complexes and to initially detect possible degradation of spironolactone through deacetylation. The effect of temperature on spironolactone stability was studied in solutions of HPβCD and SBE7 to estimate shelf-lives of potential liquid preparations intended for paediatric patients.

2. Materials and methods

2.1. Materials

Spironolactone (SP) (Fig. 1) was kindly sup-

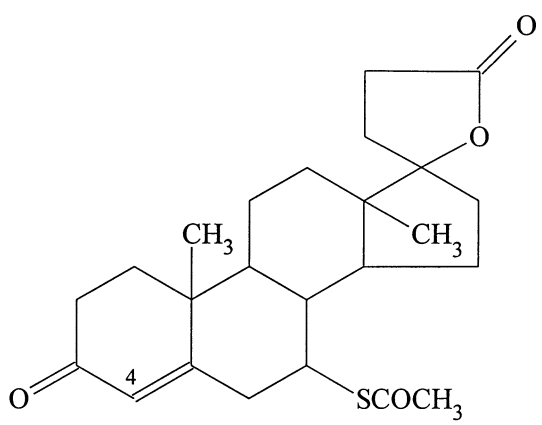

Fig. 1. Molecular structure of spironolactone.

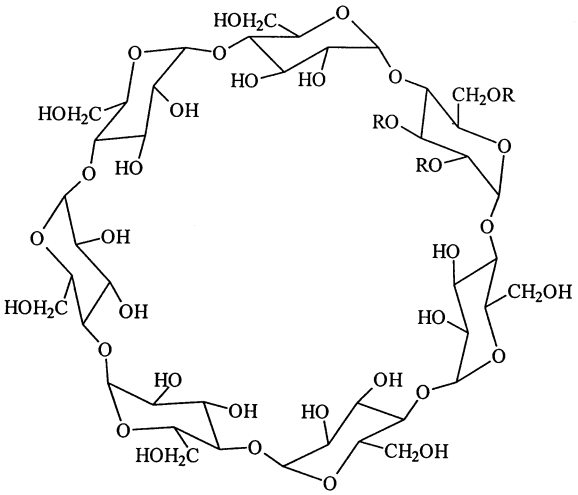

Fig. 2. Molecular structures of modified β-cyclodextrins; 2-hydroxypropyl-β-cyclodextrin, variably substituted at 2-, 3- or 6-positions with R=[$-CH_2CH(CH_3)OH$]; dimethyl-β-cyclodextrin, specifically substituted at 2- and 6-positions with R=[$-CH_3$]; sulphobutyl ether β-cyclodextrin, variably substituted at 2-, 3- or 6-positions with R = [$-CH_2CH_2CH_2CH_2SO_3\ Na^+$].

plied by Orion Pharmaceuticals, Turku, Finland. Degradation products of spironolactone, 7α-thiospirolactone (TSP) and canrenone (CAN), were obtained from Searle (Skokie, IL) and Searle (Morpeth, UK), respectively. Testosterone was purchased from Fluka (Buchs, Switzerland) and HPLC-grade tetrahydrofurane (THF) and acetonitrile from Rathburn. 2-Hydroxypropyl-β-cyclodextrin (molar substitution 0.44; degree of substitution (DS) 3.1) (Encapsin™ HPB) (HPβCD) was kindly donated by Janssen, Biotech, Beerse, Belgium and sodium sulphobutyl ether β-cyclodextrin (DS 7) (Captisol™) (SBE7) by Cydex (Kansas City, KS) (Fig. 2). Heptakis 2,6-di-*O*-methyl-β-cyclodextrin (DS 14) (DMβCD) was purchased from Sigma (St. Louis, MO).

2.2. ^{1}H-NMR spectroscopy

The ^{1}H-NMR spectra were obtained at 27°C on a Varian Unity 500 NMR-spectrometer using deuterium oxide (D_2O) as solvent and referenced to water signal (4.70 ppm). DMβCD, HPβCD and SBE7 were prepared as 1 mM solutions, where spironolactone was added and allowed to solubilize for one day prior to measurements.

2.3. Quantitation by HPLC

The HPLC equipment consisted of a Waters 510 Solvents Delivery System, an In-Line Degasser, a 717 Plus Autosampler, a Nova-Pak Phenyl Steel Cartridge Column (4 μm, 3.9 × 150 mm) with an integrated Sentry Guard Column (4 μm, 3.9 × 20 mm), and a 490 Programmable Multiple Wavelength UV/Visible Detector. Data and system management was handled by a Millennium 2010 Chromatography Manager.

Quantitative analysis was performed according to a separately published method (Kaukonen et al., 1997), where the detector was operated at 250 nm (SP and TSP) and 280 nm (CAN) using a mobile phase consisting of THF and water (21.5:78.5). The temperature of the autosampler was set at 15°C. At a flow rate of 1.0 ml/min and a sample volume of 30 μl the retention times of CAN, TSP and SP were 31.0, 35.3 and 40.0 min, respectively. Linear calibration plots were obtained for SP and TSP using testosterone (2 μg/ml) as internal standard in the concentration ranges 5.0–30.0 and 0.5–4.0 μg/ml, respectively. Calibration plots for CAN were generated without the use of internal standard in the range 0.5–4.0 μg/ml. Coefficients of variation ($n = 6$) for SP, TSP and CAN at highest and lowest concentrations were in the range 0.4–2.3%.

2.4. Solubility study

Solubility measurements were carried out according to a method of Higuchi and Connors (1965). Excess amounts of SP were added to aqueous solutions (3.0 ml) containing 0–30 mM of DMβCD, HPβCD or SBE7 and the suspensions were shaken for 24 h at ambient temperature (19–21°C). Samples of 100 μl ($n = 4$) were taken after filtering (Millex SJGV, 0.22 mm, ø 13 mm, Millipore) and analyzed by HPLC. Apparent stability constants, $K_{1:1}$ (M^{-1}) were calculated according to the following equation (Higuchi and Connors, 1965):

$$K_{1:1} = \text{slope}/C_0 \times (1 - \text{slope}) \quad (1)$$

using the slope of the phase-solubility diagram and the determined SP solubility in water for C_0.

2.5. Stability study

Solutions containing 3 mg/ml of spironolactone (SP) were prepared aseptically in autoclaved HPβCD and SBE7 solutions. SBE7 solutions were prepared at 29 mM and HPβCD solutions at 18, 22 and 29 mM to give molar ratios of βCD:SP of 2.5, 3 and 4. The autoclaved βCD solutions were stored at appropriate temperature prior to adding SP. At all temperatures solubilization of SP required vigorous shaking and the first samples (t_0) (100 ml; $n = 4$) were taken after 20–40 min. The solutions were then kept in an incubator (KBK 4330, Emmendingen, Germany) at room (22 ± 0.5°C), cool (13 ± 0.5°C) or cold (6 ± 0.5°C) temperature. At 22 and 13°C further samples were taken at 3, 6 and 9 h after t_0. At 6°C sampling was done up to 24 and 48 h after t_0 for HPβCD and SBE7 solutions, respectively. Furthermore, SP concentration at 72 h after preparation was determined separately at 6°C in SBE7 solution.

2.6. Preparation of samples for quantitation

Samples from the stability and solubility studies were diluted to appropriate concentrations by first adding half of the final volume of pure acetonitrile. HPβCD and SBE7, having low solubilities in acetonitrile, precipitated at this stage. This was taken as the endpoint to the degradation process as SP, TSP and CAN are highly soluble and chemically stable in acetonitrile (Varin et al., 1992). HPLC-grade water was added to make up the desired volume before quantitation.

2.7. Calculation of kinetics of spironolactone degradation

The fraction (%) of remaining spironolactone was calculated against the theoretical concentration of 3 mg/ml. Observed pseudo-first-order degradation rate constants (k_{obs}) (h^{-1}) were calculated from semilogarithmic plots of fraction remaining spironolactone (%) vs. time (h). Half-lives ($t_{50\%}$) and estimated shelf-lives ($t_{90\%}$) were also calculated from these plots.

3. Results and discussion

3.1. ^{1}H-NMR spectroscopy

DMβCD, HPβCD and SBE7 formed true inclusion complexes with spironolactone in solution as evidenced by observed NOE enhancement effects between spironolactone and cyclodextrin protons. Integration of anomeric protons of the cyclodextrins and H-4 protons of spironolactone suggested a stoichiometry of 1:2 for each of the SP:βCD complexes. No degradation of spironolactone was detected in the presence of DMβCD as no changes in the signal intensities of the hydrogen atoms of the thioacetate-group in spironolactone were detected even after several weeks of storage. In HPβCD and SBE7 solutions the decrease of the thioacetate signal was observed together with the appearance of a signal for the H-4 atom of deacetylated spironolactone next to the diminished H-4 signal of undegraded spironolactone (Fig. 3), indicating extensive degradation of spironolactone. Deacetylated spironolactone, 7α-thiospirolactone, also formed inclusion complexes with HPβCD and SBE7. New signals in the spectra indicated that 7α-thiospirolactone was further degraded by oxidation, which is in accordance with a previous NMR-study on spironolactone and βCD complexes (Wouessidjewe et al., 1989).

Spironolactone was also studied in glucose solution to exclude the possibility of a general glucose catalysis in spironolactone deacetylation. No degradation of spironolactone was observed in the presence of glucose, supporting the concept of a specific catalytic action of β-cyclodextrin with spironolactone (Szejtli, 1988; Wouessidjewe et al., 1989). Secondary hydroxyls have previously been suggested as the catalytic site of β-cyclodextrin (Wouessidjewe et al., 1989). Spironolactone stabilily in the presence of DMβCD, with specifically substituted hydroxyls at the 2- and 6-positions,

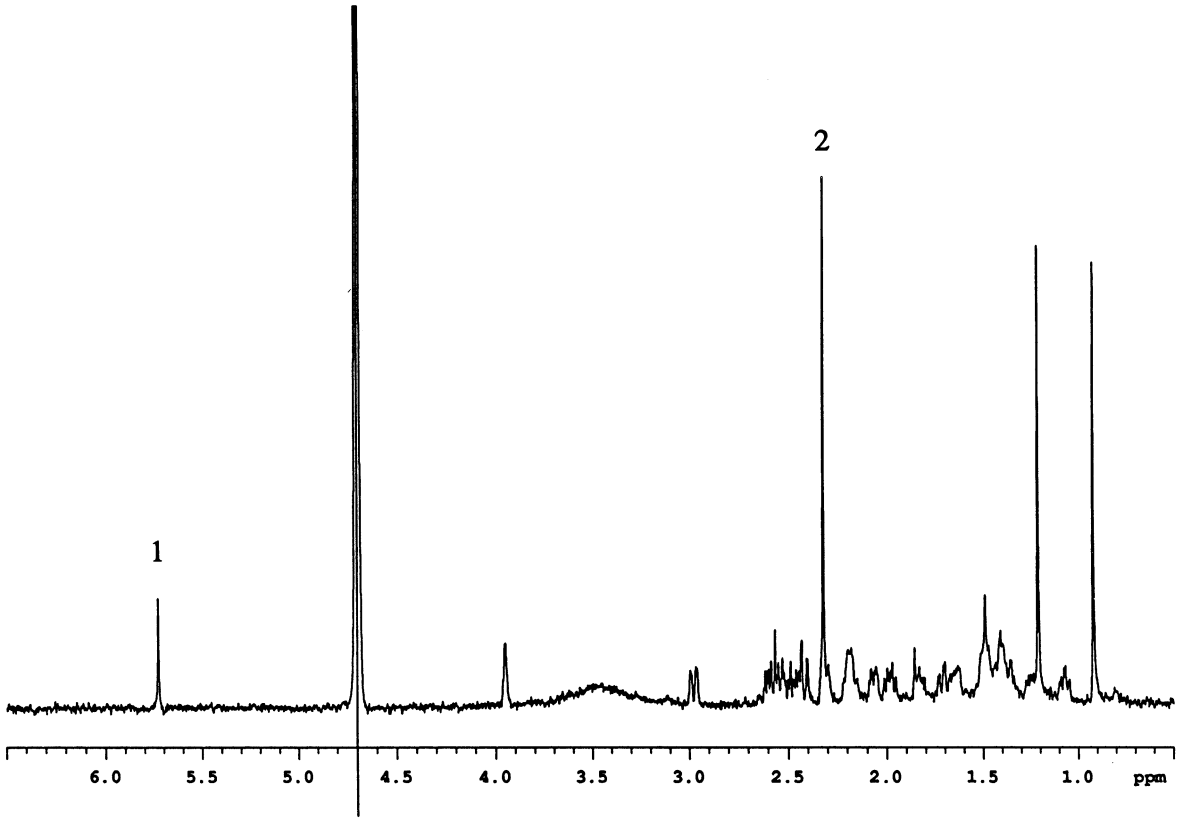

Fig. 3. ^{1}H-NMR spectra in D_2O measured at 27°C and 500 MHz of A) Spironolactone; B) Spironolactone:HPβCD complex. Numbered signals indicate the following protons: 1) H-4 proton of undegraded spironolactone; 2) protons of the acetate-group in spironolactone; 3) H-4 proton of deacetylated spironolactone.

would appoint the catalytic activity to either or both of these hydroxyls.

3.2. Solubility study

The water-solubility (C_0) of spironolactone determined in this study was 0.044 ± 0.003 mM, which was slightly lower than previously reported (0.067 mM) (Sutter and Lau, 1975). However, the two C_0 values are of the same order of magnitude and the short equilibration period (24 h) used in the solubility study may explain the observed difference. In solutions of DMβCD spironolactone solubility increased linearly as a function of DMβCD concentration (Fig. 4), giving a phase-solubility curve of A_L-type (Higuchi and Connors, 1965). According to the linear expression of this curve, a 10 mM (or 1.3%) solution of DMβCD would be able to solubilize the desired 3 mg/ml of spironolactone. Calculation according to Eq. (1) gave an apparent $K_{1:1}$ stability constant of 50 200 M^{-1} for SP:DMβCD complexes.

In solutions of HPβCD and SBE7 the degradation of spironolactone to 7α-thiospirolactone made the results more difficult to interpret. In HPβCD solutions the concentration of 7α-thiospirolactone was higher than that of spironolactone at all HPβCD concentrations studied, whereas the situation was reversed in the case of SBE7 (Table 1). The phase-solubility diagrams of undegraded spironolactone gave linear expressions of A_L-type (Fig. 4). Apparent $K_{1:1}$ values

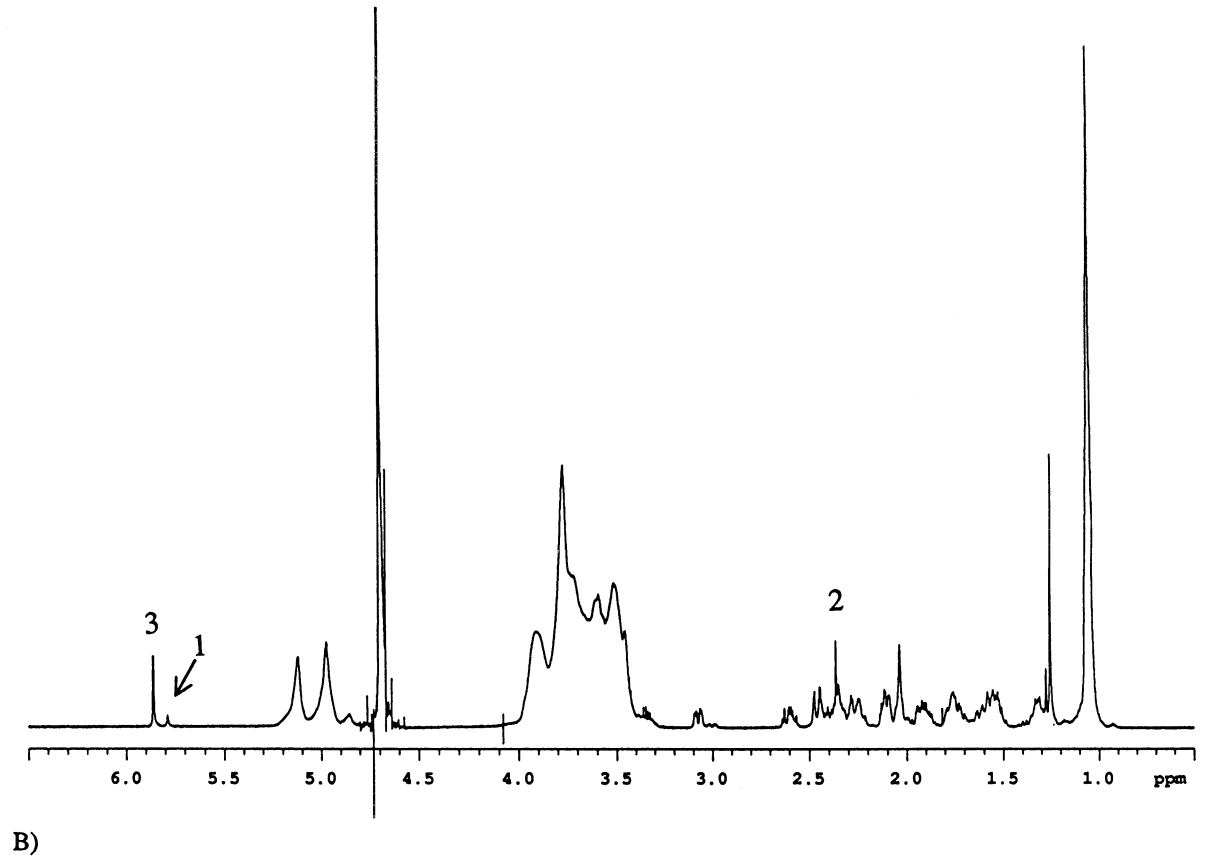

Fig. 3. (*Continued*)

calculated on these results were 9300 and 18 200 M^{-1} for SP:HPβCD and SP:SBE7 complexes, respectively. In the case of an inclusion catalyzed reaction, as seems to be the case with spironolactone and βCDs, the use of Lineweaver–Burk plots (Szejtli, 1988) would yield more valid stability constants than the phase-solubility method. The above mentioned $K_{1:1}$ values for SP:HPβCD and SP:SBE7 complexes have, therefore, been taken as suggestive. The solubilizing efficiencies of the cyclodextrins would seem to follow the order DMβCD > SBE7 > HPβCD when comparing the $K_{1:1}$ values and the phase-solubility curves on undegraded spironolactone (Fig. 4). According to the NMR-results 7α-thiospirolactone also formed inclusion complexes with HPβCD and SBE7 and the added molar concentrations of spironolactone and 7α-thiospirolactone could therefore be used to represent a more accurate measure of the solubilizing capacities of HPβCD and SBE7 (Table 1). This approach gives comparable total solubilized amounts for each of the cyclodextrins studied.

3.3. Stability study

Spironolactone degraded according to pseudo-first order kinetics in the presence of HPβCD and SBE7. The observed degradation rate constant (k_{obs}) is a sum of the degradation rates of free and complexed spironolactone according to the equation:

$$K_{obs} = k_f \cdot [SP] + k_c \cdot [SP \cdot CD] \quad (2)$$

where k_f and k_c are the degradation rate constants of free and complexed spironolactone, respec-

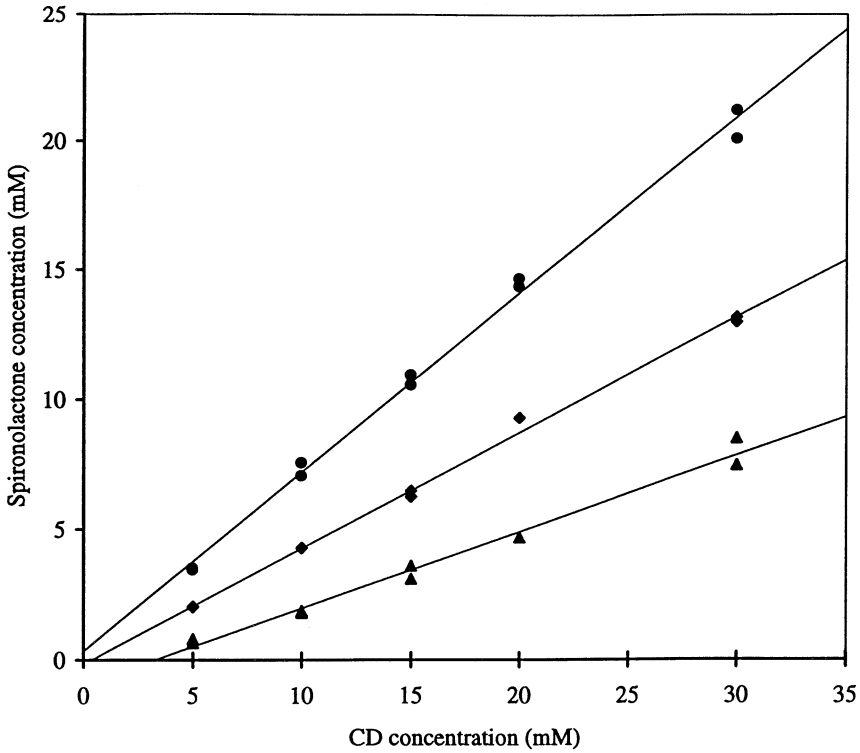

Fig. 4. Phase-solubility diagrams of spironolactone in the presence of DMβCD (●), HPβCD (◆) and SBE7 (▲) at ambient temperature.

tively. The lack of spironolactone degradation through deacetylation in pure water (Wouessidjewe et al., 1989) as well as in glucose solution suggests a specific catalysis reaction where $k_{obs} \cong k_c$. Semilogarithmic plots of spironolactone and 7α-thiospirolactone concentrations vs. time show that, in contrast to spironolactone degradation, the kinetics of 7α-thiospirolactone appearance in the solution did not follow first order kinetics (Fig. 5). This supported the NMR-results that 7α-thiospirolactone was not the end-point of spironolactone degradation but was further degraded. Canrenone, the non-sulphur degradation product of spironolactone, could not be detected in any of the samples of the stability or the solubility studies.

The stability study was performed in 29 mM solutions of the cyclodextrins, although the solubility results indicate that much lower concentrations would suffice. Spironolactone solutions in HPβCD were first prepared at 22°C with βCD:SP molar ratios of 2.5, 3 and 4. The solubilization of spironolactone required about 40 min at ratios 2.5 and 3, whereas 20 min was sufficient at ratio 4. The high molar ratio of βCD vs. spironolactone was chosen to speed up the dissolution process and to minimize the rise in solution temperature during preparation of solutions especially at lower temperatures. At each temperature studied, spironolactone degradation was slower in solutions of SBE7 than in HPβCD (Fig. 6, Table 2). In accordance with the NMR-results, this could be explained by the higher degree of substitution (DS 7 vs. 3.1) of SBE7 leaving less free hydroxyl groups to partake in the catalysis of spironolactone degradation. Estimated shelf-lives ($t_{90\%}$) in the presence of HPβCD were below 2 h even at 6°C. The molar ratio of βCD to SP did not have any clear effects on spironolactone stability in HPβCD-solutions (Table 2). The $t_{90\%}$-values in

Table 1
Solubilization of spironolactone (SP) in solutions of HPβCD, SBE7 and DMβCD

βCD (mM)	Amount of SP (mM)			Amount of SP+TSP (mM)			βCD (mM)
	HPβCD	SBE7	DMβCD	DMβCD	HPβCD	SBE7	
5	0.72 ± 0.09	2.03 ± 0.02	3.48 ± 0.05	3.48 ± 0.05	4.48 ± 0.19	3.57 ± 0.15	5
10	1.83 ± 0.06	4.29 ± 0.05	7.32 ± 0.30	7.32 ± 0.30	7.96 ± 0.44	6.38 ± 0.19	10
15	3.35 ± 0.27	6.36 ± 0.16	10.7 ± 0.2	10.7 ± 0.2	11.0 ± 0.2	9.67 ± 0.34	15
20	4.70 ± 0.20	9.29 ± 0.07	14.5 ± 0.2	14.5 ± 0.2	13.9 ± 0.2	12.7 ± 0.1	20
30	7.98 ± 0.58	13.1 ± 0.2	20.7 ± 0.7	20.7 ± 0.7	19.7 ± 0.6	18.9 ± 0.3	30

Solubilized amounts (mean $\pm$ S.D.; $n = 8$) presented as SP and the sum of SP and its degradation product 7α-thiospirolactone (TSP).

the presence of SBE7 were 4.1, 8.3 and 24.5 h at 22, 13 and 6°C, respectively. The stability study of spironolactone in SBE7 solution at 6°C was repeated three times, giving a spironolactone concentration of 2.68 ± 0.03 mg/ml or $89.7 \pm 0.5\%$ at 24 h. The separately determined concentration of spironolactone at 6°C after 72 h of storage was 2.49 ± 0.06 mg/ml or $83.0 \pm 1.9\%$. The degradation product of spironolactone, 7α-thiospirolactone, is also the metabolic precursor to thiomethylspirolactone, which is considered the main active metabolite of spironolactone after a single oral dose (Overdiek et al., 1985). Orally administered 7α-thiospirolactone and thiomethylspirolactone have shown antimineralocorticoid activity, the potencies relative to spironolactone being 0.33 and 0.26, respectively (McInnes et al., 1980). These low potencies have been explained by the low oral bioavailability of these compounds (Overdiek et al., 1985). In view of the therapeutic potential of 7α-thiospirolactone, extension of the shelf-life of spironolactone solutions beyond $t_{90\%}$ could be considered.

Generally, cyclodextrins exhibit good oral safety profiles as the absorption of intact β-cyclodextrins is very limited due to the hydrophilic nature of the outer surface of the molecule (Thompson, 1997). In humans, the oral bioavailability of HPβCD (Encapsin HPB) based on urinary excretion has been estimated at less than 0.5% (Heykants et al., 1996). With 50–62% of an oral dose excreted intact into the faeces about 40% is metabolized by the intestinal flora. Considering the polyanionic nature of SBE7 and its high degree of substitution, SBE7 should exhibit low oral bioavailability and high excretion in the faeces (Thompson, 1997). In the case of DMβCD, absorption values in rats of 6.3–9.6% have been reported with a cumulative excretion in the faeces of 96% in 72 h (Szatmári and Vargay, 1988). It would seem that the gastro-intestinal tissues are subject to the highest exposure to intact modified β-cyclodextrins and their biodegradation products. The effects of HPβCD, DMβCD and SBE7 on intestinal epithelial cells (Caco-2) have been previously investigated, to establish their local intestinal safety (Tötterman et al., 1997). HPβCD and SBE7 showed little effects on epithelial integrity and appear to be safe additives in this respect. DMβCD, despite superior stability of spironolactone, would not be recommendable for use in paediatric enteral formulations, due to its cytotoxic effects on intestinal epithelial cells (Tötterman et al., 1997).

The results of the present study would point at SBE7 as a possible candidate for spironolactone solubilization, provided the solutions were prepared and stored at 6°C or below. Assuming a spironolactone dose of 3 mg/day per kg bodyweight prepared in 29 mM SBE7, the premature infant would receive 63 mg of SBE7 per dose and about 0.2 mmol of Na^+ ions, originating from the sulphated substituents of SBE7. This amount of sodium ions should not present a problem, as treatment with spironolactone, which is a potassium sparing diuretic, may be associated with abnormal losses of sodium (Atkinson et al., 1988) requiring salt supplementation. Furthermore, pre-

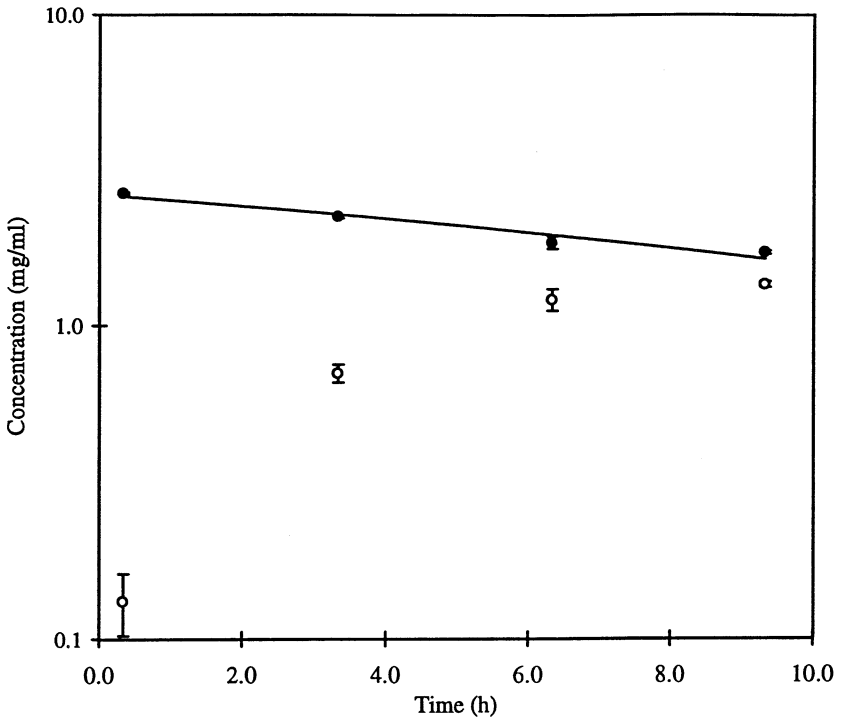

Fig. 5. Concentration (mean ± SD; $n = 4$) vs. time profiles of spironolactone (●) and 7α-thiospirolactone (○) in 22 mM HPβCD solution stored at 22°C plotted on a semilogarithmic scale; initial spironolactone concentration 3 mg/ml.

vious bioavailability studies with spironolactone:βCD complexes (Seo et al., 1983; Debruères et al., 1985; Vila-Jato et al., 1986; Yusuff et al., 1991) indicate that the dose of spironolactone, and thereby the dose of SBE7, could probably be reduced from the original because of increased oral bioavailability of spironolactone.

4. Summary

HPβCD, DMβCD and SBE7 form true inclusion complexes in solution with spironolactone, the NMR-results indicating a stoichiometry of 1:2 (SP:βCD). No degradation of spironolactone was detected in the presence of DMβCD, whereas spironolactone degraded through deacetylation according to pseudo-first order kinetics in the presence of HPβCD and SBE7. Spironolactone degradation was slower in solutions of SBE7 than in HPβCD at all temperatures studied, the degradation rate of spironolactone decreasing at decreasing temperature. According to these results, SBE7 is considered for the solubilization of spironolactone in paediatric enteral solutions, provided the solutions are prepared and stored at 6°C or below.

Acknowledgements

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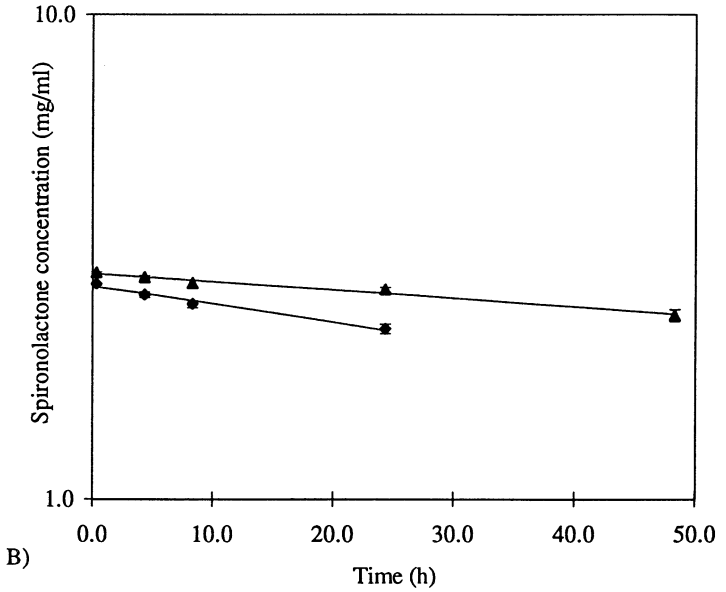

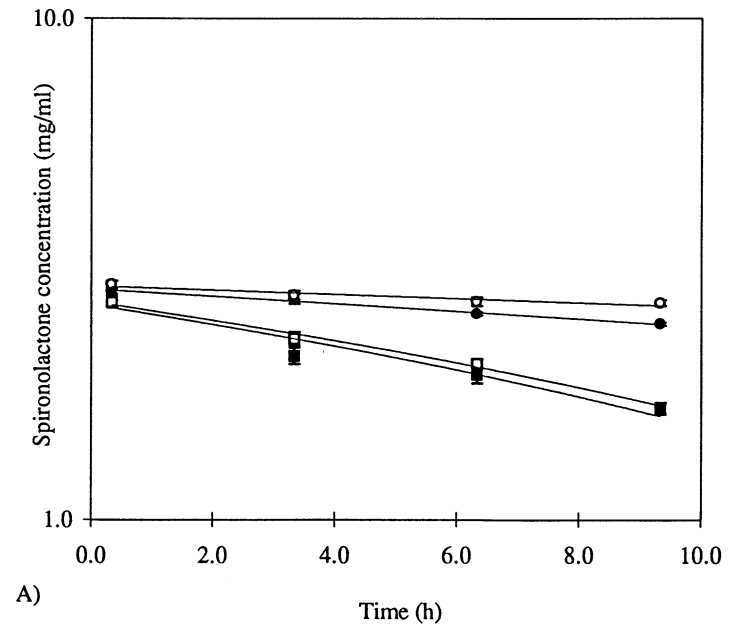

Fig. 6. Semilogarithmic plots of spironolactone concentration (mean ± SD; $n = 4$) vs. time in 29 mM solutions of A) HPβCD (■); SBE7 at 22°C (●); HPβCD (□); SBE7 at 13°C (○) and B) HPβCD (◆); SBE7 at 6°C (▲); initial spironolactone concentration 3 mg/ml.

Table 2
Apparent degradation rate constants (k_{obs}) and half-lives ($t_{50\%}$) for the degradation of spironolactone as a function of cyclodextrin derivative, cyclodextrin concentration and temperature

βCD	βCD (mM)	βCD:SP	T (°C)	$k_{obs} \cdot 10^2$ (h^{-1})	$t_{50\%}$ (h)
HPβCD	18	2.5	22	5.2	11
	22	3	22	5.0	11
	29	4	22	5.3	11
	29	4	13	2.8	17
	29	4	6	0.86	69
SBE7	29	4	22	1.7	38
	29	4	13	0.93	65
	29	4	6	0.26	237

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