INVESTIGATIONS ON THE SOLUBILITY--BEHAVIOUR OF SPIRONOLACTONE IN COMPLEXATION WITH CYCLODEXTRINS

H. VIERNSTEIN[1]
E. HOFER[1]
P. WEISS–GREILER[2]
P. WOLSCHANN[2]

[1]Institute of Pharmaceutical Technology and Biopharmaceutics, University of Vienna, Austria

[2]Institute of Theoretical Chemistry and Structural Biology, University of Vienna, Austria

Investigations of spironolactone (SP) and its solubility–behaviour in water and in complexation with cyclodextrins (CDs) at different temperatures will be presented with the aim to get detailed information about the reaction mechanism in dependence on the geometry of the complexes and the forces responsible for the association.

SP is a partial synthetic steroid–analogue of aldosterone and works as competitive aldosterone–antagonist. Its structure is given in Figure 1.

SP belongs to the group of potassium saving weak diuretics. Because of its weak effect, SP is often given in combination with other stronger diuretics like thiazides. SP is almost insoluble in water, but soluble in most of the organic solvents. It is of high interest to enhance the bioavailability (increase of solubility) by complexation with CDs (Soliman, 1997, Kaukonen, 1997).

CDs are widely used as excipients and additives in pharmacy as hosts to form inclusion complexes with small and medium sized organic molecules. A very important property of most CDs should be mentioned here, the low toxicity for humans, which enables the application in a wide field of pharmacy and pharmaceutical technology (Larsen, 2000). Beyond the increase of solubility and the subsequent bioavailability, the increase and efficiency of the active substance and the permission of its controlled release are the advantages of inclusion complexes, which lead to a broad field of various applications (Duchene, 1987).

CDs are cyclic macromolecules obtained by the degradation of starch [α(1→4) linked polyglucoseĆ by α–1,4–glucan–glycosyltransferases. Depending on the respective transferase, different types of CDs result, consisting of 6 (α–CD), 7 (β–CD) or 8 (γ–CD) α(1→4) linked glucose units (Sicard, 1987). The molecular shape of CDs resembles that of cones. They have a hydrophobic cavity with an average diameter of 5 Å (α–CD), 6.2 Å (β–CD) and 7.9 Å (γ–CD), respectively, and a thickness of 8 Å (Le Bas, 1987).

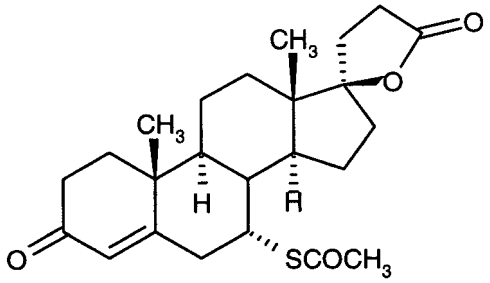

Figure 1. Structure of Spironolactone (SP)

The guest molecules are surrounded completely or partly by the mainly hydrophobic cavity, which varies in dependence of the number of the glucopyranose units and the derivatisation at the CD rim and they are shielded against the external moiety, e.g. highly polar aqueous solvents. As a consequence of the inclusion reaction the reactivities of the guest molecules, their solvent dependent spectroscopic and physicochemical properties are strongly influenced. The solubility studies on SP have been performed with β–CD and dimethyl–β–CD (DMCD) to investigate the influence of the methyl groups on the complexation reaction. Their structures are given in Figure 2.

MATERIAL AND METHODS

SP 7α–(Acetylthio)–17–hydroxy–3–oxopregn–4–ene–21–car boxylic acid–γ–lactone (IUPAC), 4–Pregnen–21–oic acid–17α–ol–3–one–7α–thiol–γ–lactone–7–acetate (C.A.), CAS Nr.52–01–7 was provided by Kwizda Pharma (Austria) (Ch. Nr.: A0846). b–CD was obtained from Roquette Frč res (Lestrem, France) as Kleptose® with a humidity of 14% (w/w). DMCD was obtained from PMCD Ringdex (Syntapharm Ref. Nr.: 1712, the water used in this study was bidistilled.

Solubility measurements and the determination of the saturation concentrations were carried out adding excess amounts of SP (35 mg/10ml) to water, β–CD and DMCD solutions. After stirring the samples (300 rpm) in a temperature controlled water bath until equilibrium was reached (for DMCD generally 48 hours and at 10°C

Author address: H. Viernstein, Institute of Pharmaceutical Technology and Biopharmaceutics, University of Vienna, Austria
E–mail: Helmut.Viernstein@univie.ac.at

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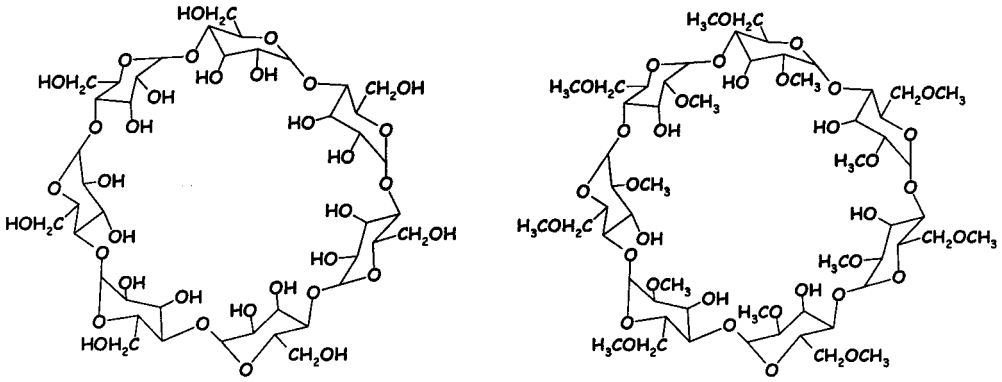

Figure 2. Structure of β–CD and DMCD

24 hours and for β–CD generally 72 hours) after sedimentation of the excess of SP a filtration was done and after 1:100 dilution with water the concentrations of dissolved SP were determined by electron absorption spectroscopy using a Perkin Elmer UV/VIS Spectrometer LAMBDA 16 (Perkin Elmer, Norwalk, CT, USA) at a wavelength of 242 nm. The saturation concentrations were estimated at different temperatures (10°C, 25°C, 35°C and 40°C). The temperatures were kept constant ±0.5°C. Nine measurements were taken at each temperature and the average value was used. Stock solutions of β–CD and DMCD were prepared and used for the solubility measurements as well as for the determination of the equilibrium constants. Due to the instability of the solutions, the spectra were recorded immediately after dilution and filtration.

The overall complexation constants K were estimated by the solubility method, assuming an one step equilibrium, varying the CDs concentration from 0–9 x 10^{-3} mol/L, according to the method of Higuchi and Connors (Higuchi, 1965).

RESULTS AND DISCUSSION

The solubility enhancement of SP in water and in complexation with DMCD and β–CD in dependence on the temperature has been studied extensively. At 10°C the concentration of SP in pure water is 0.0567 x 10^{-3} mol/L. DMCD at a concentration of 9 x 10^{-3} mol/L dissolves the 125.7 fold amount of SP in comparison of pure water. The complexation with β–CD was not investigated in detail, because a microcrystalline precipitate occurs at a β–CD concentration of 3 x 10^{-3} mol/L.

The solubility enhancement of SP at 40°C in dependence on the concentration of β–CD and DMCD is given in Figure 3.

DMCD at a concentration of 9x10^{-3} mol/L dissolves the 73.2 fold amount of SP in comparison of pure water, whereas β–CD dissolves the 51.6 fold amount of SP, which means that the solubility enhancement in the case of DMCD is 1.4 times higher compared with β–CD.

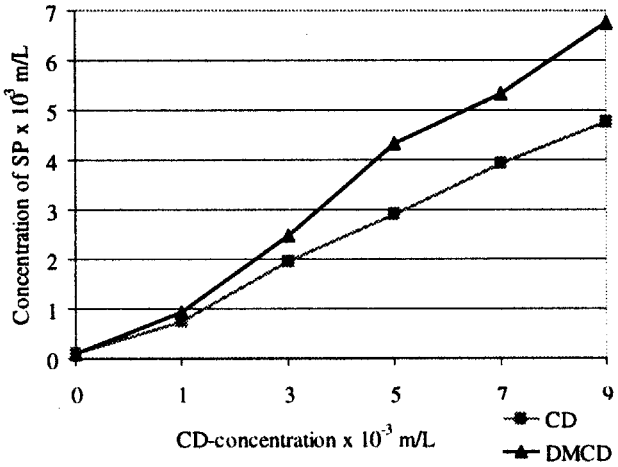

Figure 3. Concentration of SP in complexation with DMCD and β–CD at 40°C

The calculation of the overall complexation constants shows that the stability of both inclusion complexes is decreasing with raising temperature, which is depicted in Figure 4.

In a further step the thermodynamic parameters of the SP inclusion complexes with β–CD and DMCD have been calculated. The results are and presented in Table 1.

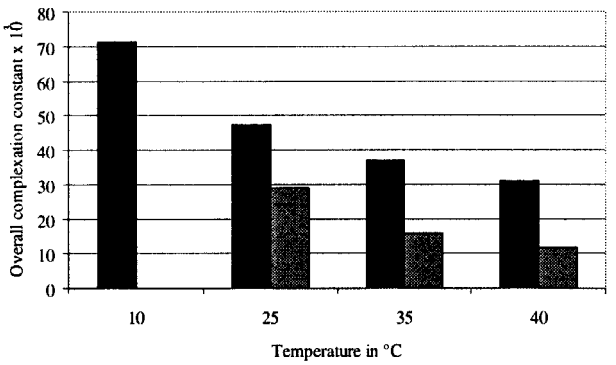

Figure 4. Comparison of the overall complexation constants at different temperatures (DMCD as black and β–CD as pink columns)

Table 1. Thermodynamic parameters of the cyclodextrin inclusion complexes with SP

	DG (kJ/mol)	ΔH (kJ/mol)	ΔS (J/molK)
β–CD	–24.8	–45.9	–21.2
DMCD	–26.67	–20.36	+6.32

CONCLUSION

The complexation of the almost water insoluble SP with CDs leads to a significant enhancement of the solubility not only with raising concentrations of the CDs but also with raising temperature. The stability of the complexes is decreasing with raising temperature and the calculation of the thermodynamic parameters shows that the reaction entropy is highly important for the inclusion reaction. The complexation with β–CD shows enthalpy – entropy compensation whereas the complexation with DMCD shows a positive contribution of the reaction entropy. Similar results have been reported for the inclusion reaction of triflumizole (Viernstein, 2002).

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