# Interaction between Surface Active Drug (FK906:Rennin Inhibitor) and Cyclodextrins in Aqueous Solution

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**Abstract** □ The aggregation behavior of FK906, which is a peptide like hypertensive agent, in aqueous solution was studied by static light scattering, $^1$H-nuclear magnetic resonance (NMR), and surface tension. These experiments showed a clear critical micelle concentration (cmc) at around $6.3 \times 10^{-3}$ to $1.3 \times 10^{-2}$ M of FK906 aqueous solution. The result of $^1$H NMR experiments revealed that FK906 aggregates primarily by hydrophobic interactions involving the benzyl moiety. The Debye plots from light-scattering studies showed that the apparent molecular weight of aggregated FK906 molecule is 1670 which corresponds to 2–3 molecules of FK906. The effect of α- and β-cyclodextrins on the surface tension of FK906 aqueous solution was investigated. It appeared that the addition of α-cyclodextrin showed very small shift of cmc, but that of β-cyclodextrin shifted the cmc to much higher concentration. The investigation on the surface tension of FK906 aqueous solution in the presence of β-cyclodextrin indicated that FK906 forms a 1:1 complex with β-cyclodextrin. On the basis of these experiments, it appears that β-cyclodextrin has an ability to change the surface active property of FK906 in its aqueous solution. Therefore, it is expected that the addition of β-cyclodextrin to FK906 aqueous solution may prevent the adsorption onto container walls and/or reduce the local irritancy.

## Introduction

Pharmaceuticals with both hydrophilic and hydrophobic groups show a surface active property. Surface active pharmaceuticals usually show critical micelle concentration (cmc) above that at which they tend to aggregate to form micelles in their aqueous solutions. This aggregation behavior of pharmaceuticals sometimes causes a completely different activity below and above the cmc, because the drug molecules exist as monomer below cmc and form aggregates above cmc. For example, the anesthetic potency as a function of drug in solution shows a decrease in activity at the cmc.[1] Furthermore, these surface active drugs sometimes present problems in pharmaceutical formulation. Typical examples are the adsorption of surface active drugs on to container walls, which may result in a loss of free drug at low concentration,[2] and erythrocytes from hemolysis.[3]

To overcome these pharmaceutical problems, many additives have been studied to change the molecular interaction of surface active drugs in their aqueous solutions. Methyl *p*-hydroxybenzoate is one of the example which has been regarded as an effective additive to prevent the gel formation caused by stacking self-association of doxorubicin at the aromatic rings and to shorten the dissolution time of its freeze-dried product.[4] Cyclodextrins also have been regarded as beneficial additives in the pharmaceutical area because they have an ability to protect erythrocytes from hemolysis and shape changes induced with surface active drugs.[5,6] Furthermore, some hydrophilic cyclodextrins, including maltosyl-β-cyclodextrin, inhibit the adsorption of bovine insulin to containers and its aggregation by interacting with hydrophobic regions of the peptide.[7]

Recently, the number of new drug candidates which have peptide-like structure has been increasing due to their specific biological activities. However, they have a risk of the above-mentioned problems in aqueous solution because they have hydrophilic as well as hydrophobic moieties in their structures.[8] Therefore, whether newly developed drugs are surface active or not is particularly significant in early stage since the concept of the preformulation should be established by taking into account the correlation between biological activities and physicochemical properties for these compounds.

In this report, FK906 ($C_{40}H_{63}N_7O_7$·HCl; mw: 790.44), which is a peptide-like synthetic rennin inhibitor, was used as a model compound since FK906 has polar and nonpolar groups, and its aqueous solution foams readily upon agitation. Thus, the aqueous solution properties of FK906 as a surface active drug were investigated by surface tension measurement, a light scattering study, and chemical shift observations in NMR spectra of samples in aqueous solution. The interaction between FK906 and cyclodextrins was also investigated to know whether cyclodextrins have an ability to change the surface active property of FK906.

## Experimental Section

**1. Materials**—FK906 ($C_{40}H_{63}N_7O_7$·HCl; mw: 790.44), (2*S*,3*S*)-cyclohexyl-3-hydroxy-6-methyl-2-[Na-methyl-[(*S*)-2-[*N*-methyl-N-[2-(*N*-methyl-*N*-morpholinocarbonylamino)ethyl]-3-phenylpropinoil]-L-histidyl]aminoheptane hydrochloride, was synthesized at Fujisawa Pharmaceutical Co., Ltd. and used without further purification. The purity determined by area % with HPLC was 99.1%. All other reagents used were reagent grade.

**2. Measurement of Static Light Scattering**—Static light scattering of FK906 aqueous solution was measured at 25 °C using a light-scattering spectrophotometer (model DSL-7000, Otsuka Electronics Co., Osaka) equipped with an argon laser (488 nm). FK906 solution filtered through a membrane filter (pore size 0.2 μm) was used for static light scattering at a scattering angle of 45−135°. The refractive index increment of FK906 was measured at 25 °C using a differential refractometer (model DRM-1021, Otsuka Elecrtrocics Co., Osaka) at 633 nm. Solutions of FK906 ranging in concentration from 4 μg/mL to 80 mg/mL were prepared for static light-scattering measurement.

**3. Measurements of $^1$H-Nuclear Magnetic Resonance Spectra**—$^1$H NMR spectra were measured at 25 °C on a 200 MHz nuclear magnetic resonance spectrometer (model AC 200P, Bruker) in deuterium oxide ($D_2O$) over a concentration range from 1 mg/mL to 50 mg/mL.

**4. Measurement of Surface Tension**—The surface tension was measured based on the Wilhelmy plate method with an automatic surface tensiometer (model CBVP-Z, Kyowa Seimitsu, Tokyo). The platinum plate was heated by an oxidizing flame before use. For the calculation of the surface tension of aqueous solution, the value of 72.0 mN/m was used as the surface tension

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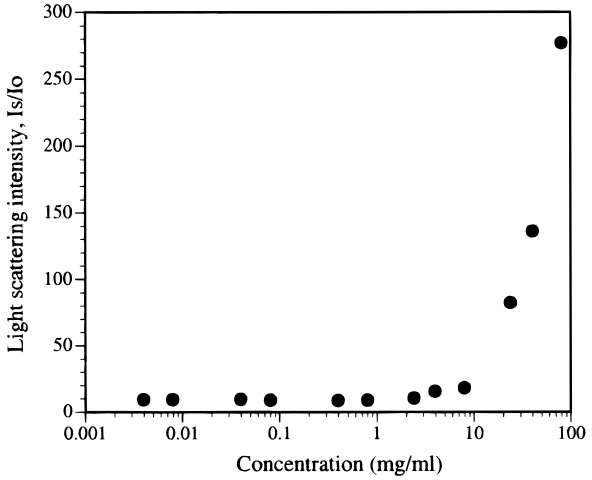


**Figure 1**—Effects of FK906 concentration on light-scattering intensity.

of pure water at 25 °C. The surface tension data for FK906 aqueous solution ranging from 1 $\mu$g/mL to 25 mg/mL were plotted against the logarithm of the concentration as the abscissa, and the intersection of the descending line and another one close to the horizontal was taken as the cmc. The effect of α- and $\beta$-cyclodextrins on the surface tension data for FK906 aqueous solution was also investigated according to the above-mentioned conditions.

## Results and Discussion

**1. Light Scattering Studies on Micelle Formation**—Figure 1 shows the effects of FK906 concentration on light-scattering intensity at an angle of 90°. A rapid rise in the curve at around 10 mg/mL ($1.3 \times 10^{-2}$ M) is evidence of the aggregate formation of FK906 molecule.

This light-scattering method has another advantage of being able to obtain the molecular weight of a self-associated drug substance. Debye plots which are obtained from the light-scattering experiments were used to evaluate the apparent molecular weight of aggregated FK906. In this calculation, the following equations were used.

$$R\vartheta = A(n^2 I / {n_b}^2 I_0) \quad (1)$$

Where $R\vartheta$ is the Rayleigh ratio, $A$ is the calibration constant of the apparatus, $n$ and $n_b$ are the refractive indices of water and toluene used for calibration, and $I$ and $I_0$ are the intensities of scattered and introduced laser light, respectively. The apparent molecular weight of FK906 aggregates, mw, was determined according to eq 2:[9]

$$K(C - C_0)/(R\vartheta - R_0) - 2A_2(C - C_0) = 1/\mathrm{mw} \times P(\vartheta) \quad (2)$$

Where $K$ is the optical constant, $R_0$ is the Rayleigh ratio at critical micelle concentration, $C_0$ is the second virial coefficient, and $P(\vartheta)$ can be assumed to be 1 when the particle size is small enough.

The apparent molecular weight of aggregated FK906 molecules obtained from Debye plots was 1670 which corresponds to 2 or 3 molecules of FK906. Although the aggregation number of FK906 molecules was much smaller than that of a reported drug substance such as a leukotriene $D_4$ receptor antagonist which aggregates more than 16000 molecules,[10] similar results to those from FK906 have been reported for some antibiotics[11] and nicotinamide.[12]

**2. $^1$H-Nuclear Magnetic Resonance Study**—Figure 2 shows the $^1$H NMR spectrum of FK906 in $D_2O$. Signal assignments of main moieties are also shown in Figure 2.

To evaluate the effect of aggregation on chemical shifts of the proton signals, the protons of benzyl, imidazoil, and

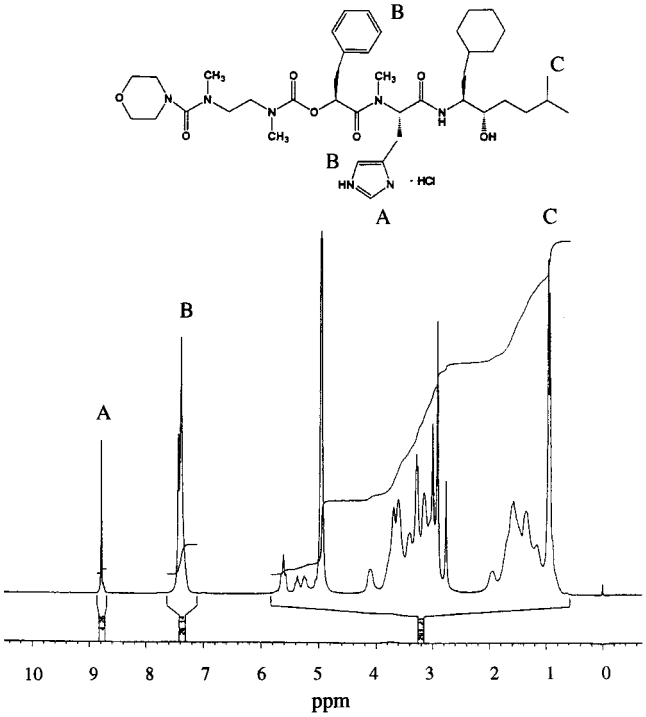


**Figure 2**—$^1$H NMR spectrum of FK906.

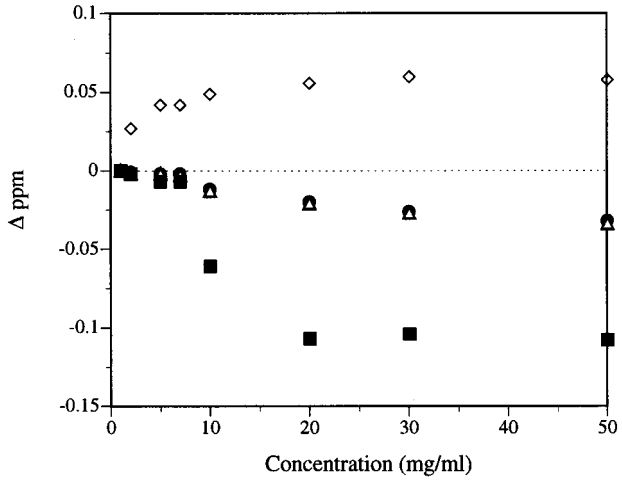


**Figure 3**—Concentration dependence of proton chemical shifts of FK906 in $D_2O$. ●, 0.76 ppm; △, 0.79 ppm; ■, 7.33 ppm; ◇, 8.52 ppm.

methyl groups were selected, since they are considered to have an important role to form micelle.[13,14] The concentration dependence of chemical shifts of above-mentioned protons are shown in Figure 3.

It appeared that by increasing the concentration of FK906 in solution, large upfield shifts were found for aromatic ring protons as compared with other protons. Furthermore, NMR studies also proved the existence of cmc for FK906 aqueous solutions, since plots of proton chemical shifts in Figure 3 showed a marked break at 10 mg/mL FK906 aqueous solution. From these chemical shifts of protons, the cmc value seems to be about $1.3 \times 10^{-2}$ M. These results suggest that FK906 aggregates primarily by hydrophobic interaction involving the benzyl moiety.

**3. Surface Tension Studies**—Figure 4 shows the relation between the surface tension and the concentration of the aqueous FK906 solution.

The curve shows a general decline in surface tension with increase in drug concentration. The value of cmc was estimated as the point of intersection of the extension of

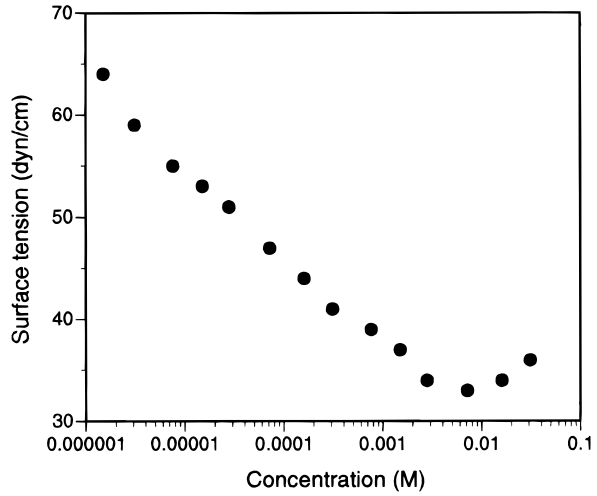


**Figure 4**—Effects of FK906 concentration on surface tension.

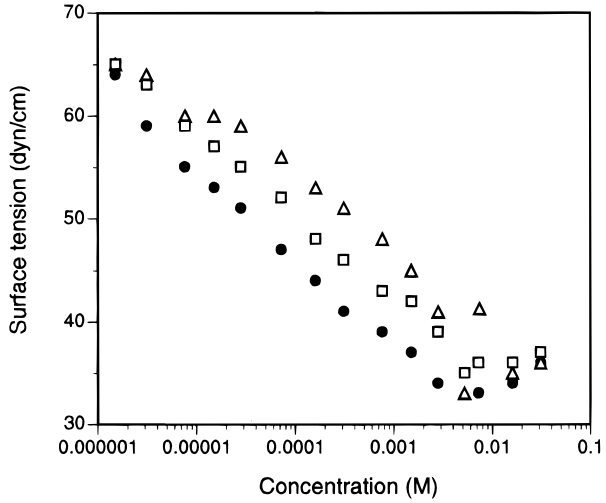


**Figure 5**—Dependence of FK906 concentration on the surface tension in the presence of cyclodextrins. ●, CyDs (0 M); △, $\beta$-Cy ($5.0 \times 10^{-3}$ M); □, α-CyD ($6.7 \times 10^{-3}$ M).

the gentle curve and the horizontal line. The cmc of FK906 in water was about 5 mg/mL ($6.3 \times 10^{-3}$ M) by the surface tension measurement.

These static light-scattering, NMR, and surface tension measurements apparently indicated that FK906 is a typical surface active product, and these experiments showed similar cmc values for FK906 in aqueous solution.

**4. Effects of Cyclodextrins on Self-Association Behavior**—It was expected that the surface tension measurement would indicate the formation of an inclusion complex of FK906 with cyclodextrins, because Funasaki et al.[15] reported that the binding constants of cyclodextrin–surfactant complexes were evaluated by surface tension data. Thus, surface tension measurements were used to evaluate the effects of α- and $\beta$-cyclodextrins on the surface tension property for FK906 aqueous solutions. Figure 5 shows the dependence of surface tension on the FK906 concentration in the presence of α- and $\beta$-cyclodextrins.

From Figure 5, some shifts of the surface tension curve can be seen when $\beta$-cyclodextrin was added to FK906 aqueous solution. Therefore, it was proved that $\beta$-cyclodextrin has an interaction with FK906 molecule. On the contrary, α-cyclodextrin showed only small shifts of surface tension curve, which indicated that the cavity size of α-cyclodextrin is too small to form a inclusion complex with FK906.

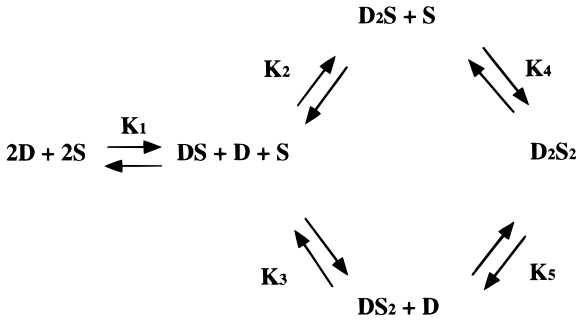


**Figure 6**—Scheme for the formation of the inclusion complex.

The following scheme (Figure 6) with regard to the complex formation between FK906 and $\beta$-cyclodextrin was introduced from a paper describing the complex formation between cyclodextrin and surfactants.[15]

In this scheme, $\beta$-cyclodextrin and FK906 were designated as D and S, respectively, and $K_1$ through $K_5$ represent the stability constants.

The each stability constant presented in Figure 6 is defined as follows:

$$K_1 = [DS]/[D][S] \quad (3)$$

$$K_2 = [D_2S]/[DS][D] \quad (4)$$

$$K_3 = [DS_2]/[DS][S] \quad (5)$$

$$K_4 = [D_2S_2]/[D_2S][S] \quad (6)$$

$$K_5 = [D_2S_2]/[DS_2][D] \quad (7)$$

where [D] represents the concentration of $\beta$-cyclodextrin and [S] is the concentration of FK906. In the case of FK906–$\beta$-cyclodextrin complex, a 2:2 complex of FK906 and $\beta$-cyclodextrin was assumed to be excluded because the binding constants of $K_4$ and $K_5$ reported for surfactant–cyclodextrin complex were almost zero. Thus, the total concentrations of $\beta$-cyclodextrin, $C_D$, and FK906, $C_S$, in the testing solution are expressed as follows:

$$C_D = [D] + [DS] + 2[D_2S] + [DS_2] = [D] + K_1[D][S] + 2K_1K_2[D]^2[S] + K_1K_3[D][S]^2 \quad (8)$$

$$C_S = [S] + [DS] + 2[D_2S] + [DS_2] = [S] + K_1[D][S] + 2K_1K_2[D]^2[S] + K_1K_3[D][S]^2 \quad (9)$$

By using the above equations, the next four cases are represented as follows:

Case 1: only DS is present.

$$\{C_D[S] - (C_S - [S])[S]\}K_1 = C_S - [S] \quad (10)$$

Case 2: DS and $D_2S$ are present.

$$\{1 + 4K_1K_2C_D[S] - K_1^2[S]^2 - (1 - K_1[S])^2 + 8K_1K_2C_D[S]^{1/2}\}/8K_1K_2[S] + [S] - C_S = 0 \quad (11)$$

Case 3: DS and $D_2S$ are present.

$$K_1K_3[S]^3 - K_1[1 + K_3(2C_D - C_S)][S]^2 + [1 + K_1(C_D - C_S)][S] - C_S = 0 \quad (12)$$

Case 4: DS, $D_2S$, and $DS_2$ are present.

$$\{1 + 4K_1K_2C_D[S] - K_1(K_1 + 2K_3)[S]^2 - 4K_1^2K_3^2[S]^4 - 3K_1^2K_3^2[S]^4 - (1 - K_1[S] - 3K_1K_3[S]^2) - [(1 + K_1[S] + K_1K_3[S]^2)^2 + 8K_1K_2C_D[S]^{1/2}\}/8K_1K_2[S] + [S] - C_S = 0 \quad (13)$$

Since the addition of $\beta$-cyclodextrin to the FK906 aqueous solution does not decrease the surface tension of water, the inclusion complexes can be expected to show less surface action than free FK906. Therefore, it can be assumed that the surface tension depends on only the concentration of free FK906 [S] even in the solution including $\beta$-cyclodextrin. As there is a good linearity between the surface tension and the concentration of FK906 below the cmc (see Figure 4), the concentration of free FK906 [S] is obtained from the surface tension of the sample solution.

From Figure 4, the concentration dependence of the surface tension for FK906 solution is written as the following equation.

$$\sigma = -3.77 \ln[S] + 11.5 \quad (14)$$

In this equation, $\sigma$ represents the surface tension.

As the total concentration of $\beta$-cyclodextrin ($C_D$), the total concentration of FK906 ($C_S$), and the concentration of free FK906 [S] are known values, the best fit value for each stability constant was estimated from Akaike information criterion (AIC).[15]

The estimation of binding constants was carried out by minimizing the AIC value which is expressed in the following equation.

$$AIC = n \times \ln(ss) + 2r \quad (15)$$

$$ss = \sum_{i=1}^{n} (\sigma_{i,obsd} - \sigma_{i,calcd})^2 \quad (16)$$

In this equation, *n*, *ss*, and *r* represent that the number of data, sum of squares, and the number of constants, respectively. The minimum value of AIC obtained from this equation was selected and tabulated in Table 1.

It appeared that type 3 is the best of them in the case of $\beta$-cyclodextrin. However, this is an unrealistic model, since the value of $K_3$ is negative. Thus, it is concluded that FK906 forms a 1:1 inclusion complex with $\beta$-cyclodextrin, since type 1 is the second fit model among these models ($K_1$ = 2000). Spectroscopic (circular dichrorism spectra) analysis was performed to confirm the formation of inclusion complex of FK906 with $\beta$-cyclodextrin. As a result, the obtained spectra strongly supported the complex formation of FK906 with $\beta$-cyclodextrin. However, it was difficult to determine the exact molar ratio of FK906/$\beta$-cyclodextrin complex, since there were some deviations in the magnitude of circular dichrorism spectra. On the contrary, it is expected that surface tension method gives us rather detailed information to discuss the model of complex formation, since quantitative treatment for cyclodextrin−FK906 interactions including 1:1, 1:2, 2:1, and 2:2 complexes was taken into account.[15]

## Conclusion

It was confirmed that FK906 shows aggregation behavior in aqueous solution. Three different experiments, $^1$H NMR spectrometry, light scattering, and surface tension, showed almost the same cmc at $6.3 \times 10^{-3}$ to $1.3 \times 10^{-2}$ M. It appeared that the addition of $\beta$-cyclodextrin strongly affected the surface tension of FK906 aqueous solution. Furthermore, the surface tension measurement suggested that the aggregation of FK906 was prevented by the formation of an inclusion complex with $\beta$-cyclodextrin.

Therefore, it is expected that the addition of $\beta$-cyclodextrin to FK906 aqueous solution may solve the pharmaceutical problems such as toxicity which comes from the surface active property of FK906.

**Table 1. Best Fit Value of Binding Constants for FK906 and $\beta$-Cyclodextrin**

| complexes | $K_1$ | $K_2$ | $K_3$ | ss | *A* |
|---|---|---|---|---|---|
| (1) DS | 2000 | – | – | 0.284 | –11.8 |
| (2) DS, $D_2S$ | 1000 | 0.11 | – | 1.26 | 606 |
| (3) DS, $DS_2$ | 2500 | – | –85 | 0.0119 | –44.8 |
| (4) DS, $D_2S$, $DS_2$ | 1700 | 0.11 | 24 | 0.425 | –3.4 |

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