level in the intestinal tissue, and a significant decrease in RNS in mucosal cells can result in disruption of the intestinal membrane integrity.

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Change of Phase-Solubility Behavior by Gamma-Cyclodextrin Derivatization

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Abstract: The effect of γ-cyclodextrin and its four derivatives on the solubility of progesterone in phosphate buffer pH 7.4 was investigated. γ-Cyclodextrin forms a complex precipitating from solution at low cyclodextrin concentrations. No precipitation of complexes was observed with the γ-cyclodextrin derivatives. This change in phase-solubility behavior is probably due to low crystallization tendencies of the derivatives.

The application of cyclodextrin complexes in the formulation of pharmaceutical preparations is usually limited to the use in solid formulations (1) because of the low aqueous solubility of β-cyclodextrin (2). On the other hand, both β-cyclodextrin as well as the highly water soluble γ-cyclodextrin often form complexes with limited aqueous solubility, thus resulting in solubility curves of the type B_S (3). For example, γ-cyclodextrin forms B_S type solubility curves with a large number of steroids and benzodiazepines (4, 5), which imposes serious limitations towards the use of γ-cyclodextrin in the formulation of liquid preparations.

We have previously shown (6) that alkylation of β-cyclodextrin with different substituents results not only in a better aqueous solubility of the derivatives compared with the parent compound, but also changes the type of solubility curves from the B_S-type with β-cyclodextrin to the A-type curve with the derivatives.

In the present paper, the effect of four different γ-cyclodextrin derivatives on the solubility of progesteron is examined. Derivatives of γ-cyclodextrin may be useful for the solubilization of drugs that form B_S-type solubility curves with non-substituted γ-cyclodextrin.

Materials and Methods

γ-Cyclodextrin derivatives (Table I) were kindly donated by Kalle AG (Wiesbaden, FRG) and dried in vacuo before use. γ-Cyclodextrin was purchased from Lehmann & Voss (Hamburg, FRG). Progesterone (Merck, Darmstadt, FRG) was used in pharmaceutical grade without any further purification. All other materials and solvents were of analytical reagent grade.

Table I. γ-Cyclodextrin derivatives++

Substituent	R	DS+
Methyl-	$-CH_3$	1.49
Hydroxyethyl-	$-CH_2-CH_2-OH$	0.77
Hydroxypropyl-	$-CH_2-CH(OH)-CH_3$	0.66
Carboxymethyl-	$-CH_2-COO\,Na$	0.86

+degree of substitution (average number of substituents per glucose subunit).
++For a detailed description of the γ-cyclodextrin derivatives, see (6, 7).

Solubility studies were carried out according to the methods of Higuchi and Connors (3). Excess amounts of progesterone were added to solutions containing various concentrations of cyclodextrins in phosphate buffer pH 7.4 (Ph. Eur.) and were shaken at 25 ± 0.5°C in the dark for one week. An aliquot of the suspensions was then centrifuged and pipetted through a 0.45 μm membranous filter.

An HPLC method was used for the quantitation of progesterone in the resulting solutions. The separation utilized a Shandon ODS Hypersil RP – 18 column (5 μm in 5 mm × 25 cm, Shandon, Runcorn, GB) with acetonitrile – water (70:30) as the mobile phase. The eluent was monitored spectrophotometrically at 240 nm. Progesterone was quantitated by measuring peak areas and comparing the areas with that of known amounts of extern standards.

The powder X-ray diffraction patterns were taken by a Diffrac 11 diffractometer (Siemens AG, München, FRG) after a three month storage of the dry substances at room temperature.

Results and Discussion

Figure 1 shows the results of the solubility studies with γ-cyclodextrin and the four γ-cyclodextrin derivatives. With γ-cyclodextrin, a solubility curve of the B_S-type was obtained. The complex reaches its maximum solubility at a rather low cyclodextrin concentration (0.4 % w/v). A further addition of γ-cyclodextrin results in precipitation of the complex from solution and subsequently in a decrease of progesterone concentration (4).

On the other hand, with all γ-cyclodextrin derivatives solubility curves of the A_L-type were obtained. However, the slope of the solubility curves obtained with the derivatives is significantly smaller than that obtained with

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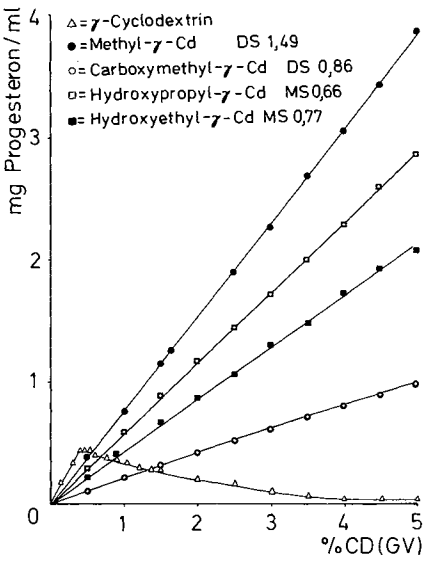

Fig. 1 Phase solubility diagram of progesterone with γ-cyclodextrin and four γ-cyclodextrin derivatives.

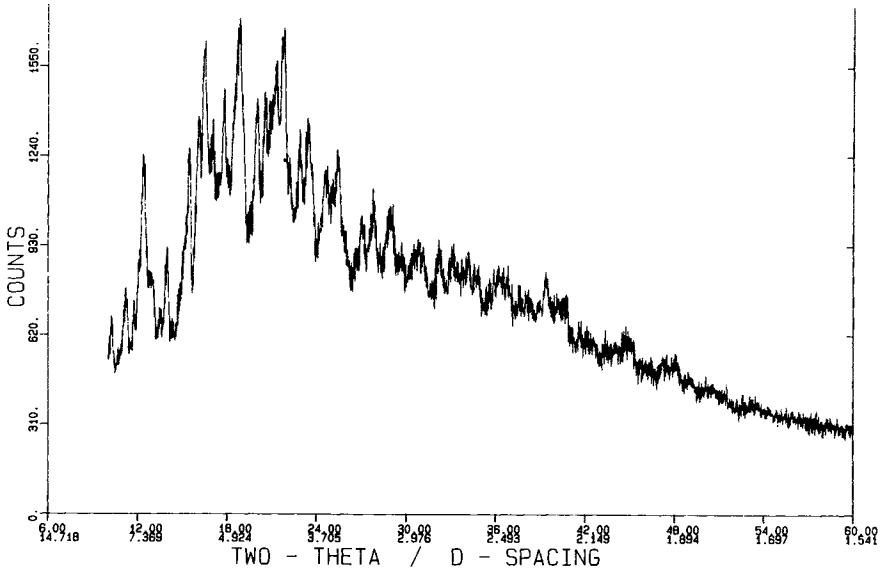

Fig. 2 Powder X-ray diffraction pattern of γ-cyclodextrin.

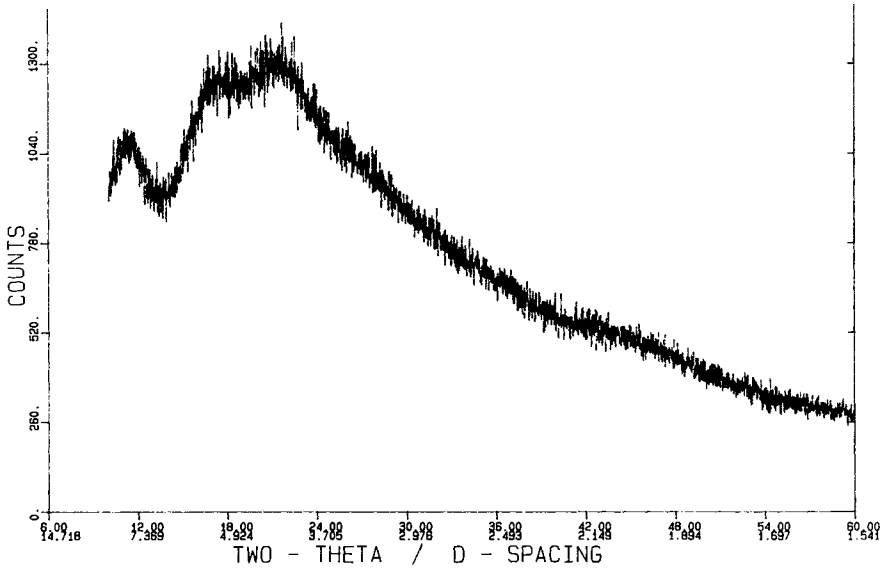

Fig. 3 Powder X-ray diffraction pattern of hydroxypropyl-γ-cyclodextrin.

pure γ-cyclodextrin in the initial linear portion of the phase – solubility diagram; hence, the complexing abilities of the derivatives are smaller than those of γ-cyclodextrin. This result may be due to steric hindrance of the cyclodextrin cavity by the substituents, as previously, observed with some β-cyclodextrin derivatives (6). Again, the type of substituent and the degree of substitution seem to have a large influence on the complexing properties of cyclodextrin derivatives. With all the derivatives, however, higher concentrations of progesterone in solution may be achieved, because no precipitation of the complexes occurs. This change in phase solubility behavior may be related to differences in the solid state properties of γ-cyclodextrin and its derivatives. Fig. 2 shows the powder X-ray diffraction pattern of γ-cyclodextrin, indicating its crystalline properties. However, attempts to crystallize the derivatives from aqueous solutions were unsuccessful. The derivatives were therefore prepared from solutions by freeze-drying. After a storage time of three month at room temperature, the dry substances showed no crystallinity. Figure 3 gives an example of a powder X-ray diffraction pattern of a derivative, in this case the hydroxypropyl derivative. The fact that the derivatives have little or no tendency to crystallize, may be related to the change in phase-solubility behavior, because the complexes formed by these derivatives displayed similarly low tendencies to crystallize. As only A-type solubility curves were formed, the γ-cyclodextrin derivatives may prove useful for the solubilization of drugs that form B_S-type solubility curves with γ-cyclodextrin (7).

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