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SOLID AND LIQUID STATE INVESTIGATIONS OF MANDELIC ACID CYCLODEXTRIN COMPLEXES

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Abstract

In the present study the solid and liquid phase behaviour of mandelic acid cyclodextrin systems were studied. The samples were prepared using dry grinding/kneading technique in the absence of any solvent. Thermoanalytical methods (TG, DSC, EGD) were used to characterise the solid compounds. In liquid phase the stoichiometry and the stability constants of the complexes formed were determined using UV spectrophotometry. Partial complex formation was found in case of all cyclodextrins used. The amount of uncomplexed mandelic acid varied between 10–20% of the total guest content.

Keywords: cyclodextrin, mandelic acid, stability constant, stoichiometry, UV photometry

Introduction

Cyclodextrins (CD-s), which, as a consequence of their size, shape and chemical characteristics, are suitable for the inclusion of molecules with appropriate geometry, were firstly used in the pharmaceutical and food industry, in the 1970-s. The formation of the special host-guest molecule system brought unexpected improvements in the field of solubility, stability, storage and bioavailability [1–2].

The first publications dealing with the chiral nature of natural and chemically modified CD-s first appeared in the late 1970-s and the early 1980-s. The cyclodextrins were applied for the separation of structural and optical isomers [3–5].

The literature overwieved mentions some chromatographic methods for tracing the selective complex formation [6–13], because of the nature of the investigated problem and the small sample demand. Cyclodextrins as host molecules, might be used either as stationary or mobile phases in these chromatographic methods [9–11].

Several compounds, e.g. drugs [4], fragrance and aroma components [13–16], polar and non-polar carbohydrates [8] and some other model compounds which contain an aromatic ring, an OH or an COO functional group and thus fit the requirements of inclusion complex formation are used as guest molecules. Mandelic acid and its esthers are suitable guest molecules [4, 9–10].

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A number of publications dealt with the separation of the enantiomers of mandelic acid (Fig.1) and its derivatives [17–18], and gave a description about the crystalline structure of the complexes [19–20]. A few authors approached the problem of complex formation from the theoretical side, with the aid of molecule-modelling [21].

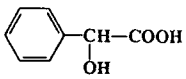

Fig. 1 Structure of mandelic acid

The CD complexes of mandelic acid and its derivatives occur as powder. According to the studied literature [17–18] they may also exist in their dissolved forms. There are no reports about the direct production of the CD complexes of mandelic acid and its derivatives, their solid state characteristics (including thermal properties). No data have been found about the liquid state behaviour (stoichiometry, stability) of the systems mentioned above either.

In a previous paper, the preparation of the α-, β- and γ-CD inclusion complexes of racemic mandelic acid and its esther derivatives with a suspension technique and their solid state characterization using different thermoanalytical methods (TG, DSC, EGA) were discussed [22].

Now, our aim was to prepare the inclusion complexes of racemic mandelic acid with three natural CD modifications using the kneading/grinding preparation technique. Complex formation was followed by thermoanalytical and X-ray power diffraction methods.

Stoichiometries, stability constants of the complexes in liquid phase and the amount of the entrapped guest were determined by UV spectrophotometry.

Materials and methods

Celdex A-100, B-100, G-100 (Nihon Shokuhin Kako Co. Ltd. Tokyo, Japan) and analytical grade racemic mandelic acid (Reanal, Hungary) were used.

Preparation of complexes have been performed with the following procedure:

Fritsch Pulverisette 02102 ball mill was used for the complexation experiments. The one to one initial host:guest molecule ratio was kept constant for all our investigations. The solid products were prepared in the absence of any solvent. The grinding experiments lasted 3 h. The mill was stopped from time to time in order to take samples from the obtained powder mixture, which were directly used for thermal analysis.

For the solid state characterisation of products the following thermoanalytical techniques were used:

TG and DSC curves were recorded on a DuPont 951 thermobalance and a DuPont 910 differential scanning calorimeter (5–6 mg sample mass, 5°C min^{-1} heating rate, 10 l h^{-1} flowing argon atmosphere). Evolved gas detection, EGD (cca. 2 mg of initial sample mass, 8°C min^{-1} heating rate, 1.8 l h^{-1} flowing nitrogen atmosphere)

was carried on a DuPont 916 (Carle 3000) Thermal Evolution Analyser. X-ray diffraction measurements were carried out using a Zeiss HZG-4 powder diffractometer (CuK_α radiation $\lambda = 1.5405$ Å).

In the liquid phase the determination of the stoichiometry of inclusion complexes was determined by the continuous variation (Job's) method [23]; the stability constant was measured according to the Higuchi-Connors [24] and Benesi-Hildebrand [25] methods using GBC 916 UV/VIS spectrophotometer (25°C, $\lambda = 257$ nm, against 1:1 ethanol:water reference solution).

Results and discussion

Thermoanalytical investigations

The knowledge of the thermal behaviour of pure components and their mechanical mixture is necessary for the evaluation of the thermoanalytical curves of the solid preparations. In addition, the curves of the mechanical mixtures give information about the possible interactions (chemical reaction, complexation, etc.) occurring between the host and guest components during heating.

In Fig. 2, the DSC curves of racemic mandelic acid, β-CD and the mechanical mixture are drawn. The endothermic peak at 122°C on the DSC curve of pure mandelic acid is due to the melting. Above this temperature the melt evaporates and decomposes. The DSC curve of β-CD shows that during the heating process up to 120°C the sample looses its water content (which was 12.4% according to the TG measurements).

The curve of the mechanical mixture is considered as the superposition of the pure components. The peak indicating the loss of water is well separated from the melting of mandelic acid. By the evaluation of the curve one can state that no interaction occurred between mandelic acid and cyclodextrin. For example no chemical reaction such as complexation took place. (The same measurements were carried out with mandelic acid–α-CD and mandelic acid–γ-CD samples as well. The obtained curves are not presented here, but their evaluation led to the same conclusion.)

Figure 3 indicates the DSC curves of mandelic acid–β-CD ground samples. The characteristic endothermic peak of the melting of free mandelic acid decreases with the time of grinding, moreover, after an hour of grinding the peak disappeared.

In general, the disappearance of the melting peak is the indirect proof of complex formation. However, this assumption is true only if the free part of the guest is present in its crystalline form in the system.

When the inclusion complex has an amorphous character, e.g. produced using spray drying, freeze-drying or kneading/grinding techniques, the uncomplexed guest may remain also in its amorphous state. In such cases the DSC method is usually not suitable to prove the inclusion complex formation.

In our case, however – as it has already been observed by Nakai [26] and Lin [27] – a crystalline → amorphous transformation takes place during the process of grinding. Besides complex formation this causes the disappearance of the melting peak of mandelic acid. (The amorphisation can be followed by the change of the

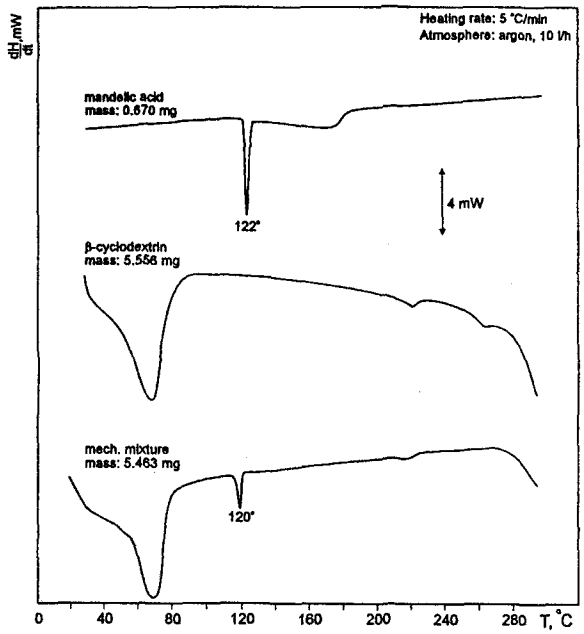

Fig. 2 DSC curves of pure mandelic acid, β-cyclodextrin and their mechanical mixture

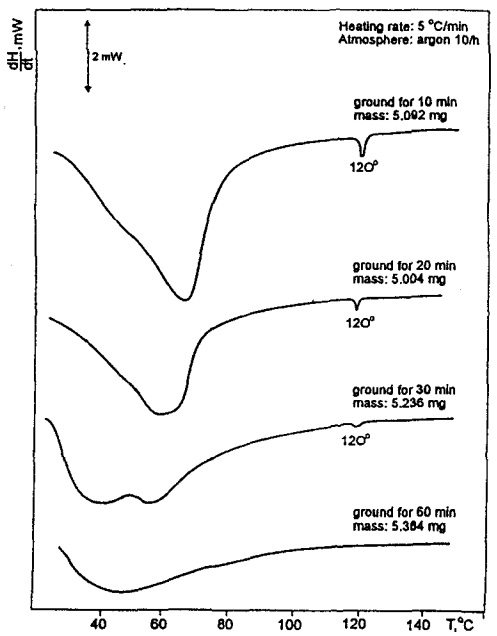

Fig. 3 DSC curves of mandelic acid–β-cyclodextrin samples (Preparation with dry grinding)

shape of DSC curve, in the range of water evolution from CD.) This also means that in such cases the DSC method is not suitable to give an evidence about the complex formation alone.

X-ray diffraction patterns of the pure components, the mechanical mixture and the samples treated by dry grinding are presented in Fig. 4. As can be seen, the diffraction lines become more diffuse after 2 h of grinding, and in 3 h almost the whole sample has transformed into an X-ray amorphous substance.

The X-ray amorphous behaviour of the substance may be attributed to the occurrence of a compound with a disordered microscopic structure. The other possibility is the existence of very small crystallites in the product. In order to prove the process of complexation, a method was developed for the EGD technique. In Fig. 5 the EGD curves of mandelic acid–β-CD samples are shown, where the dotted line be-

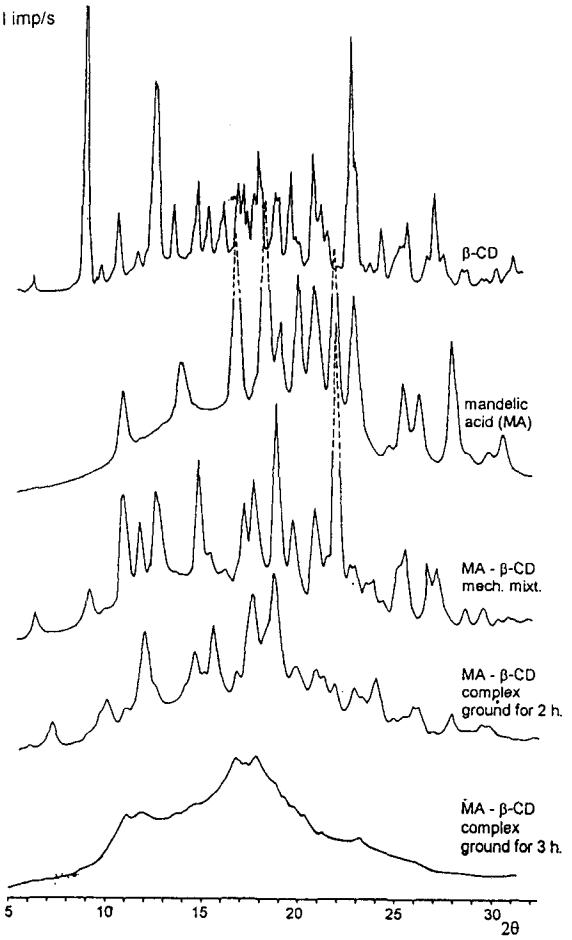

Fig. 4 X-ray powder diffractograms of mandelic acid–β-cyclodextrin samples (Preparation with dry grinding)

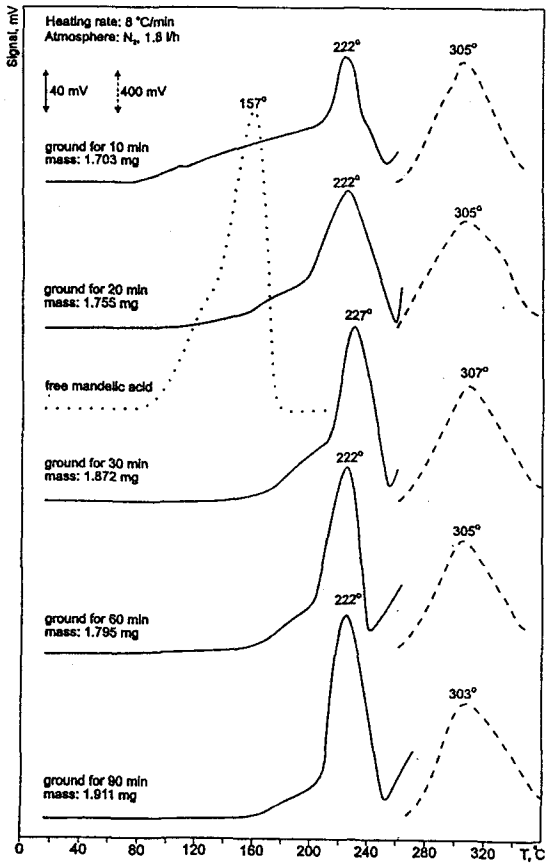

Fig. 5 EGD curves of mandelic acid–β-cyclodextrin samples (Preparation with dry grinding)

longs to the pure guest. The sublimation of mandelic acid starts at about 80°C. Then, between 120–160°C the evaporation/decomposition of the melt takes place. The solid lines indicate the samples taken at the given time.

In the EGD curves, the signal in the range of the evaporation/decomposition peak of free mandelic acid decreases with the time of grinding. After a one-hour treatment the curves are running at the base line, showing that no evaporation of organic material occurred. This means that the complex formation became complete in 30–60 min.

The area under the EGD curves is proportional to the amount of evaporated substance in the given temperature interval. Therefore the amount of uncomplexed mandelic acid that had left the samples can be calculated by integrating the EGD curves from room temperature to about 160°C. The values obtained this way are related to the amount of mandelic acid, thus the curves obtained in this way can be directly compared, Fig. 6. (The decrease of the free guest content is indirectly proportional to the amount of formed mandelic acid–CD complexes.)

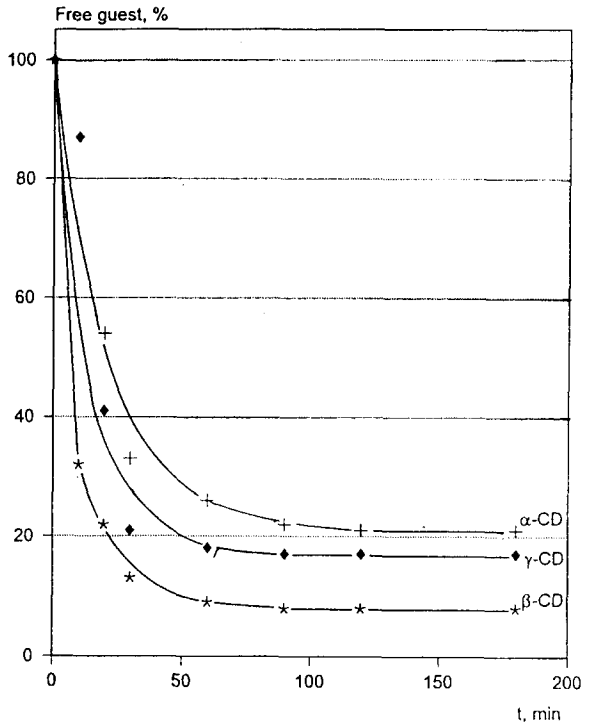

Fig. 6 Decrease of uncomplexed mandelic acid as a function of the grinding time (on the basis of EGD profiles)

The change in the amount of free mandelic acid in the products with the 3 different CD-s are described in the same figure. It can be seen that the main part of the inclusion complexes has already formed in the first 30 min of treatment. The transformation is the fastest in case of mandelic acid–β-CD system. After 120 min of grinding the curves become horizontal. This indicates the end of inclusion complex formation. It is also observed that, in spite of the 1:1 initial molar ratio, not the whole amount of mandelic acid is complexed. The transformation leads to an equilibrium, therefore the amount of inclusion complexes formed cannot be increased by lengthening the time of grinding/kneading. When β-CD was applied as host, 10% of the total quantity of mandelic acid remained outside the CD cavity, while in case of α-CD and γ-CD this amount was about 20%. The different degree of complexation can be explained by the different cavity diameter of the host molecules. According to the results of these experiments, the complexation carried out with β-CD seems to be the most suitable.

For solid products, due to the formation of an amorphous phase, the DSC and X-ray diffraction methods did not give any evidence of inclusion complex formation. The EGD method was found to be suitable to prove the complex formation. By the integration of the EGD curves the amount of the uncomplexed part, which remained in X-ray amorphous state in the sample, could be estimated.

UV spectrophotometric investigations

Determination of stoichiometry

The stoichiometry of the inclusion complexes of racemic mandelic acid formed with different cyclodextrins was determined using the Job (continuous variation) method. The molar fraction of the guest molecule varied between 0 and 1. The nearly constant total concentration was [MS]+[CD]=4.875 mM in the experiments.

In Fig. 7, the Job plots of mandelic acid–CD system are described. The maximum of the curves is found at $r = 0.5$ molar fraction. This leads to the conclusion that in liquid phase between mandelic acid and all the three types of CD complexes with 1:1 molar ratio are formed.

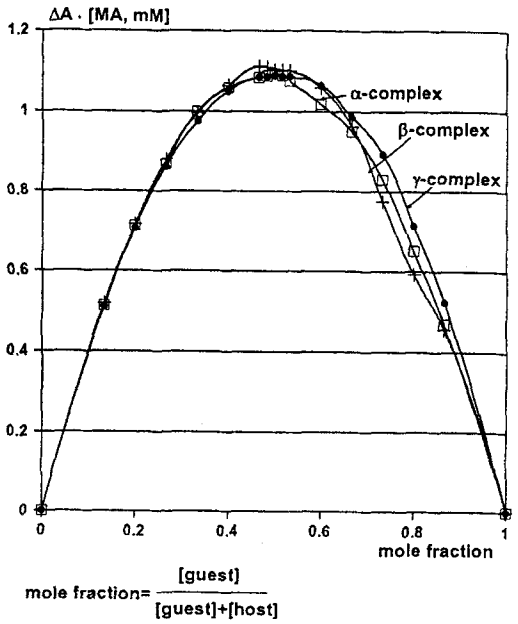

Fig. 7 Job plots of mandelic acid with α-(□), β-(+) and γ-(•) cyclodextrin

From the shape of the Job curves the stability of the complexes may be deduced – the steeper the Job curve is the higher the stability constant of the complex. No significant differences have been observed between the individual curves obtained, which suggest that there is no considerable difference between the stabilities of the complexes. The arcs of our curves are rather low, which means, that low values of the stability constants can be expected.

Determination of stability constants from UV spectral shifts

The literature mentions two methods for UV spectrophotometry to determine the stability constants of the inclusi n complexes in liquid state. One is the Higuchi-

Connors (solubility isotherm) method, where the change in the solubility of the guest molecule is measured with respect to CD concentration. From the slope of the tangent drawn to the initial part of the solubility isotherm, the solubility constant might be calculated with Eq.(1).

$$K = \frac{S_t - S_0}{S_0\{[CD]_t - (S_t - S_0)\}} = \frac{\mathrm{tg}\alpha}{S_0(1 - \mathrm{tg}\alpha)} \quad (1)$$

S_0= solability of guest in liquid state (mol dm^{-3}),
S_t= dissolved amount of guest in the given sample (mol dm^{-3}),
$[CD]_t$= total CD concentration in the given sample (mol dm^{-3}),
$\mathrm{tg}\alpha$= slope of the c_{MS}–c_{CD} solubility isotherm,
K= stability constant (dm^3 mol^{-1}).

In the investigated concentration range (Table 1) straight lines were obtained (Fig. 8). It suggests A_L type isotherms, which also refer to the formation of complexes with 1:1 stoichiometry.

Table 1 Composition of cyclodextrin and mandelic acid solutions

Cyclodextrin used	c_{CD}/mmol cm^{-3}
α-CD	$9.3 \cdot 10^{-3} - 9.3 \cdot 10^{-2}$
β-CD	$1.44 \cdot 10^{-3} - 1.44 \cdot 10^{-2}$
γ-CD	$9.3 \cdot 10^{-3} - 9.3 \cdot 10^{-2}$

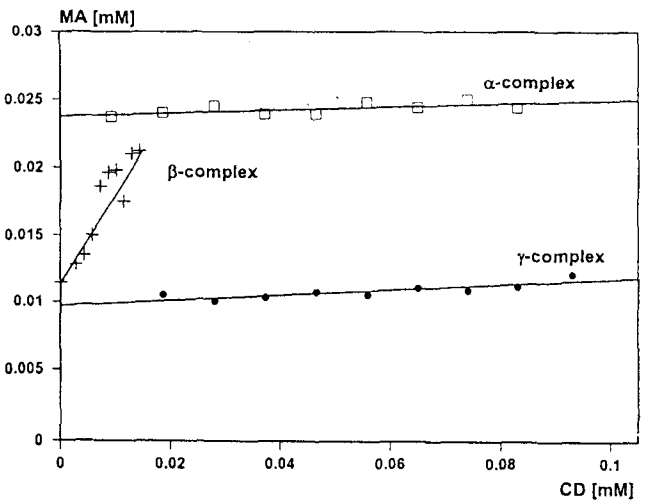

Fig. 8 Solubility isotherms of mandelic acid with α-(□), β-(+) and γ-(•) cyclodextrin

On the basis of our experiments extremely low stability constants were calculated, as shown in Table 2. Among the three different CD complexes, mandelic acid–β-CD has the highest stability constant.

Table 2 Stability constants of mandelic acid with various cyclodextrins

Cyclodextrin used	$K_{[MS\text{-}CD]}/dm^3\ mol^{-1}$
α-CD	$1.1 \cdot 10^{-2}$
β-CD	2.5
γ-CD	$2 \cdot 10^{-2}$

The values obtained support our previous statement about the Job-curves. However, while the study of the Job-curves has led to the conclusion that presumably similar stability constants can be expected for all inclusion complexes, the results obtained by the Higuchi-Connors method definitely demonstrate the difference. The values obtained for all stability constants are so low, that in such the differences cannot be detected by the Job method.

The advantage of the Benesi-Hildebrand method is its ability to follow the change of absorbance of the guest molecule–CD system in a wide concentration range. In our case the values obtained could not be evaluated, which is probably the consequence of the extremely small stability constants.

Conclusions

The thermoanalytical measurements have given evidence that mandelic acid is able to form inclusion complex with α-, β- and γ-cyclodextrins using the kneading/grinding method. It has also been proven that the process leads to equilibrium. The amount of uncomplexed mandelic acid adsorbed on the outer surface of the cyclodextrin varied between 10–20% of the total mandelic acid content.

X-ray powder diffraction measurements have supported the amorphisation of the initially crystalline samples during the process of grinding.

The DSC method alone cannot be applied to follow the complex formation if amorphisation may occur. The EGD technique have proven to be suitable to detect the complexation, in addition the amount of the free guest can also be estimated.

The results of the liquid phase experiments have shown, that the investigated aromatic guest molecule forms CD inclusion complex. The stoichiometric ratio of the formed complex was found to be 1:1 by both the Job method and the evaluation of the solubility isotherms. The complex of mandelic acid in liquid state is characterised by an extremely low stability constant. This phenomenon may be explained by the excellent water solubility of mandelic acid, therefore the equilibrium of complex formation is shifted significantly in the direction of dissociation. Complexation using β-CD has been found to be the most appropriate among the three cyclodextrin modifications used. The possible explanation is the following: probably the β-CD

has the optimal cavity diameter and provides the tightest fit, which is an important requirement from the aspect of complex formation.

* * *

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