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The Inclusion Compound of Emulsified Cetostearyl Alcohol with β-Cyclodextrin and a Competitive Reaction with a Hydrocortisone/β-Cyclodextrin Inclusion Compound in an Oil-in-Water Cream

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Abstract. β-cyclodextrin can form a solid inclusion compound with emulsified cetostearyl alcohol (ECA) by coprecipitation. This was proved by differential scanning calorimetry (DSC), X-ray diffractometry, IR spectrometry and the determination of the foaming ability according to the German Pharmacopeia (DAB 10) for ECA in the coprecipitate. The DSC result shows that both ingredients, cetostearyl alcohol and cetostearyl sulfate, are included in the β-CD cavity. The coprecipitate is therefore a mixture of inclusion compounds. ECA as a constituent of Hydrophilic Ointment (DAB 10) can substitute up to 10% hydrocortisone in Aqueous Hydrophilic Ointment (DAB 10) containing 1% HC as β-CD inclusion compound under the conditions of preparation.

Key words: Emulsified cetostearyl alcohol, β-cyclodextrin, inclusion compound, o/w cream, competitive reaction

1. Introduction

In the manufacture of formulations containing cyclodextrin (CD), interactions with other constituents of the formulation must be considered. Such competitive reactions can occur, e.g. with constituents of ointments and suppositories. CDs form inclusion compounds with adeps solidus [1], fatty acids [2], mono-, di- and triglycerides [3] and sodium alkyl sulfates [4]. It is therefore obvious that ointment constituents such as surfactants can compete for occupation of the β-CD cavity.

In recent experiments we reported that hydrocortisone (HC), which was incorporated as an inclusion compound with β-CD or hydroxypropyl-β-cyclodextrin (HPβCD), was released *in vitro* faster from an oil-in-water (o/w) cream (Aqueous Hydrophilic Ointment DAB 10) than uncomplexed HC [5]. HC becomes more hydrophilic when complexed by CD as the affinity for the inner lipophilic phase of the o/w cream is decreased and the dissolution in the outer aqueous phase is increased. In addition, it was questioned whether the enhanced HC release was

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favored by a competitive reaction between the HC inclusion compound and the emulsifying cetostearyl alcohol (ECA) of the cream. The Aqueous Hydrophilic Ointment of the German Pharmacopeia (DAB 10) contains a little less than 10% ECA. ECA is a four-component mixture of at least 88.0% fatty alcohols and 7.0% sodium cetostearyl sulfate.

The first hint as to the general existence of such competitive reactions was reported by Tokumura *et al.* [6]. The inclusion of cinnarizine in β-CD achieved only enhanced drug bioavailability in dogs if the inclusion compound was administered together with DL-phenylalanine or L-leucine. Both compounds can displace cinnarizine from the CD cavity. Frijlink *et al.* concluded that displacement of diazepam by lipids from the complex can play an important role in rectal absorption [7]. The addition of small amounts of an ethoxylated hydroxystearinic acid ester (Solutol® HS 15) as surfactant to a dissolved inclusion compound of diazepam/β-CD decreases the amount of dissolved diazepam, with partial precipitation of the drug [1]. An inclusion compound with the surfactant had been formed. In adeps solidus suppositories which contained indomethacin and β-CD the formation of a solid inclusion compound with constituents of the fat could be demonstrated [1]. The formation of an inclusion compound between β-CD and ECA has not previously been reported.

The purpose of this paper is to determine whether β-CD can form an inclusion compound with ECA. This could be the prerequisite for a possible competitive interaction in Aqueous Hydrophilic Ointment DAB 10 containing HC as a β-inclusion compound.

2. Experimental

2.1. MATERIALS AND METHODS

β-Cyclodextrin was a gift of Rhône Poulenc, Melle (Rhodocap® N, > 98% β-CD), hydrocortisone (98% purity, Sigma, Deisenhofen), emulsifying cetostearyl alcohol (Lanette N®), cetostearyl alcohol, cetostearyl sulfate (Caelo, Hilden) and white vaselin (Mainland, Frankfurt/M.) were purchased. Vaseline and the ointment constituents were of pharmaceopeial grade.

2.2. PREPARATION OF THE INCLUSION COMPOUND AND THE PHYSICAL MIXTURE

The ratio of ECA and β-CD for the preparation of the inclusion compound by coprecipitation was chosen according to the ratio of the substances in Aqueous Hydrophilic Ointment containing 1% HC (w/w) as the HC/β-CD inclusion compound. The preparation and characterization of HC/β-CD is described in [8]. A 10% aqueous emulsion of ECA (37 g) was added to 200 mL of a saturated β-CD solution (1.85%) and stirred by magnet agitator at 40 °C for 48 h. The same experiment was performed with a sample containing no β-CD. Emulsions were formed

in both cases. In addition a colorless precipitate was formed in the sample which contained β-CD.

After standing for 3 h the precipitate was separated by filtration through a sintered glass filter and dried at 40 °C in a vacuum oven. The dried powder was washed two times with 50 mL boiling ethanol using the same glass filter to remove nonincluded ECA and dried again. ECA is readily soluble in boiling ethanol, whereas the ECA/β-CD inclusion compound is largely stable under the conditions used. This preparation of the inclusion compound was repeated three times. The yield was 41.2 ± 0.9%.

The physical mixture of ECA and β-CD was prepared by mixing 0.50 g ECA and 6.07 g β-CD in a Turbula-Mixer, type T 2C (Bachofen, Basel) for 20 min.

2.3. PHYSICAL METHODS

In order to isolate ECA from the complex, dry residues were prepared by heating the ECA/β-CD inclusion compound to boiling in 100 mL ethanol (96%) for at least 5 min. After hot filtration the filtrate was evaporated to dryness in a rotary evaporator (Büchi, Göppingen) and dried in a hot-air cabinet.

The X-ray diffractograms were recorded with a powder diffractometer type 1700 (Philips, Eindhoven), using Cu $K_{\alpha 1}$ and Cu $K_{\alpha 2}$ radiation. The film was evaluated with a counting tube. DSC measurements were performed with the TA 3000 Thermoanalytic System (Mettler, Giessen) with TA processor TC 10 and the DSC 20 cell; heating rate 5 K min^{-1} from 30 to 210 °C, 5 mL min^{-1} N_2. Sample weights: ECA, cetostearyl alcohol and sulfate, residue of ethanolic extract of ECA/β-CD 2 mg, physical mixture (molar ratio ECA : β-CD = 1 : 2), inclusion compound 10 mg. The IR spectra were recorded with a PU 9716 IR spectrophotometer (Philips, Cambridge).

The experiments for the determination of the foaming behavior were performed according to the German Pharmacopeia (DAB 10). 10 mL of 0.5% (w/w) aqueous solution of ECA or of 1.5% (w/w) corresponding solution of the β-CD complex were vigorously shaken for 10 s. The formed foam must remain stable for at least 30 min.

2.4. HC DETERMINATION IN A MIXUTRE OF ECA, HC/β-CD AND ECA/β-CD INCLUSION COMPOUNDS

The concentration ratio of ECA, HC/β-CD and water corresponds to that in the Aqueous Hydrophilic Ointment (DAB 10) which contains 1% HC as its inclusion compound. ECA emulsion (13.3 g, 15.7% concentration) was added to 1.51 g HC/β-CD inclusion compound in small portions. The emulsion obtained was dried in a vacuum drying oven at 40 °C for 24 h. Nonincluded ECA was removed by washing twice with 50 mL boiling ethanol (96%). A colorless powder was obtained. To demonstrate how far the washing procedure with boiling ethanol can change the HC content in the β-CD inclusion compound, 1.51 g of the complex alone was

washed with boiling ethanol under the experimental conditions. The HC content of the inclusion compound was determined by UV spectrometry at 248 nm after dissolution in water. It diminished after washing from 13.2 ± 0.2% to 12.9 ± 0.1% HC. Considering this HC loss, the mixture of HC/β-CD and ECA/β-CD inclusion compounds contains 11.7% ± 0.1% HC. This indicates indirectly that 8.6% HC was displaced from the HC/β-CD inclusion compound by ECA.

3. Results and Discussion

3.1. INCLUSION COMPOUND OF ECA WITH β-CD

A colorless precipitate was obtained by stirring a 10% aqueous o/w emulsion of ECA with a saturated β-CD solution. The separated precipitate was used for further characterization. The same experiment without a β-CD solution did not show any precipitation.

The DSC thermogram of ECA shows three endothermic peaks at about 40, 50 and 80 °C, respectively (Figure 1). A much weaker peak is seen at 110 °C. The two main peaks at 50 and 80 °C are also recorded with a weaker intensity with the physical mixture of ECA and β-CD. The weakness of the peak at 80 °C and the missing peak at 110 °C are caused by overlapping by the broad thermal rise between 80 and 120 °C, corresponding to the release of water. None of these peaks, with the exception of the broad water inflection, can be detected with the ECA/β-CD complex. This observation implies that the molecular arrangement of ECA in the solid complex is different from that in their own crystal habit. It probably indicates that the disappearance of the melting peak of ECA in the complex is due to the molecular inclusion.

Figure 2 shows the X-ray powder diffractograms for ECA, β-CD, the complex and the corresponding mixture. The diffractogram of ECA has only one characteristic peak at 23°. The diffractogram of the complex does not correspond to that of the physical mixture: it shows the typical diffraction pattern which is characteristic of channel inclusion compounds [9].

Further proof of the existence of ECA in the complex is the identity reaction of the German Pharmacopeia concerning the foaming ability of ECA. A 0.5% solution of ECA must form a foam which is stable for at least 30 minutes. The solution of our precipitate fulfills this condition although β-CD is ascribed some antifoaming effect dependent on concentration [10]. A 1% or a saturated β-CD solution forms no foam under these experimental conditions.

DSC examination of the dry residue of the ethanolic extract of the ECA/β-CD inclusion compound shows two endothermic melting peaks at 48 and 80 °C which can also be detected in the thermogram of ECA alone (Figure 3). ECA is composed of about 9 parts (w/w) cetostearyl alcohol and 1 part cetostearyl sulfate. Each of these two parts is again a mixture of two constituents, cetyl alcohol and stearyl alcohol, and the corresponding sulfates. The DSC peaks at 40 and 50 °C are caused by cetostearyl alcohol, and the peak at 80 °C by cetolstearyl sulfate. The peaks of

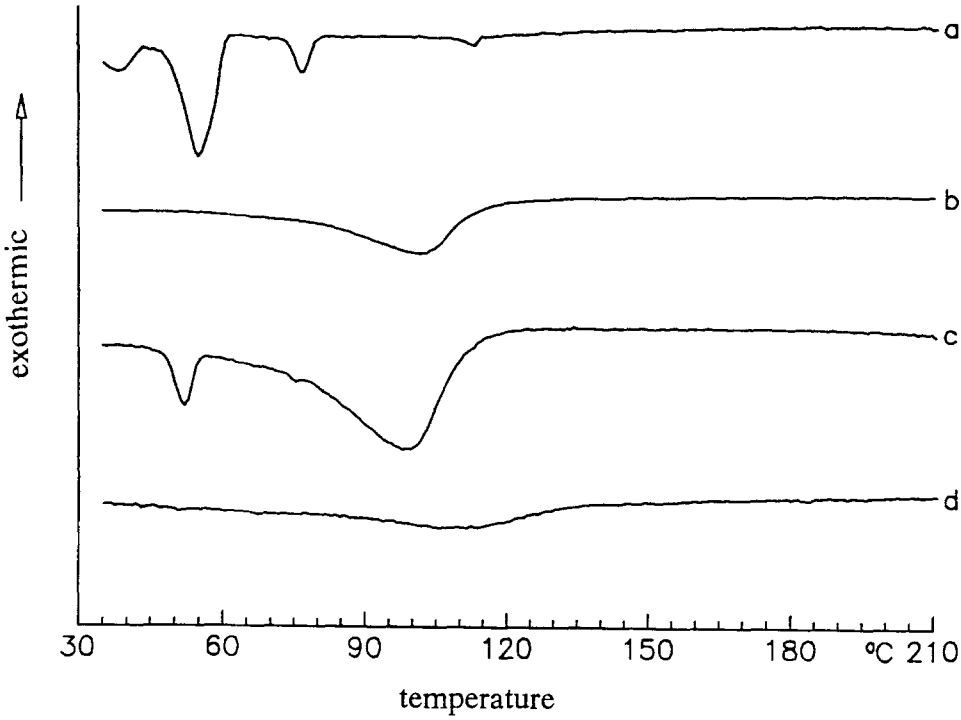

Fig. 1. DSC thermograms of (a) Emulsifying Cetostearyl Alcohol (DAB 10); (b) β-CD; (c) Physical mixture; (d) Complex.

both constituents can be observed in the residue. Therefore it can be concluded that both constituents are included in the β-CD cavity.

The IR spectra of the residues of the ethanolic extract and ECA show identical bands (Figure 4). Primarily the signals at about 2900 cm^{-1} (CH stretching vibration), 1500 cm^{-1} (asymmetric deformation vibration of the CH_2 groups) and the band in the finger print region at 700 cm^{-1} coincide in the two samples.

3.2. COMPETITIVE REACTIONS BETWEEN ECA AND HC FOR INCLUSION INTO THE β-CD CAVITY

The possibility that ECA can substitute HC in a HC/β-CD inclusion compound when formulated in o/w cream needs to be checked. One can assume that the four constituents of ECA can be included into the β-CD cavity as long alkyl chains are favourite candidates for inclusion [11]. Guo *et al.* [4], for example, reported the formation of soluble inclusion complexes of β-CD with anionic sodium alkyl sulfates, $C_nH_{2n+1}OSO_3Na$ (n = 5–12). These authors reported that the association constants of these surfactants reported in the literature differ widely. It seemed impractical to determine the association constants in the four-component mixture

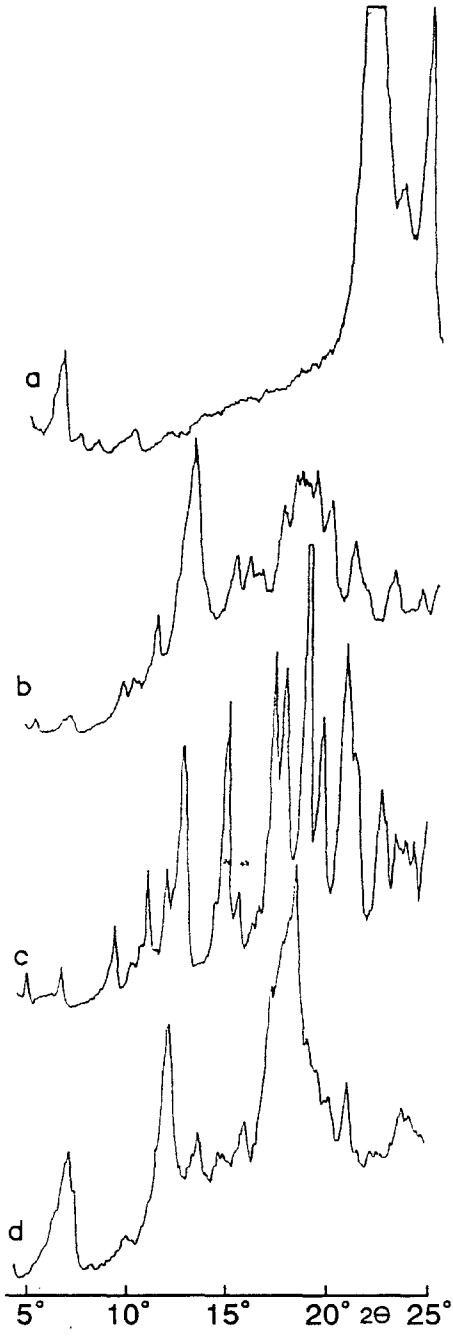

Fig. 2. X-ray diffractograms of (a) Emulsifying cetostearyl alcohol; (b) β-CD; (c) Physical mixture; (d) Complex.

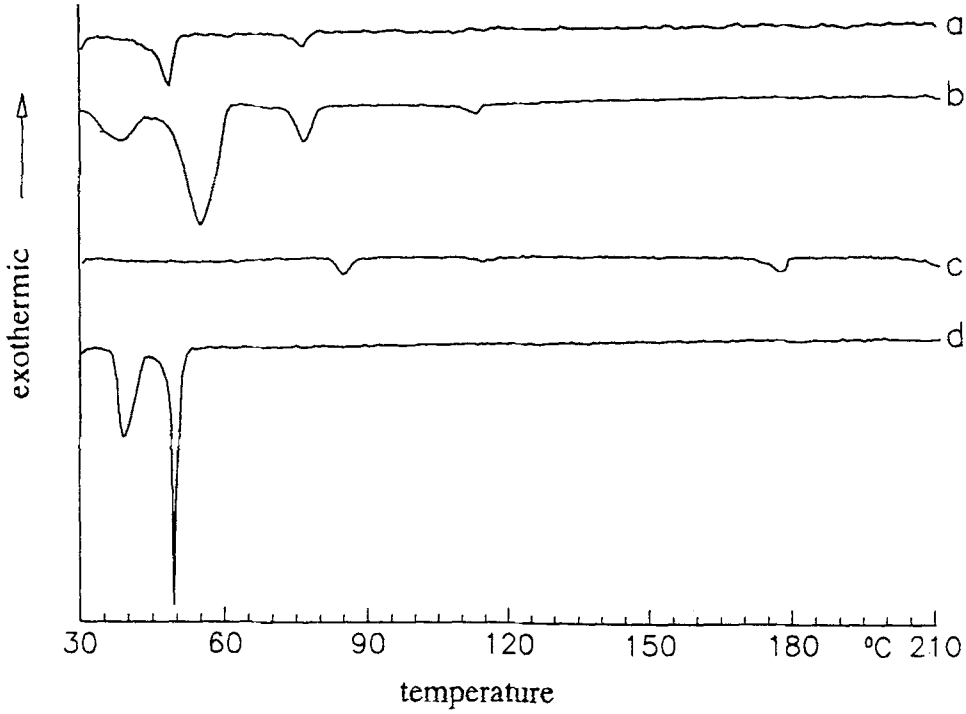

Fig. 3. DSC thermograms of (a) Product isolated from the β-CD complex; (b) Emulsifying cetostearyl alcohol; (c) Cetostearyl sulfate; (d) Cetostearyl alcohol.

ECA. The examination of the substitution of HC by ECA in the β-CD cavity should therefore be performed indirectly by the determination of the precipitate.

In order to do this an aqueous emulsion of ECA is added to the HC/β-CD inclusion compound in small portions, as is done during the preparation of the ointment. The amounts of HC/β-CD, ECA and water used are those occuring in a 1% HC o/w cream containing the HC/β-CD inclusion compound. After washing twice with 50 mL boiling ethanol, filtration and drying, a colorless powder is obtained. The following experiments were done with this powder:

- The DSC thermogram of the powder shows neither a melting peak of ECA nor of HC. That indicates that both substances can be included in β-CD.
- The determination of the foaming behavior results in a stable foam. Although no melting peak of ECA could be detected thermoanalytically, the powder must contain ECA.
- The IR spectrum of the powder does not show any bands due to ECA. But these bands are also not seen in the IR spectrum of the ECA/β-CD inclusion compound because the ECA concentration is very small so that its bands are overlapped by the bands of β-CD. The characteristic bands of HC are recorded.

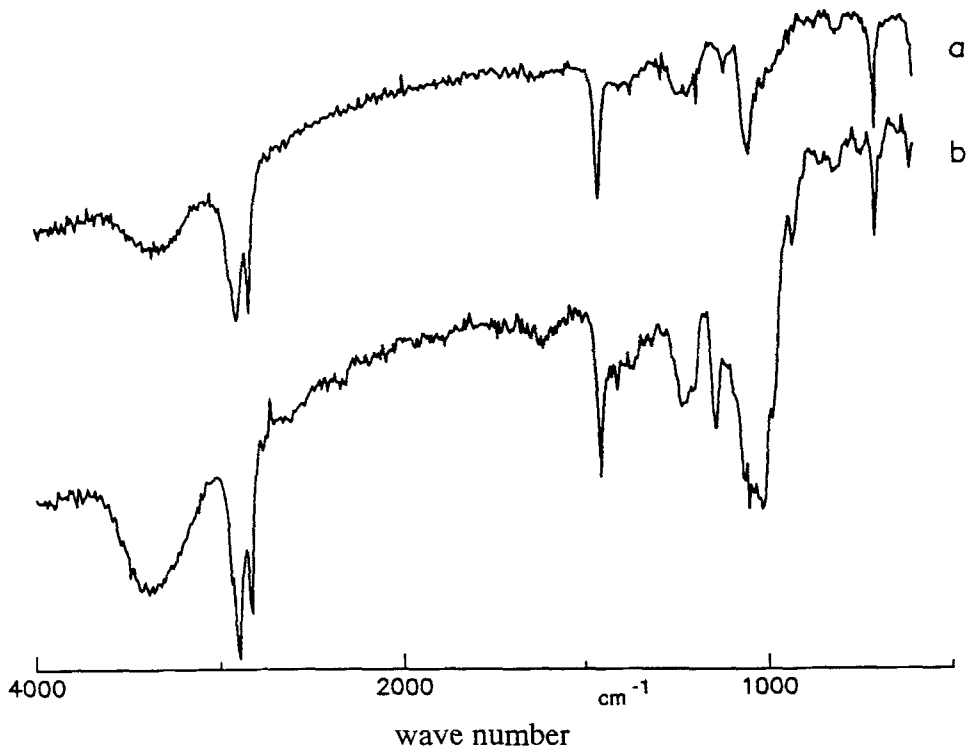

Fig. 4. IR spectra of emulsifying cetostearyl alcohol. (a) Commercial product; (b) Product isolated from the β-CD complex.

In order to identify any ECA present an ethanol extract of the powder was prepared corresponding to the ECA/β-CD inclusion. The IR spectrum of this residue and of ECA shows identical bands.

These results indicate that the powder consists of a mixture of ECA/β-CD and HC/β-CD.

For the determination of the amount of HC which is displaced from the HC/β-CD inclusion compound by ECA the HC concentration in the mixture of both inclusion compounds plus ECA was determined. Nonincluded ECA was extracted with ethanol. Washing the HC/β-CD inclusion compound with boiling ethanol decreases its HC content from 13.2 to 12.9%.

The mixture of the ECA/β-CD and HC/β-CD inclusion compounds contains 11.7% HC. Washing with ethanol indicates that 8.6% HC is displaced from the HC/β-CD inclusion compound by ECA. While neglecting the small difference of the molecular masses of HC and ECA (362.5 and 358.5, respectively) a maximum of about 0.9% of the total ECA which is contained in the o/w cream is included in the inclusion compound.

In conclusion, all the performed physical determinations prove the formation of an inclusion compound between ECA and β-CD. Both cetostearyl alcohol

and cetostearyl sulfate are included in the β-CD cavity as shown by the DSC experiments. It could be proved that up to 10% HC can be displaced by ECA from a HC/β-CD inclusion compound under the conditions of preparation of an Aqueous Hydrophilic Ointment DAB 10 which contains 1% HC as β-CD inclusion compound.

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References

1. K.-H. Frömming, W. Mehnert, P. Bady, C. Kraus, M. Stein, and V. Wederlich: *Competitive Reactions of Cyclodextrin Inclusion Compounds* (Minutes 5th International Symposium on Cyclodextrins, Ed. D. Duchêne), pp. 392–398, Editions de Santé, Paris (1990).
2. J. Szejtli and E. Blánky-Elöd: *Stärke* **27**, 368 (1975).
3. Y. Okada, K. Koizumi, K. Ogata, and T. Ohfuji: *Chem. Pharm. Bull.* **37**, 3069 (1989).
4. Q.-X. Guo, Z.-Z. Li, X.-Q. Zhu, and Y.-Ch. Liu: *J. Incl. Phenom.* **17**, 149 (1994).
5. A. Preiss, W. Mehnert, and K.-H. Frömming: *Pharmazie* in press.
6. T. Tokumura, Y. Tsushima, M. Kayano, Y. Machida, and T. Nagai: *J. Pharm. Sci.* **74**, 496 (1985).
7. H. W. Frijlink, A. C. Eissens, A. J. M. Schoonen, and C. F. Lerk: *Int. J. Pharm.* **64**, 195 (1990).
8. A. Preiss, W. Mehnert, and K.-H. Frömming: *Arch. Pharm.* (Weinheim) in press.
9. K. Takeo and T. Kuge: *Agr. Biol. Chem.* **33**, 1174 (1969).
10. J. Szejtli: *Cyclodextrin Technology*, p. 326, Kluwer, Dordrecht (1988).
11. J. Szejtli: *Cyclodextrins and their Inclusion Complexes*, pp. 131–133, Akadémiai Kiadó, Budapest (1982).