Inclusion Complex of 3,9-Bis(N,N-dimethylcarbamoyloxy)-5H-benzofuro[3,2-c]quinoline-6-one (KCA-098) with Heptakis(2,6-di-O-methyl)-β-cyclodextrin: Interaction and Dissolution Properties

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Interactions of KCA-098 with heptakis(2,6-di-O-methyl)-β-cyclodextrin (DM-β-CyD) in solution and in the solid state were studied by the solubility method, UV and fluorescence spectroscopy, powder X-ray diffractometry, and thermal analysis. The KCA-098/DM-β-CyD system showed an Ac type solubility diagram with stability constants of 5870 and 2220 m⁻¹ in aqueous and 10% methanol solutions, respectively. Following the addition of DM-β-CyD, the maximum UV wavelength of KCA-098 was shifted to a longer wavelength and the fluorescence intensity was decreased. A similar spectral change was observed when KCA-098 was dissolved in less polar solvents, especially in proton-acceptor solvents, such as acetone and dimethylsulfoxide, suggesting that KCA-098 interacts with DM-β-CyD through not only a hydrophobic interaction but also hydrogen bonding. The solid complex of KCA-098 with DM-β-CyD in a molar ratio of 1:1 was prepared by the kneading method and the solvent evaporation method, using organic solvents. Powder X-ray diffractometric and differential scanning calorimetric studies indicated that KCA-098 was dispersed as microparticles on the DM-β-CyD complex in the solid state prepared by the solvent evaporation method although it dispersed as crystals in the sample prepared by the kneading method. The dissolution of KCA-098 from the solid complex prepared by the former method was markedly faster than that prepared by the latter method, although it slowed down with the passage of time. The reduced dissolution of KCA-098 was explained by crystallization to the hydrate form in the medium. These data indicate that poorly water-soluble KCA-098 interacts with DM-β-CyD in water and in the solid state and that a fast-dissolving form of KCA-098 can be obtained by evaporating with DM-β-CyD using organic solvents.

Key words KCA-098; DM-β-CyD; complex; dissolution

A new benzofuroquinoline derivative, 3,9-bis(N,N-dimethylcarbamoyloxy)-5H-benzofuro[3,2-c]quinoline-6-one (KCA-098, Fig. 1), has a pharmacological action useful for the treatment of osteoporosis, such as inhibition of bone resorption and stimulation of bone formation.1–3) We previously reported that KCA-098 has three different anhydrous crystal forms, forms 1, 2, and 3, which occur at 260, 152, and 93 °C from the hydrate form.4) Forms 1 and 2 have the same dissolution profile, whereas form 3, a metastable form, has a faster dissolution rate. However, because of the transformation to the hydrate, all anhydrous crystal forms of KCA-098 have the same solubility as the hydrate. Because form 2 crystals are physicochemically stable and their preparation is easy, we selected form 2 as suitable for pharmaceutical formulation. However, some pharmaceutical manipulation is required to increase the dissolution rate of form 2 in order to obtain a sufficiently high oral bioavailability for poorly water-soluble KCA-098.

Cyclodextrins (CyDs), which are cyclic oligosaccharides, and their derivatives are useful pharmaceutical excipients. The molecular structure of these glucose derivatives, which resembles a truncated cone or torus, generates a hydrophilic exterior surface and a nonpolar interior cavity. CyDs can interact with molecules of appropriate size to form inclusion complexes. These noncovalent complexes offer a variety of physicochemical advantages over the original drugs, including the possibility of increased aqueous solubility and physicochemical stability.5) There are several methods for preparing solid complexes depending on the physicochemical properties of the “guest” drug molecules: (a) precipitation based on the phase solubility;6) (b) neutralization;7) (c) spray-drying;8) (d) freeze-drying;9) (e) kneading;10) (f) solvent evaporation;11) and (g) ball-milling and sealed-heating.12) Precipitates are easily prepared with a water-soluble guest, but such preparations are difficult with guests which are slightly soluble in water. In this study, we selected two preparation methods, i.e., the kneading and solvent evaporation methods, to synthesize the solid complex, because KCA-098 is practically insoluble in water as previously reported.4) Generally, ethanol11) and acetone13) have been chosen as an organic solvent for the evaporation method. In our preliminary study, KCA-098 was extremely soluble in halogenated solvents, e.g., dichloromethane, and relatively soluble in alcohol. Further, dichloromethane has been widely used as a solvent for preparing solid dispersions.14) In view of the solubility of KCA-098, we selected a mixture of dichloromethane and ethanol as a solvent for complex formation.

In this study, we found that KCA-098 interacted with heptakis(2,6-di-O-methyl)-β-CyD (DM-β-CyD) in water and/or an aqueous organic solvent through hydrogen bonding as well as via hydrophobic interaction and that KCA-098 dispersed in solid complexes prepared by the solvent evaporation method. Furthermore, rapid dissolution of KCA-098 in

Fig. 1. Structure of KCA-098

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solid complexes was obtained by the solvent evaporation method.

**Experimental**

**Materials** KCA-098 was synthesized by Kissei Pharmaceutical Co., Ltd. Form 2 of KCA-098 was prepared by the previously reported method. DM-β-CyD and other CyDs were provided by Toshin Chemical Co. (Tokyo, Japan), and used without further purification. All other chemicals and solvents were of analytical reagent grade.

**Phase Solubility Studies** The solubility studies were performed according to the method reported by Higuchi and Connors. KCA-098, in an amount that exceeded its aqueous solubility, was accurately weighed in individual 100-ml glass-stoppered flasks to which was added 50 ml water containing various concentrations of CyDs. In addition, the solubility of KCA-098 with DM-β-CyD was also studied in 10% methanol solution. These flasks were shaken at 37 °C in a thermostatically controlled water-bath incubator for 2 h. This short period of shaking was chosen intentionally, because polymorphic transition of the drug from form 2 to the hydrate was observed after 3 h under these conditions as previously reported. The samples were filtered through a 0.45-μm membrane filter, and an internal standard (diphenylamine) was added to the filtrate, and then the KCA-098 content was measured by HPLC as described previously. The apparent 1:1 stability constant, 

\[ \text{Kc} = \frac{\text{slope}}{\text{intercept} \times (1 - \text{slope})} \] (1)

**UV Spectrometry** The UV absorption of KCA-098 was recorded at various DM-β-CyD concentrations in 4—50% methanol aqueous solutions or chloroform using a Shimadzu model UV-2500PC spectrophotometer.

**Fluorescence spectrometry** Fluorescence spectra were obtained by spectrophotometry (JASCO FP-770, Tokyo, Japan) at an excitation wavelength of 244 nm and scanning the emission over the range 300—500 nm. The fluorescence spectrum of KCA-098 was measured in the presence of DM-β-CyD in 4 and 10% methanol aqueous solutions and in organic solvents.

**Preparation of Solid Complexes** Solid complexes were obtained by the solvent evaporation method. KCA-098 and DM-β-CyD were mixed and ground and the particles able to pass through a 355 μm sieve were used for further study. For comparison, DM-β-CyD was treated in the same way. However, the other CyDs used in this study had only a slight effect on drug solubility. In the case of DM-β-CyD and 2HP-β-CyD, the phase solubility diagrams showed an Al type, as defined by Higuchi and Connors, indicating that the aqueous solubility of KCA-098 increased linearly as a function of CyD concentration and that a soluble complex was formed. The apparent 1:1 stability constant, \( \text{Kc} \), for the KCA-098/DM-β-CyD system was 5870 M⁻¹, which may contribute to improving the bioavailability of poorly water-soluble drugs. Furthermore, a solubility study of the KCA-098/DM-β-CyD system was conducted in 10% methanol solution. The system in this solution also exhibited typical Al type solubility, as shown in Fig. 2. The stability constant calculated by Eq. 1 from the initial straight-line portion of the phase solubility diagram, using the equation:

\[ \text{Kc} = \frac{\text{slope}}{\text{intercept} \times (1 - \text{slope})} \] (1)

Results and Discussion

**Interaction between KCA-098 and DM-β-CyD in**

**Aqueous and Organic Media** The solubility method is useful for investigating an inclusion complexation of drugs with CyDs in water because it gives not only the solubilizing ability of the host molecules but also the stability constant of complexes by analysis of the solubility curve. Figure 2 shows the phase solubility diagrams obtained for KCA-098 with various CyDs in water. The extremely low solubility (1.65×10⁻¹⁶ M at 37 °C) of KCA-098 increased with a rise in DM-β-CyD and 2HPβ-CyD concentrations; i.e., the solubility of KCA-098 increased 115- and 94-fold in the presence of 20 mM DM-β-CyD and 30 mM 2HP-β-CyD, respectively. However, the other CyDs used in this study had only a slight effect on drug solubility. In the case of DM-β-CyD and 2HP-β-CyD, the phase solubility diagrams showed an Al type, as defined by Higuchi and Connors, indicating that the aqueous solubility of KCA-098 increased linearly as a function of the CyD concentration and that a soluble complex was formed. The apparent 1:1 stability constant, \( \text{Kc} \), for the KCA-098/DM-β-CyD system was 5870 M⁻¹, which may contribute to improving the bioavailability of poorly water-soluble drugs. Furthermore, a solubility study of the KCA-098/DM-β-CyD system was conducted in 10% methanol solution. The system in this solution also exhibited typical Al type solubility, as shown in Fig. 2. The stability constant calculated by Eq. 1 from the initial straight line was 2220 M⁻¹, which is smaller than that in water. This phenomenon could be explained as follows: the high concentration of methanol interacted with DM-β-CyD in a competitive manner and addition of alcohol to the aqueous solution reduced the polarity of the solvent and, thus, decreased the hydrophobicity difference between the solvent and the DM-β-CyD cavity.
Figure 3 shows the effect of DM-β-CyD on the UV spectrum of KCA-098 in 4% methanol solution at 37 °C. For the spectral measurement, methanol was added to solubilize KCA-098. Both maxima at 326 and 341 nm shifted to longer wavelengths, and the maximum absorbance decreased with the addition of DM-β-CyD up to 0.61 mM, and then increased in the presence of more concentrated DM-β-CyD. The isosbestic point was observed at 299, 328, 337, and 343 nm. No change in the isosbestic points was observed with the CyD concentrations used, indicating the formation of only one species of complex. Similar spectral changes were observed for the inclusion complex of pyrene with β-CyD in aqueous solution.\(^\text{18}\) The UV spectrum of KCA-098 with the highest concentration of DM-β-CyD (2.4×10⁻² M) might be identical to that of the complex, because 90% of the drug was in the complex when 1:1 complex formation was assumed (\(K_c=4720\) M⁻¹, calculated from the linear plot of methanol concentration vs. log \(K_c\)). The UV spectra obtained with various concentrations of DM-β-CyD agreed with the sum of the UV absorption calculated from the fraction of each spectrum of KCA-098 only and its complex with DM-β-CyD. A similar spectral change in KCA-098 caused by DM-β-CyD was observed in 4—30% methanol solution, but the degree of this change was smaller at higher methanol concentrations. In 50% methanol solution, the maximum absorbance decreased upon adding DM-β-CyD with no red shift. The plot of \(1/\Delta A\) vs. \(1/[CD]\) at 343 nm was linear, suggesting the presence of a 1:1 complex. The apparent 1:1 stability constant, \(K_c\), was determined by the Benesi–Hildebrand plot,\(^\text{19}\) and was 90 M⁻¹ at 50% methanol concentration. In 20 and 30% methanol solutions, the \(K_c\) values were similarly calculated to be 1760 and 850 M⁻¹, respectively. A reduction in the stability constant in methanol solution was observed, as in the solubility study described above.

The effect of DM-β-CyD on the fluorescence spectrum of KCA-098 is shown in Fig. 4. The fluorescence intensity of both peak maxima (349.5 and 366 nm) decreased with the addition of DM-β-CyD and shifted to a longer wavelength, from 349.5 nm to 354.5 nm and from 366 nm to 369 nm in 4% methanol solution. The fluorescence spectral change in KCA-098 caused by the addition of DM-β-CyD was smaller in 10% methanol solution than in 4% methanol due to a weaker interaction in the higher concentration of methanol. On the other hand, no change in the fluorescence spectrum of KCA-098 in 4 or 10% methanol solution was seen when 3-O-methyl glucose was added in place of DM-β-CyD. These data suggest that KCA-098 interacts with cyclic glycosides. We also measured the fluorescence spectrum of KCA-098 in various solvents. Table 1 lists the maximum wavelength and fluorescence intensity of KCA-098 in these solvents. The fluorescence intensity of KCA-098 decreased with increasing methanol concentration, i.e., with a decrease in dielectric constant (\(\varepsilon\)). Further, the spectral change in ethanol (\(\varepsilon=24.5\)) was similar to that in methanol (\(\varepsilon=32.7\)), where methanol and ethanol were proton-donor solvents. On the other hand, a dramatic reduction in the fluorescence intensity and a pronounced red shift in the maximum wavelength were observed in proton-acceptor solvents, such as acetone (\(\varepsilon=20.6\)), dimethylsulfoxide (\(\varepsilon=46.5\)) and dioxane (\(\varepsilon=2.2\)). The fluorescence spectral change in KCA-098 caused by the addition of DM-β-CyD was similar to that in proton-acceptor solvents in comparison with proton-donor solvents. The above data suggest that KCA-098 is included in the cavity of DM-β-CyD.
Characterization of Solid Complexes  
From the results of the above experiments, we concluded that KCA-098 formed the most water-soluble complex with DM-β-CyD among the five kinds of CyDs used in this study. Because the phase solubility diagram of KCA-098 with DM-β-CyD was of the A_L type and, thus, a precipitate caused by complexation could not be obtained by adding a high concentration of DM-β-CyD, it is mainly hydrogen bonding. Hydrogen bonding may play an important role in the interaction of the drug with methylated cyclodextrins as well as the hydrophobic interaction.

The powder X-ray diffractograms of KCA-098 and its solid complexes are shown in Fig. 5. KCA-098 and DM-β-CyD showed different crystal states by the kneading and solvent evaporation treatments. After the kneading treatment, KCA-098 maintained the form 2 crystal, whereas KCA-098, after treatment with a mixture of dichloromethane and ethanol in the same manner used for the preparation of the solid complex, showed a different diffraction pattern (Fig. 5(e) and (g)) corresponding to the superimposed diffractograms of KCA-098 (form 2 and the new crystal form) and DM-β-CyD obtained by the same treatment. The diffraction pattern of the kneaded sample (trace (f)) was nearly the same as that of the physical mixture (trace (e)), i.e., one characterized by a peak for form 2 of KCA-098 at 12.7°. These data suggest that KCA-098 and DM-β-CyD separately dispersed as crystalline forms. On the other hand, the complex prepared by the solvent evaporation method showed a small and broad peak, indicating the reduced crystallinity of KCA-098 and DM-β-CyD.

DSC was employed to evaluate the crystal state of KCA-098 with DM-β-CyD. The obtained DSC curves are shown in Fig. 6. The thermogram of intact KCA-098 (form 2) showed an endothermic peak at 269°C, corresponding to the melting point with a transition peak from form 2 to form 1 at 256°C (trace (a)). The new crystals of KCA-098, obtained by the solvent evaporation method, showed an exothermic peak at 130°C corresponding to the transformation to form 2 (trace (b)). The crystal forms of DM-β-CyD, before and after treatment in organic solvent, showed no peak. In the case of the physical mixture of kneaded samples, the broad endothermic peak of KCA-098 was found around 261°C (trace (c)) with the transition peak at 248°C. The DSC thermogram of the kneaded sample (trace (f)) showed the same peaks as that of the physical mixture. These results indicate that KCA-098 is dispersed as a crystal in the kneaded sample, in agreement with the conclusion made from the powder X-ray diffractogram. However, the solid complex obtained by the solvent evaporation method (trace (h)) showed a weak and broad fusion peak, although the physical mixture (trace (g)) showed the characteristic peak of the new crystal of KCA-098. Moreover, this broad endothermic peak was slightly shifted to a

### Table 1. Maximum Fluorescence Intensity and Wavelength of KCA-098 in Various Organic Solvents and Methanol Aqueous Solutions

<table>
<thead>
<tr>
<th>Solvent</th>
<th>Wavelength (nm)</th>
<th>Intensity</th>
</tr>
</thead>
<tbody>
<tr>
<td>4% Methanol solution</td>
<td>349.5</td>
<td>0.4314</td>
</tr>
<tr>
<td></td>
<td>366</td>
<td>0.4906</td>
</tr>
<tr>
<td></td>
<td>366.5</td>
<td>0.4769</td>
</tr>
<tr>
<td>10% Methanol solution</td>
<td>350</td>
<td>0.4194</td>
</tr>
<tr>
<td></td>
<td>368</td>
<td>0.454</td>
</tr>
<tr>
<td>50% Methanol solution</td>
<td>351</td>
<td>0.4076</td>
</tr>
<tr>
<td></td>
<td>368</td>
<td>0.454</td>
</tr>
<tr>
<td>100% Methanol solution</td>
<td>353</td>
<td>0.3214</td>
</tr>
<tr>
<td></td>
<td>370.5</td>
<td>0.3528</td>
</tr>
<tr>
<td>Ethanol</td>
<td>354</td>
<td>0.329</td>
</tr>
<tr>
<td></td>
<td>371</td>
<td>0.3522</td>
</tr>
<tr>
<td>Acetone</td>
<td>354</td>
<td>0.0065</td>
</tr>
<tr>
<td></td>
<td>354</td>
<td>0.0068</td>
</tr>
<tr>
<td>Dioxane</td>
<td>357</td>
<td>0.0608</td>
</tr>
<tr>
<td></td>
<td>375</td>
<td>0.0614</td>
</tr>
<tr>
<td>Dimethylsulfoxide</td>
<td>359</td>
<td>0.0131</td>
</tr>
<tr>
<td></td>
<td>377</td>
<td>0.0134</td>
</tr>
<tr>
<td>Chloroform</td>
<td>357</td>
<td>0.0570</td>
</tr>
<tr>
<td></td>
<td>374</td>
<td>0.0608</td>
</tr>
</tbody>
</table>
Dissolution Studies  The dissolution profiles of the solid complex samples prepared by the two methods were compared with those of the physical mixture and KCA-098 alone. As shown in Fig. 7, the release profile of the kneaded sample was not improved, compared with that of the physical mixture whose dissolution rate was faster than that of the drug alone. On the other hand, dramatically more rapid dissolution was obtained with the complex prepared by the solvent evaporation method, reflecting the microparticle dispersion. The maximum concentration, which was reached within 2 min, was about 2.5 times higher than the maximum dissolution level of the physical mixture. After reaching the highest dissolution level, the concentration of KCA-098 dissolved from the complex decreased and became less than that of the physical mixture after 60 min. After the dissolution study, the precipitate was filtered and its crystal form was characterized by powder X-ray diffractometry. The precipitate, after dissolution of the complex prepared by the solvent evaporation method, showed the hydrate crystal structure of KCA-098, which has the lowest solubility (0.2 μg/ml) among the five known crystal forms.5 In contrast, both the physical mixture and the kneaded sample maintained the crystal form (form 2) of the drug up to 60 min after the dissolution study. From these results, the reduction in the drug concentration of KCA-098 dissolved from the solid complex prepared by solvent evaporation was ascribed to transformation to the hydrate, although a few percent of the dissolved KCA-098 interacted with DM-β-CyD to form a complex in accord with the hydrate constant \((5870 \text{ M}^{-1})\). The relatively small increase in the dissolution rate observed for the physical mixture may be explained by the wetting effect of the DM-β-CyD on the drug particle surface. Since DM-β-CyD dissolves more rapidly in the dissolution medium than the pure drug, it can be assumed that, in the early stages of the dissolution process, the DM-β-CyD molecule will operate locally on the hydrodynamic layer surrounding the drug particles, this action resulting in an in situ inclusion process, which increases the amount of dissolved drug.24 Moreover, the dissolution rate of KCA-098 was faster for the new crystal treated by solvent evaporation than for form 2. Thus, the increase in the dissolution rate was found to be dependent on the preparation method, since the evaporated solid complex exhibited the highest initial dissolution rate. This enhancement can be attributed to microparticle dispersion and complexation in the solid state and to a reduction in the crystallinity of the product, as confirmed by powder X-ray and DSC studies. Moreover, the content of ethanol and dichloromethane in the solid complex prepared by the solvent evaporation method was 0.12% and less than 0.06%, respectively, suggesting that solvent evaporation is a useful and safe method for rapid-dissolving formulations of poorly water-soluble drugs.

In conclusion, DM-β-CyD can interact with KCA-098 in aqueous and methanol solution through hydrogen bonding as well as by hydrophobic interaction, and the solid complex prepared in organic solvent showed a rapid dissolution rate due to microparticle dispersion of the drug.

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