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Regular Article

Improving the Solid-State Photostability of Furosemide by Its Cocrystal Formation

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The photostability of three types of furosemide (FUR) cocrystal (FUR-caffeine, FUR-urea, and FUR-nicotinamide cocrystals) was studied under irradiation with a D65 fluorescent lamp. The coloration of the FUR-urea pellets was significantly faster than that of the intact FUR, whereas the coloration of FUR-nicotinamide was suppressed compared with that of intact FUR and the other cocrystals. In the case of FUR-urea, the chemical degradation of FUR increased by approximately 6.6% after irradiation for 90 d. On the other hand, FUR-nicotinamide showed better chemical stability, with only 1.3% of FUR degraded, which was significantly lower than the other cocrystals. The FUR-urea pellets showed a UV-Visible absorption spectrum similar to that of intact FUR, while the absorption range of FUR-nicotinamide shifted to a shorter wavelength. The light sensitivity of FUR-nicotinamide was improved because of the much lower emission of the D65 fluorescent lamp in the absorption range of the cocrystal.

Key words furosemide cocrystal; photostability; solid-state UV-Visible spectrum; caffeine; urea; nicotinamide

Introduction

Furosemide (FUR) (Chart 1) is used extensively as a loop diuretic or antihypertensive agent and is known to be unstable when exposed to light.[1,2] For this reason, an orange-red press-through package that is light resistant is used for the commercially available tablet form. However, when the packaging is removed to refill a prescription in a one-dose package to enhance patients' adherence, the tablets are consequently exposed to light, which could decrease the quality of the dosage form. It is therefore desirable to improve the photostability of the active pharmaceutical ingredient (API) itself.

Cocrystals are multicomponent crystals, and the components bind to one another through nonionic interaction.[3–5] The physical properties, thermal and oxidative stability, *etc*., of the API can be improved by cocrystallization with various cocrystal formers (coformers). For example, the solubility of megestrol acetate and saccharin 1 : 1 cocrystal was enhanced more than that of the API itself.[6] Miconazole hemisuccinate cocrystals have a superior intrinsic dissolution rate and stability.[7] The stability of adefovir dipivoxil gallic acid cocrystal hydrate improved compared with that of the pure API.[8]

FUR was cocrystallized with various coformers such as caffeine (CAF), urea (UREA), *p*-aminobenzoic acid, nicotinamide (NIC), *etc*.[9–12] Goud *et al.* demonstrated that the solubility of FUR-CAF was approximately 6-fold higher than that of FUR.[9] FUR is unstable under light, as described above. However, the light stability of those cocrystals has not yet been studied. Therefore, we first evaluated the light stability of three types of FUR cocrystals (FUR-CAF, FUR-UREA, FUR-NIC). The structures of the three coformers are shown in Chart 1.

Experimental

Materials Bulk FUR powder (purity >99.0%) was purchased from Tokyo Chemical Industry Co., Ltd. (Tokyo, Japan) and anhydrous CAF (purity >98.5%), NIC (purity >98.5%), and urea (purity >99.0%) were purchased from Nacalai Tesque, Inc. (Kyoto, Japan). Those samples were used without purification. The commercial solvents for cocrystal preparation and HPLC analysis were also used without further purification.

Preparation of FUR Corystals Three FUR cocrystals were prepared using the previously reported methods.[9] FUR 0.68 g and CAF 0.40 g (1 : 1 M ratio) were mixed in an agate

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mortar after the addition of 20 mL acetone until the solvent evaporated. FUR 0.99 g and NIC 0.37 g (1 : 1 M ratio) were mixed in an agate mortar after the addition of 15 mL of ethanol until the solvent evaporated. FUR 1.02 g and urea 0.18 g (1 : 1 M ratio) were mixed in an agate mortar after the addition of 1 mL of acetone until the solvent evaporated, and the mixing process was repeated 10 times. The three types of cocrystal were dried *in vacuo* at room temperature. These physical mixtures (PMs) were prepared by mixing the drug and each coformer (1 : 1 M ratio) in a vortex mixer.

Preparation of Pellets FUR 250 mg and three cocrystal powders were compressed using an accurate compression/ten-

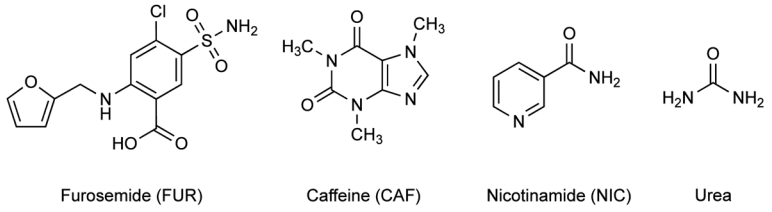

Chart 1. Structure of Furosemide and Three Coformers (Caffeine, Nicotinamide, Urea)

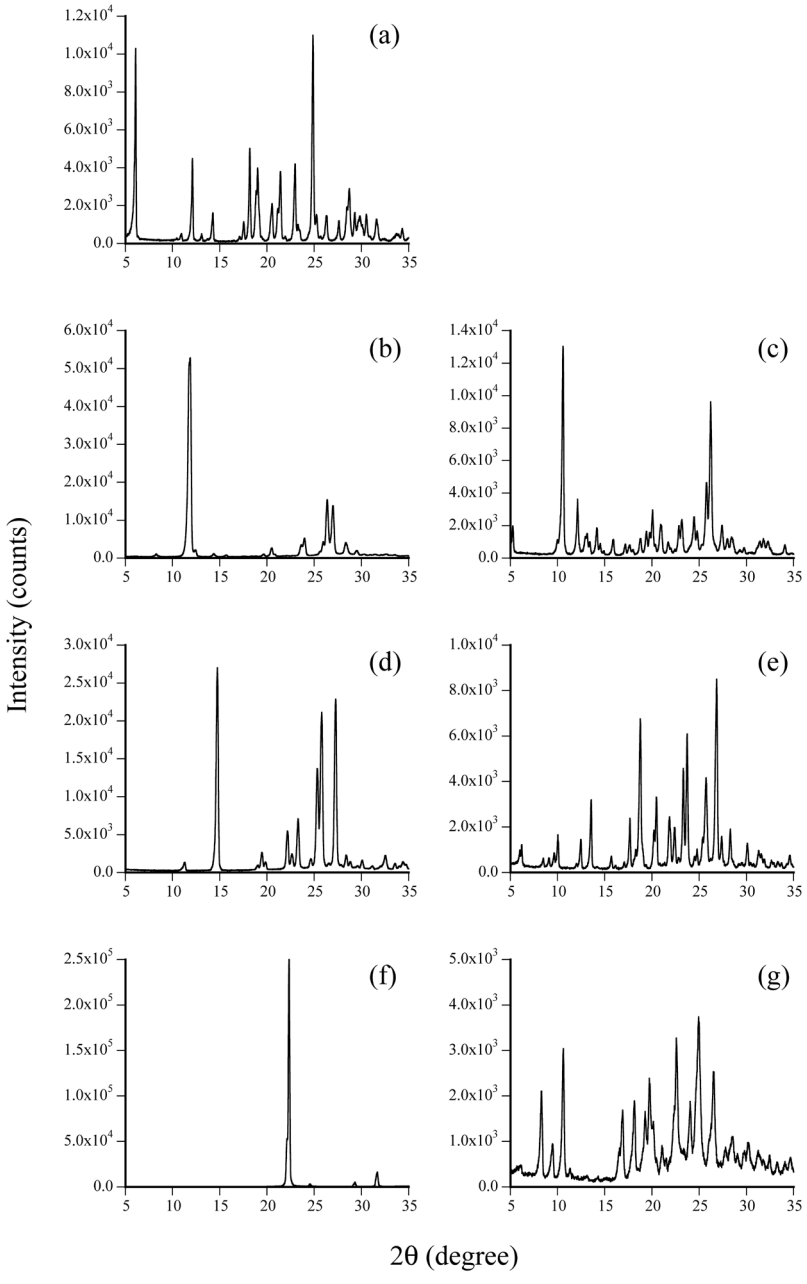

Fig. 1. Powder X-Ray Diffraction Profiles of FUR, Coformer and Cocrystal

(a) FUR, (b) CAF, (c) FUR-CAF, (d) NIC, (e) FUR-NIC, (f) UREA, (g) FUR-UREA

sion testing machine (TG-50kN, Minebea Co., Tokyo, Japan) equipped with flat-faced punches and a cylindrical die (8 mm i.d.).

Irradiation Test The pellets were stored in a light-irradiation tester (Light-Tron LT-120, Nagano Science Co., Takatsuki, Japan) equipped with a D65 fluorescent lamp for use in color comparison and inspection. The illuminance was set at 3500 lx. The irradiation tests were carried out at 25°C.

Powder X-Ray Diffractometry X-Ray powder diffraction (PXRD) patterns were measured using an X-ray diffractometer (RINT Ultima, Rigaku, Tokyo, Japan) with Cu$K\alpha$ radiation at room temperature. The operating conditions were as follows: voltage, 40 kV; current, 30 mA; diffraction angle, 5–50° (2θ); and scanning speed, 0.02°/min.

Thermal Analysis Thermograms of the solids were recorded on a differential scanning calorimeter (DSC) (3100SA, Netzsch Japan K.K., Kanagawa, Japan) from 35 to 250°C. The operating conditions in the open-pan system were as follows: sample weight, 2 mg; heating rate, 5°C/min; and N_2 gas flow rate, 30 mL/min.

Solid-State UV-Visible (UV-Vis) Spectrometry The diffuse reflectance UV/Vis absorption spectra of FUR and the cocrystals were recorded on a UV-2450 system (Shimadzu, Kyoto, Japan) equipped with an integrating sphere unit (Shimadzu ISR-2200) at room temperature. A cell was filled with sample powder, and the spectra were acquired in the wavelength range from 300 to 450 nm. Barium sulfate was used as the reference standard. The reflection spectra obtained were modified using the Kubelka–Munk function, and the spectral data were transformed by the normalized function.

Colorimetric Measurement The surface color of the compressed sample pellet was measured with a color reader (CR-13, Konica Minolta Japan, Inc., Tokyo, Japan) after the designated irradiation times. The color difference (ΔE^* ab) before and after irradiation was calculated using Eq. (1) to evaluate the degree of coloration:

$$\Delta E^* \mathrm{ab} = \sqrt{(L_t^* - L_0^*)^2 + (a_t^* - a_0^*)^2 + (b_t^* - b_0^*)^2} \quad (1)$$

where ΔE^* ab is the color difference, L^* is lightness, and a^*, b^* are coordinates. All values were the averages of three measurements.

HPLC Analysis HPLC analysis of FUR and cocrystal powders exposed to light was carried out based on the purity of the FUR entry in the 16th edition of the Japanese Pharmacopoeia in a Waters Alliance 2695 HPLC system with a 2489 UV/Vis detector equipped with Waters Empower 3 software (Waters, Milford, MA, U.S.A.). The column (LiChrospher 100 RP-18 [5 μm], 4.6 × 150 mm, Merck, Darmstadt, Germany) was operated at 35°C at a flow rate of 1.4 mL/min. The mobile phase was composed of a mixture of water, tetrahydrofuran, and acetic acid (70 : 30 : 1 v/v/v). After 335-h irradiation, 25 mg of the sample was dissolved in the mixing solvent of water, acetonitrile, and acetic acid (489 : 489 : 22 v/v/v). Subsequently, the diluted solution in the same solvent was analyzed using the above HPLC system.

Results and Discussion

Characterization of FUR Cocrystals Figure 1 shows the PXRD patterns of intact FUR, three coformers, and prepared cocrystals. Each cocrystal exhibited characteristic patterns,

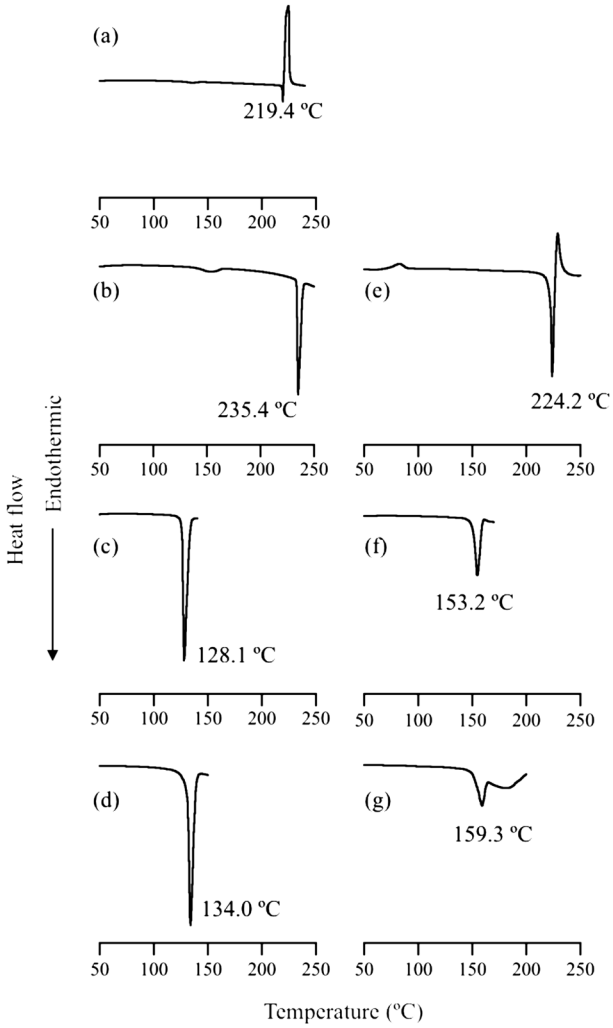

Fig. 2. DSC Profiles of FUR, Coformer and Cocrystal

(a) FUR, (b) CAF, (c) FUR-CAF, (d) NIC, (e) FUR-NIC, (f) UREA, (g) FUR-UREA

and the PXRD patterns were different from those of intact FUR and coformers. Furthermore, the PXRD patterns of the cocrystals were confirmed to be the same as those reported by Goud *et al.*[9] The DSC profiles of FUR and three cocrystals are shown in Fig. 2. The FUR used was form I based on the PXRD patterns and DSC profile.[2] The DSC profiles of FUR-CAF, FUR-NIC, and FUR-UREA cocrystals showed a unique endothermic peak at 224.2, 153.2, and 159.3°C, respectively. The temperatures of these endothermic peaks were approximately the same as reported by Goud *et al.*[9]

Appearance Change and Degradation of the Three Types of Cocrystal Figure 3A shows the time courses for the coloration of FUR, PMs of FUR and coformers, and the three types of cocrystals under D65 fluorescent lamp at 3500 lx and 25°C. The surface color of white FUR pellet turned from white to pale yellow during exposure to light and $\Delta^* E$ ab increased quickly with increasing the irradiation time. The PMs of FUR and each coformer also changed color. On the other hand, the coloration rate differed among the cocrystals. The coloration of the FUR-NIC pellet was very slow compared with that of FUR and PMs, while the surface color of FUR-UREA pellets changed more quickly than that of the others. Figure 3B shows the $\Delta^* E$ ab values of FUR, PMs, and

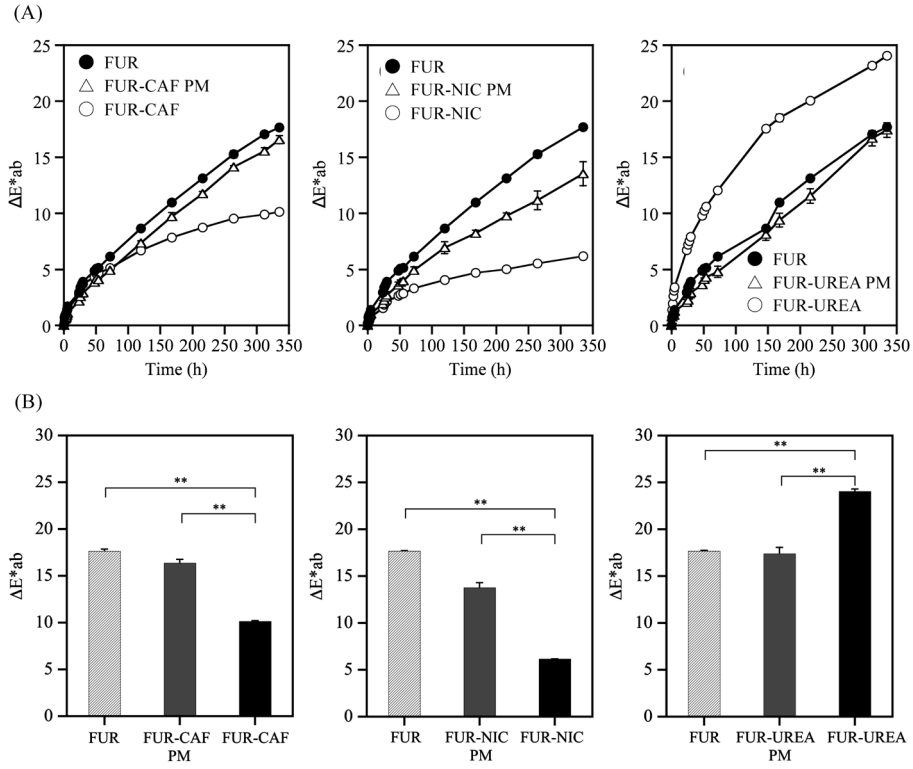

Fig. 3. Changes in Color Difference (Δ*E* ab) (A) and Δ*E* ab after 335 h-Irradiation (B) under D65 Lamp of Cocrystal, PM, and FUR

Data represent the means ± S.D. ($n = 3$). $^{**}p < 0.01$, significantly different among the data.

Table 1. FS Degradation (%) after 90 d-Irradiation by D65 Fluorescent Lamp

Coformer	Physical mixture (A)	Cocrystal (B)	B/A
	Degradation (%)		
CAF	7.03	5.30	0.754
NIC	5.17	1.28*	0.248
UREA	9.79	6.56	0.670

Data are the means of three experiments. $^{*}p < 0.05$, significantly different from the value in FS-NIC PM.

cocrystal pellets after irradiation for 335 h under the D65 fluorescent lamp. The Δ*E* ab of FUR-CAF and FUR NIC pellets was significantly decreased compared with the value of intact FUR and PMs. On the other hand, FUR-UREA seemed to be the most unstable when exposed to light, because the Δ*E* ab of those pellets showed the highest value after 335-h irradiation under the D65 fluorescent lamp.

The degrees of degradation (%) of PMs and cocrystals after 90-d irradiation with the D65 fluorescent lamp are shown in Table 1. The degradation rate of FUR in FUR-NIC was significantly decreased compared with that of the other samples, while that of FUR-UREA was markedly increased. These results suggest that FUR-UREA is more photosensitive than FUR-NIC and that it would be possible to improve the photostability of FUR by preparation of the optimal cocrystal.

Spectroscopic Profiles of FUR and Three Types of Cocrystal The solid-state UV-Vis absorption spectra from 350 to 450 nm of FUR and three types of cocrystal are shown in Fig. 4. In this figure, the Kubelka–Munk function for each sample was normalized to compare the spectroscopic profiles

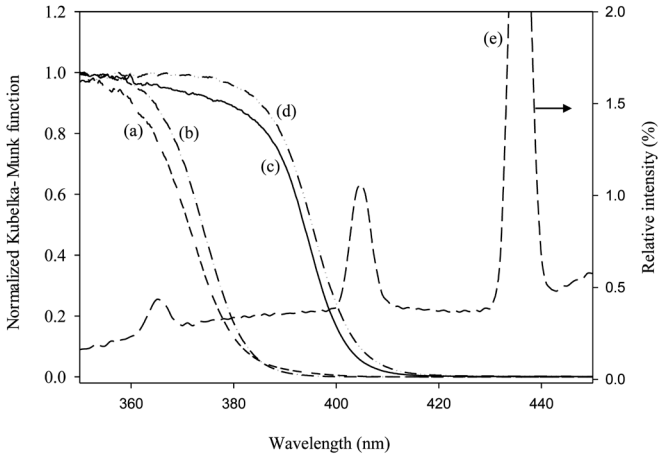

Fig. 4. Normalized Solid-State UV/Vis Spectra of FUR and Three Types of FUR Cocrystals and Relative Spectral Distribution of D65 Fluorescent Lamp

(a) FUR-NIC, (b) FUR-CAF, (c) FUR, (d) FUR-UREA, (e) Relative spectral distribution of D65 fluorescent lamp.

easily, and the profiles were compared with the relative irradiation intensity of the D65 fluorescent lamp to examine the photostability of those crystal forms. Solid-state FUR and FUR-UREA exhibited similar absorption spectra below 420 nm, whereas the spectra of FUR-CAF and FUR-NIC were observed in the shorter wavelength range than those of FUR and FUR-UREA. The UV-Vis reference spectrum of FUR in the Japanese Pharmacopoeia, 17th Edition, had no absorption between 350 and 450 nm when FUR was dissolved with sodium hydroxide solution. On the other hand, solid-state

FUR exhibited absorption over a broader range compared with FUR-NIC. It was suggested that the spectrum of solid-state FUR shifted to the longer wavelength region by relatively intensive π–π stacking interactions compared with FUR-NIC and FUR-CAF. Thus, FUR-NIC could absorb less light energy than FUR because the D65 fluorescent lamp showed intensive emission peaks at longer wavelengths. Consequently, FUR-NIC was more stable to light than FUR and FUR-UREA.

The COOH and the primary sulfonamide NH of FUR are connected to the basic CAF N and C=O groups with hydrogen bonding in FUR-CAF, respectively.[9] On the other hand, the hydrogen bonding interaction occurs between COOH and sulfonamide S=O of FUR and pyridinium N and NH_2 of NIC, respectively.[11] The crystal structure of FUR-UREA has not been entirely clarified. There might be no interaction between the sulfonamide of FUR and NH_2 of urea *via* hydrogen bonding, because FUR-UREA did not exhibit a peak shift toward shorter wavelengths from the absorption maximum. 4-Chloro-5-sulphamoylanthranilic acid (CSA) was found after FUR was exposed to sunlight.[13] We estimated that the photostability of FUR-NIC was improved more than that of FUR-CAF due to the difference in the hydrogen bonding interaction with the sulfonamide group.

Conclusion

The solid-state UV-Vis absorption spectrum of FUR-NIC shifted toward a shorter wavelength region than that of FUR, and the amount of irradiation from the D65 fluorescent lamp was much lower in this region. In conclusion, FUR-NIC was the most photostable cocrystal in this study. Our results suggest that FUR-NIC is a useful crystal form to inhibit the photodegradation of FUR and therefore cocrystallization could be a novel strategy for formulation design which would contribute to improving the photostability of the API.

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Conflict of Interest The authors declare no conflict of interest.

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