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Preparation, characterization and dissolution enhancement of mefloquine hydrochloride-βCD inclusion complex

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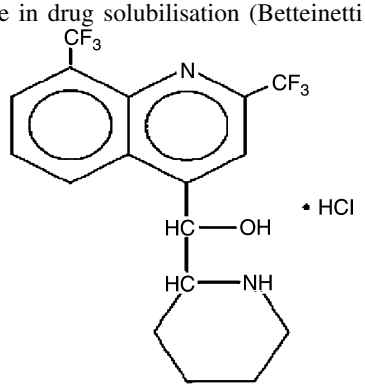
The solid state properties and dissolution behaviour of binary systems of mefloquine hydrochloride (MH) with βCD were investigated. MH-βCD interaction in the solution state was studied by phase solubility analysis and demonstrates the ability of βCD to complex with MH giving A_L type profile with 120.34 M^{-1} stability constant. The kneading method was adopted to prepare binary sytems of MH with βCD in 1:1 molar ratio. The solid inclusion was characterized by differential scanning calorimetry, fourier transformation infrared spectroscopy and X-ray powder diffractometry. Experimental results confirmed the existence of 1:1 inclusion complex of MH with βCD. Aqueous solubility of MH was found to be enhanced by 118% for inclusion complex. The dissolution properties of binary systems were studied in simulated gastric fluid without enzyme and compared with MH alone. The inclusion complex of MH prepared with βCD showed a dissolution rate several times faster than that of physical mixture and pure drug.

1. Introduction

Mefloquine hydrochloride (MH) is a 4-quinolinemethanol derivative used for prophylaxis and treatment of chloroquine resistant falciparum malaria. According to the biopharmaceutical classification system, MH is classified under BCS Class II category (Lindenberg et al. 2004). Though its widespread used for malaria, MH is a lipophilic drug (Log P = 3.10) being just very slightly soluble in water (Drug bank). MH shows polymorphism which is one of the reasons for low aqueous solubility and low bioavailability of the drug (Kitamura et al. 1994). Very low plasma concentration of MH is associated with an increased risk of prophylaxis and treatment failure (Franssen et al. 1989; Lobel et al. 1993, 1991). Its low aqueous solubility and poor dissolution can cause formulation problems and limit its therapeutic application by delaying the rate of absorption and the onset of action. Cyclodextrins (CDs) are cyclic (α-1,4)-linked oligosaccharides of α-D-glucopyranose, containing a relatively hydrophobic central cavity and a hydrophilic outer surface. They are commonly used in drug formulations as solubility enhancers because of their ability to form water soluble inclusion complexes with poorly water soluble drugs (Loftsson and Brewster 1996; Rajewski and Stella 1996). The method of complexation may play role in drug solubilisation (Betteinetti et al. 1992).



Mefloquine hydrocloride

Therefore attempt has been made to prepare and characterize an inclusion complex of MH with βCD for improving the solubility and *in vitro* dissolution rate which will further contribute to improved oral bioavailability as the rate and extent of absorption of MH is dissolution rate limited. Selective physicochemical determinations based on differential scanning calorimetry (DSC), X-ray powder diffractometry (XRD), and fourier transform infrared spectrophotometry (FTIR) were used to characterize the systems. Further the saturation solubility studies and a dissolution rate profile of the complex was evaluated.

2. Investigations, results and discussion

2.1. Preliminary studies

The complexation of MH with βCD was studied in 1:1 molar ratio. The complex was prepared by the kneading method which is an industrially feasible, economic method and can be applied on large scale. The % yield of the kneaded product was found to be 95%. The prepared complex was evaluated for dissolution studies, saturation solubility and solid state characterization like DSC, FTIR and XRD.

2.2. Phase solubility studies

Phase solubility profile of MH with βCD is presented in Fig. 1. The aqueous solubility of drug increases linearly as a function of increasing βCD concentration. Phase solubility plot can be classified as A_L - type according to the method given by Higuchi and Connors (1965). The linear host-guest correlation coefficient ($R^2 = 0.994$) with a slope of 0.464 suggested the formation of a 1:1 inclusion complex with respect to βCD concentrations. The line equation from the linear regression analysis was found to be as follows

$$y = 0.464x + 0.008 \quad (1)$$

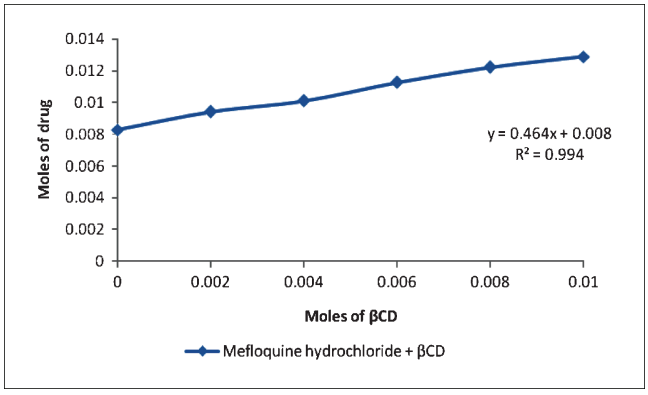


Fig. 1: Phase solubility diagram of MH in aqueous solution of BCD.
[a] mean ± SD, n = linear equation is y = 0.464x + 0.008(R^2 = 0.994).

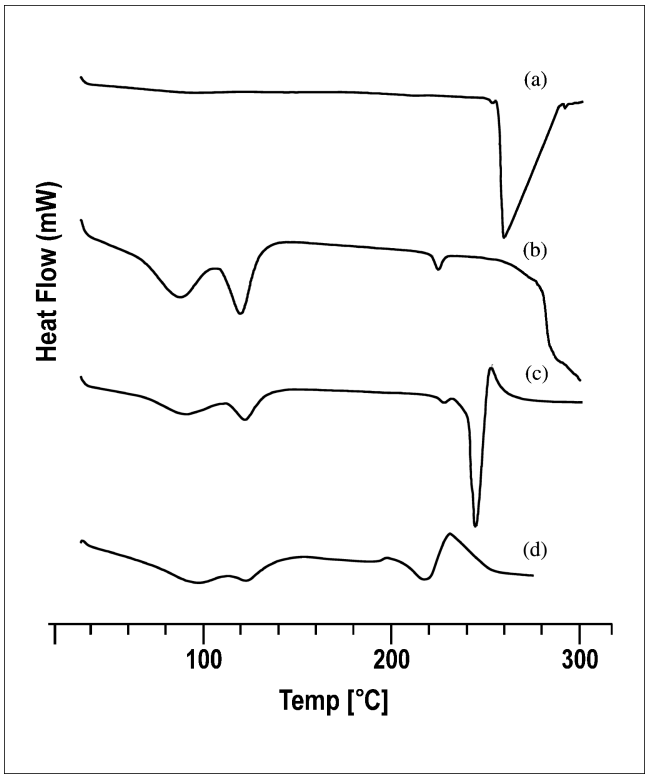


Fig. 2: DSC diagram of MH-BCD systems: (a) MH; (b) BCD; (c) physical mixture; (d) inclusion complex

Since the slope of the phase solubility plot was less than one, the complex stoichiometry was assumed to be 1:1. The apparent stability constant, K_s obtained from the slope of the linear phase solubility diagram was found to be 120.34 M^{-1} being well within the range of 100 to 1000 M^{-1} and this considered as ideal binding constant. Good stability constant suggests that MH has sufficient affinity towards the βCD cavity (Szejtli 1985). A smaller stability constant value indicates too weak interaction, whereas a larger value indicates the possibility of limited release of drug from the complex thereby interfering with drug absorption.

2.3. Differential scanning calorimetry (DSC)

The solid binary systems were analyzed by means of DSC to detect possible altered thermal properties. DSC curves of the pure MH, βCD, physical mixture and kneaded complex are shown in Fig. 2. When a guest molecule is incorporated in the CD cavity, its melting point generally shifts to a different temperature. DSC measurement for MH (a) showed an endothermic peak at 261 °C corresponding to the melting point of pure drug. In physical mixture (c), the peak corresponding to the melting point of MH shifted to 253 °C while in the inclusion complex (d), fusion peak of MH shifted to the lower value 247 °C with a reduction in intensity of the peak. This could be due to the entrapment of MH in βCD cavity resulting in a shift of the melting point to a lower temperature (Dolo et al. 1996). The DSC thermogram of βCD showed (b) peaks at 88 and 120 °C attributed to the desolvation of water molecules present in the βCD cavity. In physical mixture, the peak at 123 °C corresponds to the desolvation of the water molecules. A peak due to the desolvation of the water molecule gets broadened in the inclusion complex which indicates the formation of an inclusion complex with MH. Furthermore, βCD showed a small endotherm peak at 226 °C corresponding to irreversible solid-solid phase transition and final degradation process of βCD has been observed above 300 °C. In physical mixture, the peak of βCD at 246 °C corresponds to irreversible solid-solid phase transition while for inclusion complex broad peak observed at 238 °C. The DSC thermogram for MH in the inclusion complex showed the persistence of a melting endothermic peak of MH but with diminished in intensity demonstrating interaction between MH and βCD suggesting inclusion complex formation.

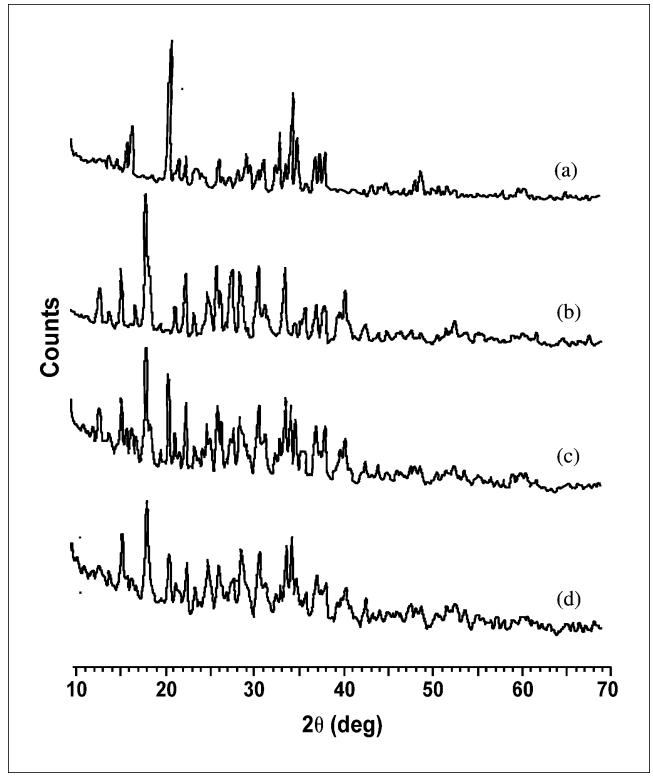


Fig. 3: XRD pattern of MH-BCD systems: (a) MH; (b) BCD; (c) physical mixture; (d) inclusion complex

2.4. X-ray powder diffractometry (XRD)

The X-ray diffraction patterns of the MH, βCD, physical mixture and kneaded complex are presented in Fig. 3. The XRD pattern of MH showed intense and sharp peaks indicating its crystalline nature. Crystallinity was determined by comparing some representative peak heights in the diffraction pattern of binary systems with those of reference (pure MH) (Ryan 1986). MH showed (a), sharp peaks at 21.120°, 34.810°, 33.610° and 35.450° with peak intensities of 164, 102, 81 and 56 respectively. The peak height at 21.120° (2θ) was used for calculating

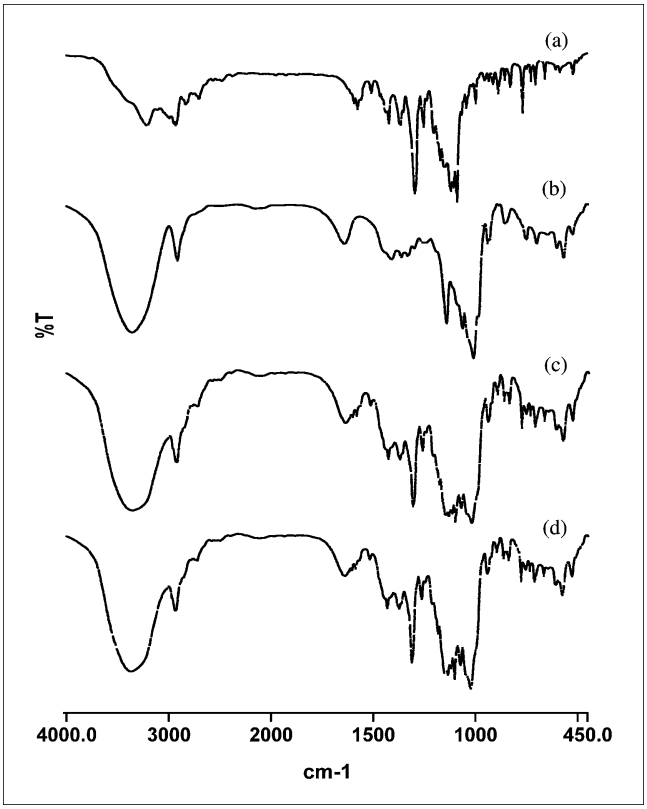


Fig. 4: FTIR spectra of MH-BCD systems: (a) MH; (b) BCD; (c) physical mixture; (d) inclusion complex

the relative decrease in crystallinity (RDC) of binary systems. The RDC value for complex was found to be 0.2987 while for physical mixture 0. 4024. The diffraction pattern of physical mixture showed (c), peaks of MH and βCD with little decrease in peak intensity demonstrating reduction in crystallinity while for inclusion complex (d), crystallinity of MH was reduced to a greater extent as compared to physical mixture and pure MH alone. Furthermore, the peak at 33.610 °C of MH for inclusion complex was completely disappeared indicating formation of inclusion complex. This result indicated that the drug in the complex form was completely in an amorphous state compared to the pure drug as well as physical mixture.

2.5. Fourier transform infrared (FTIR) spectroscopy

Fig. 4 illustrates the FTIR spectra of MH, βCD, physical mixture and inclusion complex. The FTIR spectra of pure MH showed (a), characteristic peaks at 3232 cm^{-1} (N-H stretching vibration), 1109, 1377 and 1602 cm^{-1} (quinine ring stretching vibration), 1315 and 1140 cm^{-1} (CF_3 stretching vibration), 2850 cm^{-1}(C-H bridge), 2954 cm^{-1}(CH_2), 1586 cm^{-1} (C = N/C = C), 1266 cm^{-1}(C-N), 1054 cm^{-1} (piperidine ring), 1188 cm^{-1} (C-C/N-H stretching vibration). The spectrum of pure βCD showed (b), characteristic peaks at 3392 cm^{-1} (O-H), 2925 cm^{-1} (C-H), 1647 cm^{-1} (H-O-H bending), 1157 cm^{-1} (C-O) and 1028 cm^{-1} (C-0-C). Absorption peak at 3232 cm^{-1} of MH (N-H) showed strong interaction with absorption peak at 3392 cm^{-1} assigned to βCD (O-H) and the peak is shifted to lower value for physical mixture and complex. This might be due to the intermolecular hydrogen bonding with MH. In physical mixture (c), the absorption peak due to quinine ring stretching vibration assigned at 1602 cm^{-1} is shifted to 1641 cm^{-1} while in complex (d), to 1637 cm^{-1}. Moreover, the peak assigned to the quinine ring stretching vibration at 1109 cm^{-1} reduces in physical mixture and in complex while absorption peak at 1377 cm^{-1} in MH shifted to a lower value with reduced intensity indicating a strong interaction of MH with βCD. In physical mixture, absorption peak of βCD (C-H) at 2925 cm^{-1} is shifted to 2933 cm^{-1} while in complex to 2934 cm^{-1}. Absorption peaks at 1315 cm^{-1} (CF_3 stretching vibration), 1586 cm^{-1} (C = N/C = C), 1266 cm^{-1} (C-N) appears with reduced intensities in both physical mixture and in complex. Some peaks at 2850 cm^{-1} (C-H bridge), 2954 cm^{-1} (CH_2), 1054 cm^{-1} (piperidine ring) completely disappeared in physical mixture and in complex. In physical mixture and complex, the absorption peak observed at 1188 cm^{-1} due to C-C/N-H stretching vibration of MH but with reduced intensity while absorption peak of βCD at 1157 cm^{-1}(C-O) is missing may be due to hydrogen bonding. Thus, it could be suggested that MH-βCD interactions are dominated by hydrogen bonds. The peak at 1647 cm^{-1} in IR spectra of βCD due to water of crystallization, also disappeared in both PM and inclusion complex (Mukne and Nagarsenker 2004). Some peaks disappeared, some peaks appeared and some peaks heights decreased. These changes occurred in FTIR spectra of samples indicating the formation of an inclusion complex in solid state.

Table 1: Saturation solubility data of MH, physical mixture and inclusion complex

System	Solubility in water at 25 °C (mg/ml)
MH	0.387
Physical mixture	0.432
Inclusion complex	0.457

Table 2: Dissolution time of MH in simulated gastric fluid without enzyme at 37 ± 0.5 °C

Sample source	Dissolution time (min)
MH	>60
Physical mixture	46
Inclusion complex	10

2.6. Saturation solubility studies

The prepared binary systems of MH with βCD showed enhanced solubility compared to pure drug (Table 1). The 1:1 inclusion complex of MH with βCD showed higher solubility than their physical mixture and pure drug. The enhancement in the solubility of the complex is mainly attributed to the formation of a stable inclusion complex of MH with βCD having a stability constant of 120.34 M^{-1}. This indicates that MH has sufficient affinity to bind to the CD cavity as the solubility of the complex found to be increased by 118%. This enhancement in solubility could be due to wetting property and hydrophilicity of the polymer with simultaneous reduction in the crystallinity of the MH caused by kneading process and inclusion into the hydrophobic CD cavity (Longxiao and Suyan 2006).

2.7. Dissolution studies

The *in vitro* dissolution curves of the MH, physical mixture and inclusion complex are shown in Fig. 5. The release rate profiles were expressed as % drug released (vs) time (min). The dissolution time of MH from physical mixture and inclusion complex was determined and $t_{90\%}$ values are reported in Table 2 compared to pure MH alone. The dissolution of MH-βCD inclusion com-

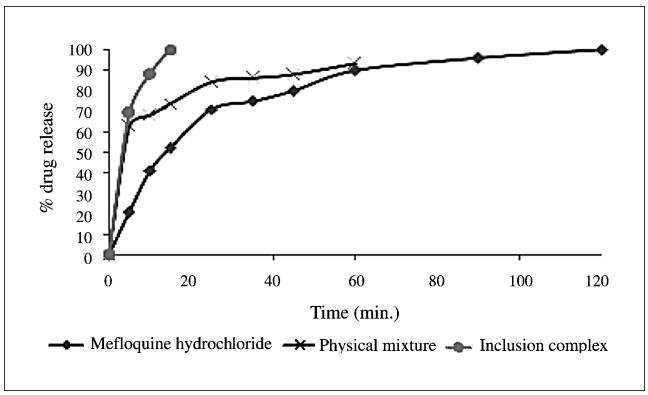


Fig. 5: Dissolution diagram of MH-BCD systems at 37 °C ± 0.5 °C
[a] mean ± SD, $n = 3$, Mefloquine hydrochloride, Physical mixture, Inclusion complex.

plex was higher compared to its physical mixture and the drug alone. The dissolution profile of the kneaded complex showed 90% drug released in only 10 min while that of the physical mixture and pure drug showed in 46 min and > 1 h respectively. This enhancement in dissolution rate for the inclusion complex could be attributed to the higher hydrophilic character of the system due to the presence of βCD, which can reduce the interfacial tension between the MH and the dissolution medium. Also formation of a stable inclusion complex in the solid state and reduction in MH crystallinity in the kneaded complex as confirmed by XRD study responsible for faster dissolution rate. Moreover, in the case of βCD, in the early stage of the dissolution process, the carrier dissolves more rapidly than the drug. Hence, it can act on the hydrodynamic layer surrounding the drug particles, resulting in an *in situ* inclusion process that improves the dissolution of the drug (Mura et al. 2001).

3. Experimental

3.1. Materials

MH was supplied as a gift sample from Macleod Pharmaceuticals Ltd., Mumbai, India. βCD was provided by Panacea Biotech Ltd., Chandigarh, India. All the reagents used were of analytical grades.

3.2. Phase solubility studies

Phase solubility studies were performed in triplicate in distilled water according to the method given by Higuchi and Connors (1965). An excess amount of MH was added to 20 ml of aqueous solution containing various concentrations of βCD (0–0.01 M). The suspensions were shaken on the rotary shaker at 25 ± 2 °C for 5 days. After equilibrium was achieved, the samples were filtered through a 0.45 μm membrane filter and appropriately diluted. The concentration of MH was determined spectrophotometrically (Schimadzu 1700, Japan) at 284 nm. The stability constant was calculated from a phase solubility diagram with assumption of 1:1 stoichiometry according to following equation.

$$K_s = \text{slope}/S_0(1 - \text{slope}) \quad (2)$$

where K_s is the stability constant, slope is obtained from the linear relationship between the concentration of MH and βCD and S_0 is MH solubility.

3.3. Preparation of physical mixture and the solid inclusion complex

For physical mixture, MH and βCD were weighed accurately in 1:1 molar ratio, mixed thoroughly by trituration in a mortar for 45 min and passed through sieve no. 80. The inclusion complex of MH with βCD was prepared in a 1:1 molar ratio by wetting the physical mixture in a mortar with a minimum volume of ethanol/water (1:1, by volume) mixture and kneaded thoroughly with the help of pestle for about 2 h to obtain a paste, which was then dried in a hot air oven at 45 °C, sieved through sieve no. 80 and stored in a dessicator until further evaluation.

3.4. Differential scanning calorimetry (DSC)

DSC measurements were performed on a Schimadzu 60 differential scanning calorimeter. The accurately weighed sample was placed in an aluminum pan. An empty aluminum pan was used as reference. The experiment was carried out in nitrogen atmosphere (flow rate 10 ml/min) at scanning rate of 10 °C/min in the range of 0–300 °C.

3.5. X-ray powder diffractometry (XRD)

The XRD patterns of MH, βCD, inclusion complex, and physical mixture were recorded by a Philips Analytical XRD – PW 3710 (Holland) diffractometer with tube anode Cr over the interval 10–70^0/2θ. The operation data were as follows: Generator tension (voltage) 40 kV, Generator current 25 mA, and scanning speed 2^0/min.

3.6. Fourier transform infrared (FTIR) spectroscopy

Infrared spectra were obtained using a Perkin-Elmer Spectrum- one FTIR spectrometer using KBr disks. The samples were previously ground and mixed thoroughly with KBr. The KBr disks were prepared by compressing the powder. The scanning range was kept from 4000 to 450 cm^{-1}.

3.7. Saturation solubility studies

Saturation solubility studies were performed according to the method described by Higuchi and Connors (1965). Excess of pure drug, physical mixture and inclusion complex were added to 20 ml of distilled water taken in stoppered conical flasks and shaken for 24 h in a rotary flask shaker at room temperature. After shaking to achieve equilibrium, appropriate aliquots were withdrawn and filtered through Whatman filter paper no. 41. The filtrate so obtained was analysed spectrophotometrically at 284 nm.

3.8. Dissolution studies

The *in vitro* dissolution rate studies for MH pure drug, its physical mixture and inclusion complex were performed in simulated gastric fluid without enzyme (900 ml) at 37 ± 0.5 °C using USP II apparatus (Electrolab, India) with a paddle rotating at 100 rpm (U.S. FDA). 250 mg of MH or its equivalent amount of MH-βCD complex was added to dissolution medium and 5 ml of samples were withdrawn at time intervals of 5, 10, 15, 25, 35, 45, 60, 90, and 120 min. The volume of dissolution medium was adjusted to 900 ml by replacing each 5 ml aliquot withdrawn with 5 ml of fresh simulated gastric fluid without enzyme. The solution was immediately filtered through a 0.45 μm membrane filter, suitably diluted and and concentration of MH in the samples determined spectrophotometrically at 284 nm.
The present investigation shows that MH forms an inclusion complex with βCD in the solid state as confirmed by DSC, XRD and FTIR study. Phase solubility study revealed the existence of stoichiometry in 1:1 molar ratio with good stability constant. From these results, it can be assumed that the formation of the inclusion complex of MH with βCD can increase the aqueous solubility of MH. The dissolution profile of the kneaded complex showed 90% drug released in 10 min. The improved dissolution rate compared to physical mixture and pure drug alone may be due to an increase in solubility, brought about by complexation which will eventually lead to faster onset of action as the rate and extent of absorption of MH is dissolution rate limited. From these evidence it can be concluded that the aqueous solubility and dissolution rate of MH can be enhanced by forming an inclusion complex with βCD.

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