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Host–Guest Interaction between Swelling Clay Minerals and Poorly Water-Soluble Drugs. 1: Complex Formation between a Swelling Clay Mineral and Griseofulvin

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Abstract. The formation of a complex between a swelling clay mineral and griseofulvin (GF), a poorly water-soluble drug, was examined. A strong host–guest interaction between the neutral drug molecules and the clay mineral was observed not only in the solid state but also in aqueous dispersion. The powder X-ray diffraction patterns revealed the disappearance of a crystalline phase of GF through host–guest interaction for samples having low GF contents. The complex formation was confirmed to be due to monolayer adsorption on the basis of quantitative thermochemical analyses. The strong interaction between GF and the clay was also detected when the complex powder was dispersed in an aqueous medium on the basis of the intensity changes of from free GF solution in CD and fluorescence spectra as compared with those observed for the free GF solution.

Key words. Poorly water-soluble drug, griseofulvin, swelling clay mineral, complex formation, amorphous, crystallinity, monolayer adsorption.

1. Introduction

Swelling clay minerals, such as montmorillonite, are well known as inorganic host compounds. They are built up of layered structures which undergo interactions with various kinds of guest compounds, especially with cationic compounds, to form intercalation complexes accompanied by an expansion of the basal spacings. Clays have exchangable cations or polar groups, such as silanol groups, on the surfaces of the inner layers, which undergo cation exchange, electrostatic interactions, and hydrogen bonding interactions with polar substances [1]. Such intercalation compounds of clays with metal ions [2, 3], organic cations [4], polymers [5], and surfactants [6], have been widely invesigated with a view to the development of new functional materials such as catalysts [7] and organogels [8, 9].

Since the inner layers of swelling clay minerals provide extremely polar environments, the formation of complexes between clays and relatively nonpolar substances has scarcely been studied to date. In the present investigation, a neutral drug having a poor solubility in water was chosen as a guest compound for incorporation into a swelling clay mineral with the aim of developing a new drug carrier. The authors have found that a swelling clay mineral can interact strongly with a neutral drug by a mechanism different from the intercalation mode exercised by cationic and polar substances. The physicochemical properties of the clay–drug complex was examined by means of powder X-ray diffraction, differential scanning calorimetry

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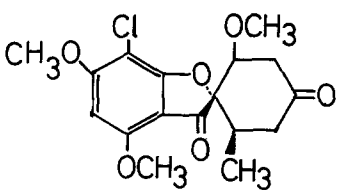
(DSC), scanning electron microscopy (SEM), as well as by circular dichroism (CD) and fluorescence spectroscopy. A neutral drug, griseofulvin (GF), was confirmed to interact strongly with a clay mineral: the basal spacing of the host was not expanded, but the crystalline GF phase disappeared. The formation of a complex was also suggested in an aqueous dispersion on the basis of the intensity increase of CD and fluorescence spectra.

2. Experimental

2.1. MATERIALS

A synthetic hectorite (Laponite XLG obtained from Laporte Industries Ltd.) was used as the swelling clay mineral. The stated chemical composition is $(Mg_{7/3}Li_{1/3})Si_4O_{10}(OH)_2Na_{1/3}$. The average particle size and the specific surface area are 5.5 μm and 330 $m^2\,g^{-1}$, respectively. Griseofulvin (GF), which was purchased from Muromachi Chemicals Co. Ltd., was used as the neutral and poorly water-soluble drug without further purification. Acetone for the preparation of the complexes (Wako Pure Chemical Industries Ltd.) was of reagent grade and was used without further purification.



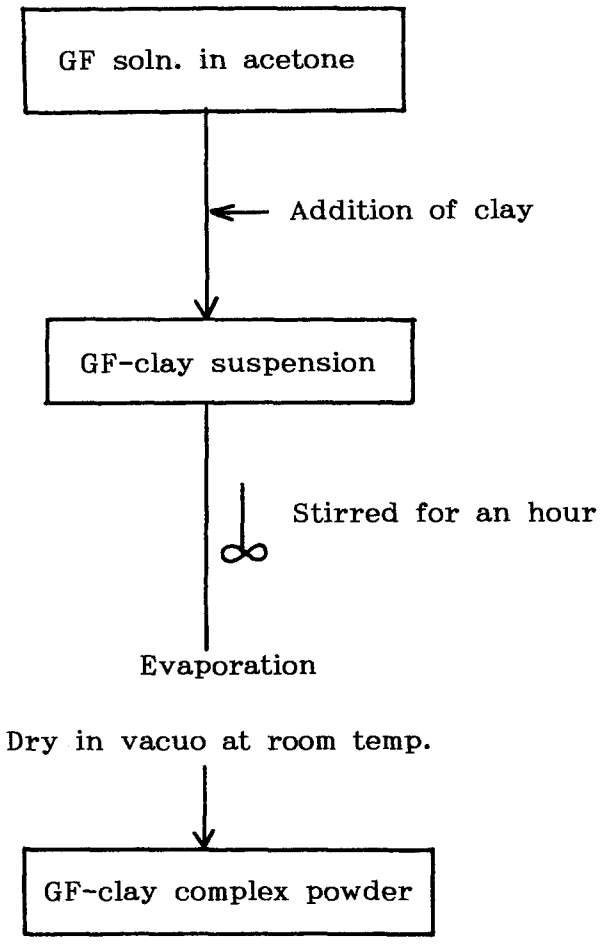
Griseofulvin (GF)

2.2. PREPARATION OF THE COMPLEX POWDER

The GF–clay complexes having different GF contents were prepared according to the procedure shown in Scheme 1 (the solvent method). GF was completely dissolved in acetone to which an appropriate amount of clay was added. This suspension was stirred for an hour, the solvent was evaporated off, and the residue was dried *in vacuo* at room temperature. The clay powder with no GF was similarly treated with acetone in order to use it as a reference material. Physical mixtures of the clay and GF were also prepared in a mortar for comparison.

2.3. X-RAY DIFFRACTION

Powder X-ray diffraction patterns of the complexes were recorded on a JEOL JRX-12VA diffractometer by using CuK_α radiation generated at 40 kV, 100 mA.



Scheme 1

2.4. SCANNING ELECTRON MICROGRAPHS (SEM)

Scanning electron micrographs were observed on a Hitachi S-510 scanning electron microscope, using gold-coated samples.

2.5. DIFFERENTIAL SCANNING CALORIMETRY (DSC)

DSC measurements were carried out on a DSC-200 instrument (Seiko Instrument Inc.) for a 10-mg sample of each solid specimen in an aluminum pan at a heating rate of 5°C min^{-1}. The degree of crystallinity of the adsorped GF was evaluated from the heat of fusion calculated from an endothermic peak area at the GF melting point.

2.6 CIRCULAR DICHROISM (CD) SPECTRA

Aqueous dispersions of the complexes having the same GF concentration ([GF] = 2.7×10^{-5} mol dm^{-3}) were prepared using deionized water ($\sigma < 1$ μs cm^{-1}) as a solvent. CD spectra were recorded at 25°C on a JASCO J-500 spectropolarimeter.

2.7. FLUORESCENCE SPECTRA

Fluorescence spectra of aqueous dispersions of the various complexes at a GF concentration identical with that for measurements of CD spectra ([GF = 2.7×10^{-5} mol dm^{-3}) were recorded at 25°C on a JASCO FP-770 fluorophotometer using an excitation wavelength of 353 nm. An equilibrium constant for the complex formation was evaluated from intensity changes of the fluorescence maximum.

3. Results and Discussion

3.1. X-RAY DIFFRACTION

The structural changes of swelling clay minerals caused by the complex formation with guest compounds were generally investigated by the low-angle X-ray diffraction patterns in the range of $2\theta < 10°$. The basal spacing (d) of the GF–clay complex prepared by the solvent method is shown in Figure 1 as a function of the weight fraction of GF (ϕ_{GF}). The basal spacing of a swelling clay obtained from the $d(001)$ diffraction peak is generally enlarged by the formation of intercalation complexes as observed for inclusion of cationic and polar substances. In the present system, however, such an intercalation was not observed, as is clear from the X-ray diffraction data. The basal spacing of the clay treated with acetone was 14.7 Å, and remained constant in the range of 14–15 Å, even after complex formation with GF, independent of the GF content.

On the other hand, the diffraction patterns in the 2θ range from 10° to 30° give us information about the degree of crystallinity of the drug. Figure 2 illustrates the X-ray diffraction patterns of GF crystals in various GF–clay complexes. As for the samples with relatively higher GF contents, clear diffraction patterns originating from the GF crystals were observed, although their intensities were less than that of GF alone. In the case of the complex containing 5% GF, however, the diffraction peaks of GF crystals disappeared completely and exactly the same patterns were observed as the clay mineral presented alone. This result indicates that part of the GF molecules is adsorbed in an amorphous phase upon the formation of a complex with clay, and all of the crystalline GF exists in an amorphous phase when the GF content is low. Since the diffraction patterns due to the crystalline GF were still observed for the physical mixture at a GF content of 5%, disappearance of the crystalline patterns is not due to the sensitivity of the instrument, but to the fact that the complexes are more readily produced by the solvent method than by the physical mixing method.

These results obtained by X-ray diffraction prove that complex formation does take place between the clay and the neutral drug. Although the constant basal

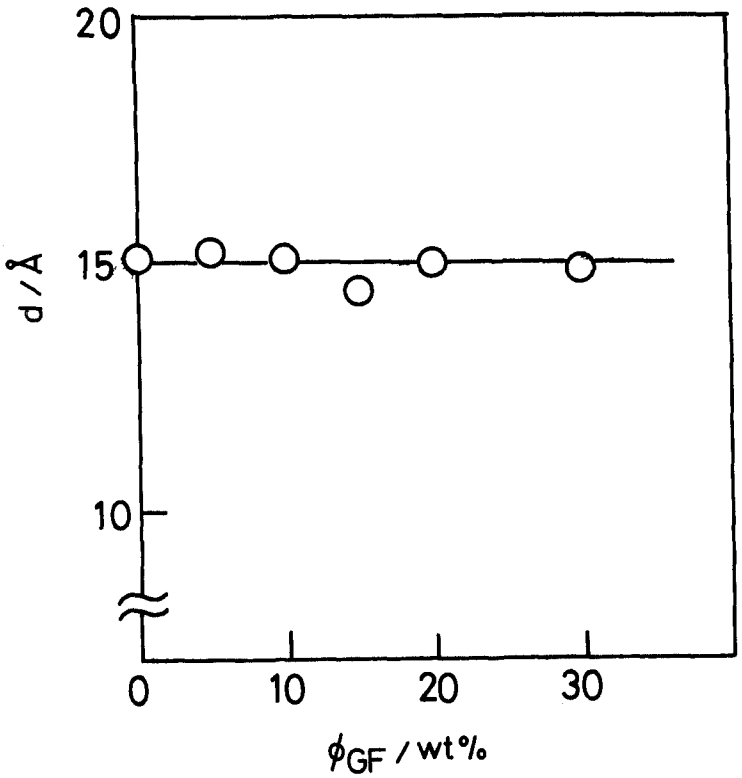


Fig. 1. Correlation of basal spacing (d) of GF–clay complexes with GF weight fraction (ϕ_{GF}).

spacing (Figure 1) suggests that intercalation complexes were not formed in the present system: the individual GF molecules must be dispersed on the surface of the clay without mutual association, based on our observation of the disappearance of the crystalline patterns due to the drug. This host–guest interaction is completely different from the intercalation mechanism which has been widely reported for inclusion of polar substances by clay minerals.

3.2. SCANNING ELECTRON MICROSCOPY

The complexes were examined by SEM in order to obtain direct confirmation of the disappearance of the drug crystals. Figure 3 shows the micrographs for spherical particles of the clay alone (a) and those including 5 wt% (b) and 20 wt% (c) of the drug, respectively. The shape of the spherical particles containing 5 wt% GF is almost identical with that of the clay particles containing no GF. On the other hand, aggregation caused by excess drug crystals was observed along with spherical clay particles when the clay contains 20 wt% of GF. These micrographic data are consistent with the results obtained by the powder X-ray diffraction method.

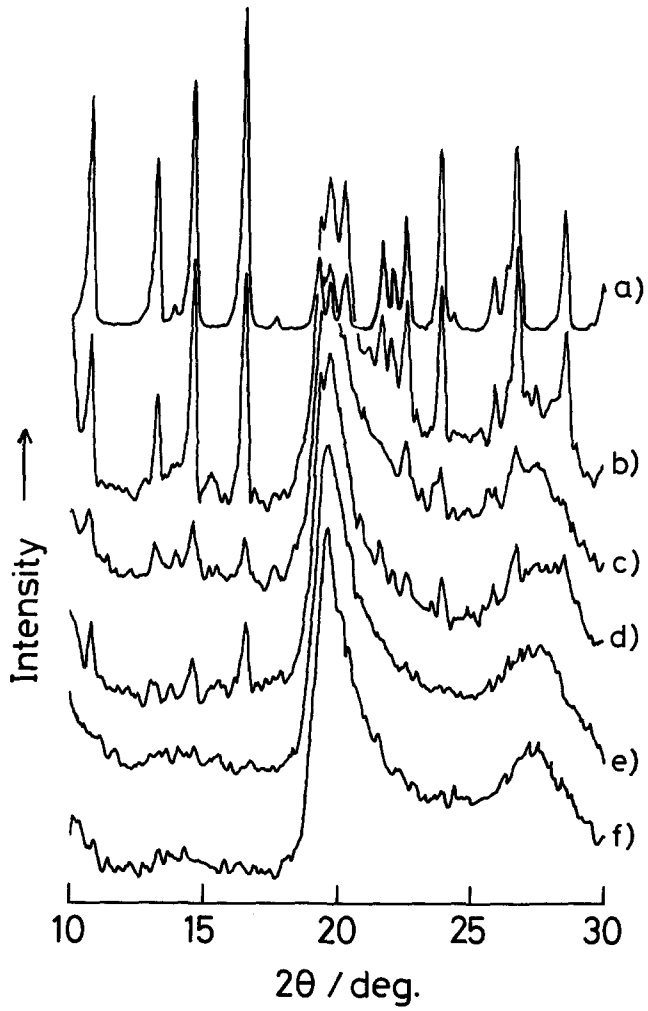


Fig. 2. X-ray diffraction patterns of griseofulvin supported by clay mineral. (a) GF alone, (b) GF/clay(w/w) = 20/80, (c) GF/clay = 10/90, (d) GF/clay = 5/95 (physical mixture), (e) GF/clay = 5/95, (f) clay alone, treated with acetone.

3.3. DSC MEASUREMENTS

The DSC thermograms of complexes containing various GF contents are shown in Figure 4. Although a shift of the melting point (220°C) was not observed upon complex formation, the endothermic peak due to GF fusion gradually decreased along with a decrease in the GF weight fraction. Finally, the peak disappeared completely for the sample containing 5 wt% GF. This result also indicates that a part of the GF molecules interacts in an amorphous state with the clay, and the molecules were completely amorphous when the GF content in the clay mineral was low. Since the endothermic peak was still detected in the physical mixture containing

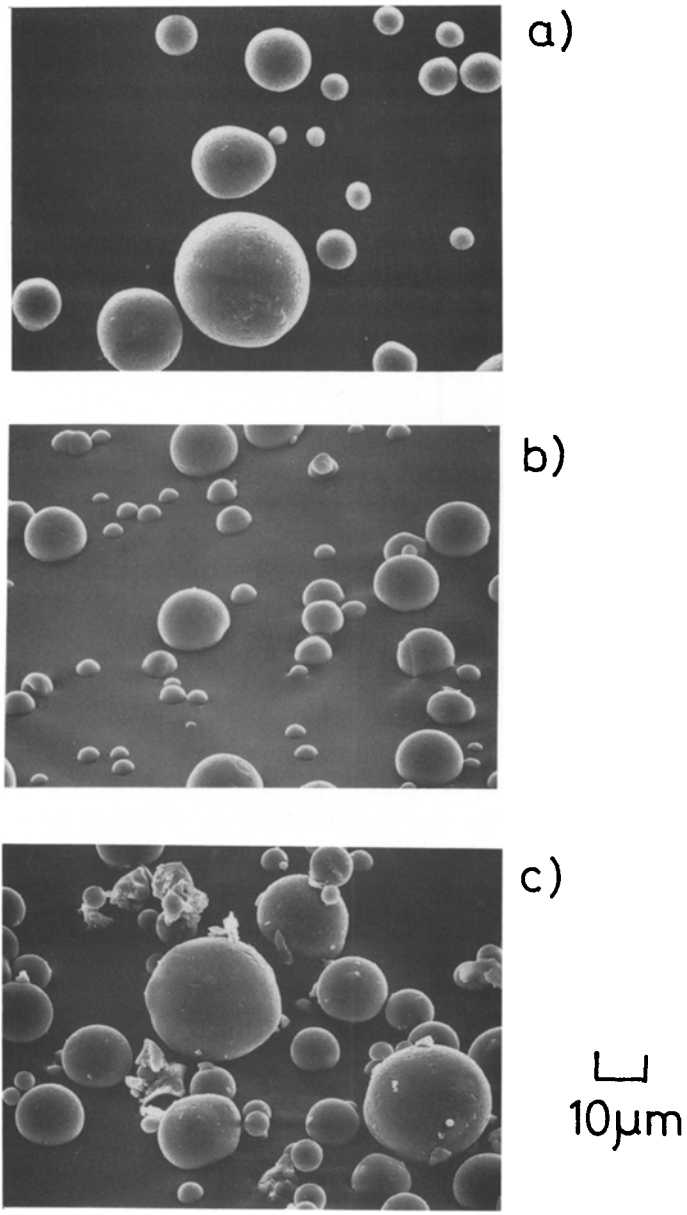


Fig. 3. Scanning electron micrographs for powder samples of GF–clay complexes. (a) clay alone, (b) GF/clay(w/w) = 5/95, (c) GF/clay = 20/80.

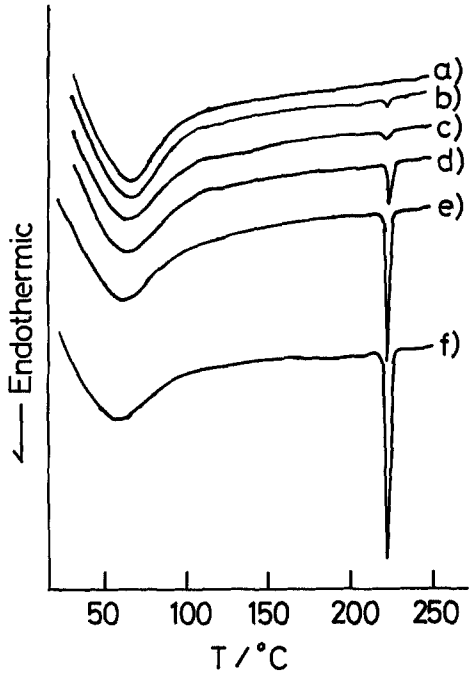


Fig. 4. DSC thermograms of GF–clay complexes. (a) GF/clay(w/w) = 5/95, (b) GF/clay = 5/95 (physical mixture), (c) GF/clay = 10/90, (d) GF/clay = 15/85, (e) GF/clay = 20/80, (f) GF/clay = 30/70.

5 wt% GF, the solvent method is seen to be superior to physical mixing for preparing a homogeneous GF–clay complex.

The crystallinity of the drug in the clay was quantitatively evaluated on the basis of the heat of fusion (ΔH) obtained from the area of the endothermic peak of GF melting. Since the endothermic peak observed at 220°C is due to the fusion of GF crystals, the ΔH decrease corresponds to the decrease in the degree of crystallinity of GF. The values of ΔH^*, ΔH per 10 mg of GF, were plotted against the weight fraction as shown in Figure 5. If all of the GF molecules are present in a crystalline state, ΔH^* must retain a constant value ($\Delta H_0 = 84.9$ mJ mg^{-1} for GF alone) independent of the GF/clay weight ratio. The actual values of ΔH^*, however, gradually decrease as the GF/clay weight ratio is reduced. This decrease in ΔH^* seems to be due to a decrease in the crystalline fraction of GF upon formation of a complex with the clay. Finally, all the GF molecules are in an amorphous state when the weight fraction becomes less than 10 wt%.

Since the degree of crystallinity of the adsorbed GF can be obtained by $\Delta H^*/\Delta H_0$, the amount of the crystalline GF (C mg) is obtained from equation (1).

$$C = (\Delta H^*/\Delta H_0) W_{GF} \tag{1}$$

Thus, the amount of amorphous GF can be expressed by Equation (2).

$$A = [(\Delta H_0 - \Delta H^*)/\Delta H_0] W_{GF} \tag{2}$$

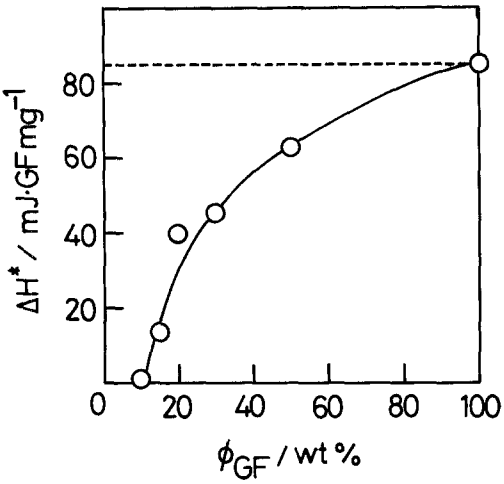


Fig. 5. Relationship between GF weight fraction and ΔH^* (ΔH per g clay).

Here, W_{GF} is the weight of GF in 10 mg of the complex powder. Figure 6 shows the A values as a function of C, which is equivalent to an adsorption isotherm of GF on the clay surface. In the case of lower GF contents, the added GF was selectively adsorbed in an amorphous phase. The A value gradually increased along with an increase in the GF weight fraction, and finally reached a constant value when the crystalline GF appeared. This behaviour is similar to a Langmuir adsorption isotherm so that the interaction between the amorphous GF and the clay can be interpreted in terms of monolayer adsorption theory.

The Langmuir equation for the present system can be written as follows:

$$C/A = 1/Kb + (1/b)C \tag{3}$$

The plots of C/A vs. C must be linear if the complex formation obeys the Langmuir adsorption theory. Figure 7 shows a linear relationship between C and C/A for the present system. This relationship means that the interaction mode between GF and the clay is referred to monolayer adsorption on the clay surface without intermolecular interactions among the individual GF molecules. The monolayer capacity for GF adsorption is represented by the b value obtained from the reciprocal of the slope of Figure 7. The b value for this system was calculated to be 0.137 (g g-mixture^{-1}). This value means the maximum amount of GF which can be adsorped in an amorphous phase is about 13.7% per g of the mixture, and 15.9% per g clay, respectively.

In summary, a part of the GF molecules ($\phi_{GF} < 14\%$) are embedded in the clay by monolayer adsorption without significant interactions among the GF molecules. The rest of the GF molecules are adsorbed as crystals, showing the same physicochemical behavior as observed for solid GF.

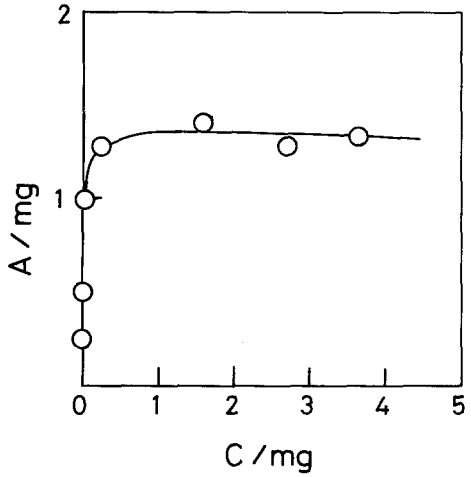


Fig. 6. Relationship between amounts of crystalline GF and amorphous GF.

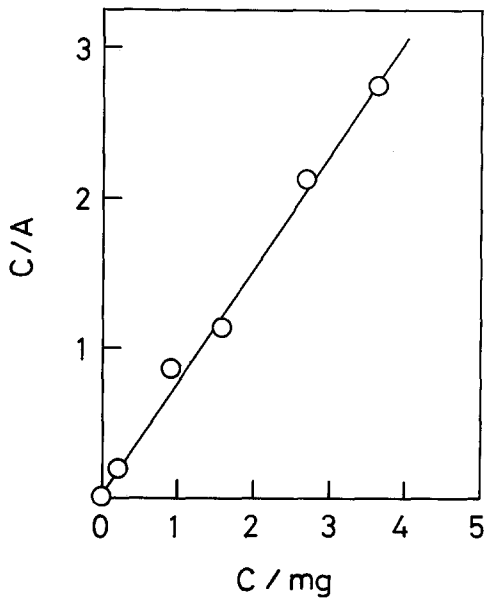


Fig. 7. Relationship between C and C/A. See text for definition of C and A.

3.4. CD SPECTRA

The stability of the GF–clay complex when it was dispersed in an aqueous solution was examined by CD and fluorescence spectroscopy. Since the GF molecule is optically active, CD spectroscopy is an appropriate method for the investigation of a change in the state of GF molecules both before and after complex formation with the clay. Figure 8 shows CD spectra of the GF–clay complexes dispersed in

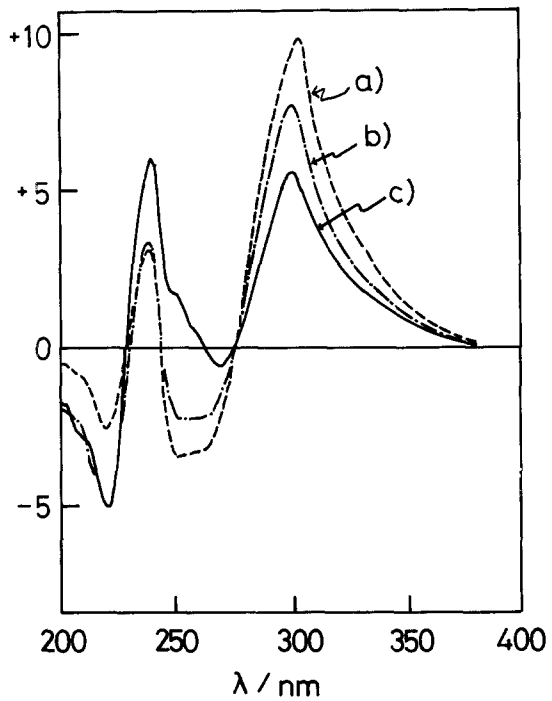


Fig. 8. CD spectra of the aqueous dispersions of GF–clay mixtures at 25°C. (a) GF/clay(w/w) = 5/95, (b) GF/clay = 5/95 (physical mixture), (c) GF alone. [GF] = 2.7×10^{-5} mol dm^{-3}.

pure water. In spite of the same GF content, the CD intensity at 300 nm of the complex prepared by the solvent method was higher than those of GF alone and the physical mixture. Clay minerals tend to recognize optically active substances, and their application to the separation of chiral materials has been investigated [10]. This increase in CD intensity is attributed to the induced CD caused by the clay, in addition to the inherent optical activity of GF. Therefore, the intensity change is taken to be a measure of the extent of the host–guest interaction, and the GF–clay interaction in the complex produced by the solvent method is stronger than the interaction in a physical mixture. This may be attributed to the absence of intermolecular interactions among the individual GF molecules, suggesting that Langmuir adsorption also takes place in an aqueous dispersion.

In the light of the above results, the physicochemical properties of the complex of GF and the clay, as dispersed in pure water, are almost identical with those of the solid sample. The GF molecules in the complex in an aqueous dispersion are also suggested to be in the amorphous state; interacting only with the clay surface, and not with the neighboring GF molecules.

3.4. FLUORESCENCE SPECTRA

Fluorescence spectra were measured in order to investigate further the interaction between the drug and the clay in an aqueous dispersion. Since GF in an aqueous solution enhances a fluorescence peak at around 440 nm by the excitation at 353 nm, this spectroscopic method is suitable for an analysis of this host–guest interaction.

Figure 9 shows fluorescence spectra of the complexes having various GF contents. The fluorescence intensity of the GF–clay complex was higher than that of the free GF, and the intensity became greater as the GF content was decreased. This behavior is similar to that observed by CD spectroscopy, and is also attributed to a specific effect provided by the clay surface. A fluorescence intensity usually increases when a guest compound is captured in an hydrophobic environment, such as a cyclodextrin cavity [11]. In the present system, however, fluorescence enhancement was not detected when GF was dissolved in hydrophobic solvents such as chloroform and acetone. Therefore, it is suggested, on the contrary, that GF molecules are included in a relatively hydrophillic environment in the clay. An increase in fluorescence intensity is often caused by suppression of the molecular motion; thus, in the present system, the GF molecules may be captured in the specific active site on the surface of the clay.

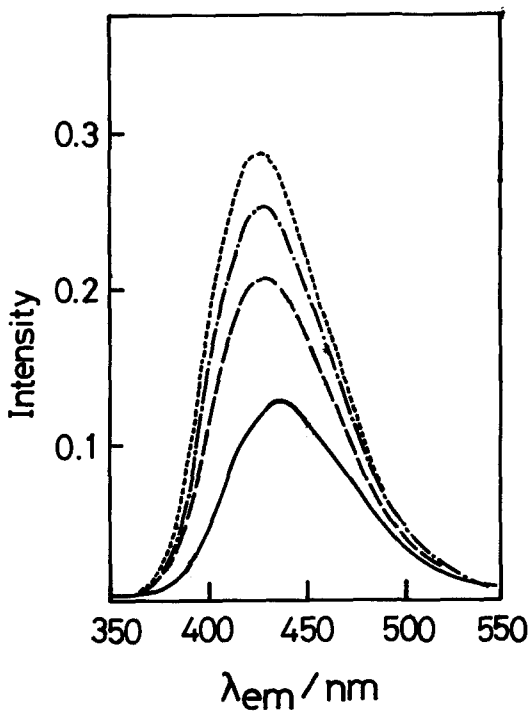


Fig. 9. Fluorescence spectra of the aqueous dispersions of GF-clay complexes at 25°C. (. . .); GF/clay (w/w) = 2.5/97.5, (– · –); GF/clay = 5/95, (– –); GF/clay = 10/90, (——); GF alone. [GF] = 2.7×10^{-5} mol dm^{-3}.

Kondo *et al.* examined a mechanism for inclusion by cyclodextrin on the basis of intensity changes of fluorescent probes [12]. This evaluation method was applied to the present system on the basis of the following assumptions: (1) one GF molecule forms a 1 : 1 molar ratio complex at a specific active site on the clay surface, and the active sites behave independently of each other. (2) GF molecules dispersed in an aqueous solution are composed of two components; GF–clay complex and free GF. (3) The intensity change (ΔF) is proportional to the concentration of the GF–clay complex.

The equilibrium of complex formation between GF and an active site on the clay (C_a) in an aqueous solution can be expressed in the form of Equation 4 based on assumption 1.

$$GF + C_a \rightleftharpoons GF \cdot C_a \tag{4}$$

Thus, the equilbrium constant K for the complex formation is defined by Equation 5, where $[GF]_0$ is the concentration of free GF.

$$K = [GF \cdot C_a]/[GF]_0[C_a] \tag{5}$$

As for assumption 2, the total concentration of GF ($[GF]_t$) is expressed as follows.

$$[GF]_t = [GF]_0 + [GF \cdot C_a] \tag{6}$$

Consequently, the following relationship is given by a combination of Equations 5 and 6.

$$[GF \cdot C_a]/[GF]_t = K[C_a]/(1 + K[C_a]) \tag{7}$$

Based on assumption 3,

$$\Delta F = q[GF \cdot C_a] \tag{8}$$

where q is a proportionality constant. The relationship between $[C_a]$ and ΔF is then given by Equation 9.

$$1/\Delta F = (1/Kq[GF]_t)(1/[C_a]) + 1/q[GF]_t \tag{9}$$

The value of $[C_a]$ is expressed in molar concentration in order to evaluate the equilibrium constant K. According to the DSC results, the molar concentration of the active site per g clay can be obtained from the value of b in Equation 3. The b value per g clay is calculated to be 0.159 (g g-clay^{-1}); $0.159/352.77 = 4.51 \times 10^{-4}$ (mol g-clay^{-1}) of GF is adsorbed on the clay surface. This value is referred to as the molarity of the active site for GF adsorption per g of clay. Thus, the values of $[C_a]$ (mol dm^{-3}) are readily calculated from the concentration of the dispersed clay. The $1/\Delta F$ value was plotted against $1/[C_a]$ obtained from Equation 9, as shown in Figure 10. The linear correlation given in Figure 10 means that the interaction between CF and the clay is consistent with assumption 1. Hence it is suggested that the adsorption of GF in aqueous solution is also the monolayer mode, in a manner similar to that observed for the solid dispersion. The equilibrium constant K is given by the slope of the line according to Equation 9. The q value, which is obtained from the intercept on the $1/\Delta F$ axis of Figure 10, is 7.60×10^3, affording $K = 1.90 \times 10^4$. This K value is extremely high for an inclusion complex. For example, the equilbrium constant for inclusion of 6-*p*-toluidine-*o*-2-naphthalene

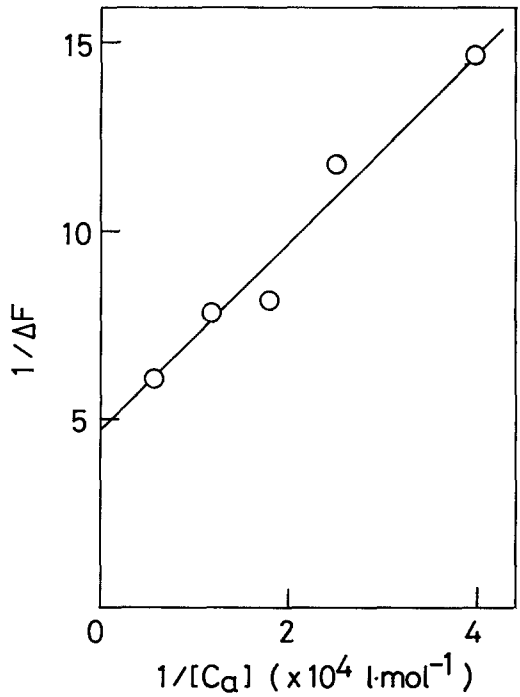


Fig. 10. Relationship between $1/[C_a]$ and $1/\Delta F$. See text for definition of symbols.

sulfonate (TNS) by β-cyclodextrin was reported by Kondo *et al.* to be 1.54×10^3 [15].

The present equilibrium constant indicates the high affinity of GF for the active site on the clay surface. In other words, the formation of the complex between GF and the clay is brought about by a specific interaction which is stronger than ordinary physical adsorption.

4. Conclusion

A swelling clay mineral was proved to interact strongly with the neutral griseofulvin. The clay–drug complex, however, is not an intercalation compound, and is different from inclusion complexes of clay minerals with cationic substances. Quantitative evaluations indicate that the GF molecules interact with specific active sites on the clay surface plausibly by monolayer adsorption, and the monolayer capacity of the clay was calculated to be about 0.16 per g of clay. The extremely high value of the equilibrium constant indicates that the affinity of GF for the active site is strong enough for crystal growth to be retarded even in the aqueous dispersion. This host–guest interaction seems to be applicable to pharmaceutical manipulations to improve the solubility of poorly water-soluble drugs which have low bioavailability in the crystalline state.

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