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Effect of Dehydration and Urea on Stability of Homosulfamine in Solid States

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In the presence of urea in solid states, the stability of unpulverized homosulfamine hydrate (phase I; UHH) is significantly decreased whereas that of unpulverized homosulfamine anhydrate (UHA) is not. The stability of UHH is decreased slightly more by pulverization (PHH). The major objective of this study was to investigate the effects of urea, dehydration, and pulverization on the stability of homosulfamine in solid states. Binary mixtures of UHH and urea, PHH and urea, and UHA and urea in a ratio of 1:1 (wt/wt) were prepared as physical mixtures and were analyzed by scanning electron microscopy (SEM), powder X-ray diffraction (PXRD), and Fourier transform infrared (FTIR) spectroscopy to study their appearance and structural changes before and after storage. PXRD analysis revealed that physical mixtures comprising UHH and urea and PHH and urea have the same diffraction pattern as that of the mixture of UHA and urea after preparation. The dehydration rate of the crystal water of UHH was accelerated by the presence of urea in addition to pulverization. Moreover, the PXRD patterns of the physical mixtures of UHH/urea and PHH/urea were significantly altered during storage, whereas that of UHA/urea was not, which was consistent with the SEM and FTIR results. The particle shape and appearance of UHH varied significantly as a result of pulverization. The stability of homosulfamine was influenced not only by the presence of urea and dehydration but also by the surface state and particle size of the crystalline form.

Keywords homosulfamine; dehydration; urea; stability; reaction product

INTRODUCTION

The presence of crystal water is common in the molecular crystals of both organic and inorganic compounds as numerous pharmaceuticals exist in both hydrated and anhydrous forms (Haleblian, 1975; Khankari & Grant., 1995), and more than 90 hydrates are listed in the *United States Pharmacopoeia* (1995). Hydrate formation and the dehydration of the product may occur during the manufacturing or storage of pharmaceuticals as the stability and dehydration behavior of hydrates is very sensitive and variable (Burger & Griesser, 1991). Knowledge of the hydration and dehydration behaviors of drugs is essential for the development of stable pharmaceutical formulations because the physicochemical and mechanical properties and the biological effects of hydrates could differ from those of anhydrates. In the case of products with a crystalline structure, these significant stability differences might be related to the thermodynamic properties conferred by the presence of crystal water. Generally, water molecules in hydrates are always involved in hydrogen bonds, which usually contribute to the coherence of the crystal structure. As the phase transition that occurs on hydration or dehydration is accompanied by a change in the physicochemical properties, it is important to understand the mechanisms of these transitions under various conditions (Morris, Griesser, Eckhardt, & Stowell, 2001; Otsuka, Hasegawa, & Matsuda, 1997; Phadnis & Suryanarayanan, 1997). It is now recognized that the stability of drugs is influenced by adsorbed water (water contents), which is formed during the dehydration of crystal water under various storage conditions. Horikoshi and Himuro (1966) reported that the coloring reaction of modified glucuronic acid was dependent on the excess free water, whereas the crystal water was not affected. Du and Hoag (2001) have reported that aspirin and niacinamide tablets containing lactose monohydrate have the same stability as tablets containing anhydrous lactose, which implies that the bound water does not influence the reaction rate. Matsunaga et al. (1993) reported that the degradation of TAT 59 (an anticancer drug) was strongly related to the water content of the tablets. Yamamoto (1959), Yamamoto and Inazu (1959a, 1995b), Yamamoto and Kawai (1959), and Yamamoto and Takahashi

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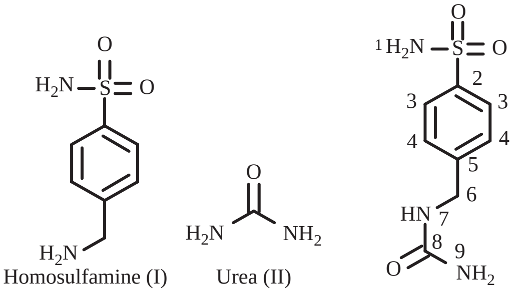
(1959) reported that the stabilities of ascorbic acid, thiamine, and aspirin were significantly affected by the extent of their water contents. Moreover, Carstensen and Attarchi (1988), Carstensen, Attarchi, and Hou(1985), and Yamamoto and Inazu (1959b) reported that the stability of aspirin was significantly affected by the extent of water content. El-Banna, Daabis, and El-Fattah (1978) reported that the stability of aspirin was reduced in its solid dispersion with urea because urea has six hydrogen binding sites with water molecules. Therefore, the stability of drugs in solid and semisolid states is affected by their capacity to retain water contents in the presence of additives.

Recently, we discovered that the stability of homosulfamine hydrate (phase I) in ointment was significantly affected by its degree of mixing with urea powder. Homosulfamine exists as a hydrate containing 2/3 molecules of crystal water (phase I) and in three anhydrate forms (phases II-IV) (Kato & Kido, 1977). Phase I is commonly purchased as a bulk powder. In contrast, it is well known that urea has hydrogen binding sites for the retention of water molecules (El-Banna et al., 1978). The stability of a medicinal compound in any dosage form must be considered and characterized before a product is marketed. However, there has been no previously reported study on the interaction between homosulfamine and urea. In this study, we attempted to clarify the effect of urea on the stability of homosulfamine in a solid state, to gain insights that could be used to predict the stability of homosulfamine in ointment formulations. It is reasonable to suppose that the stability of drugs in ointment formulations can be predicted by evaluating the behavior of free water and the change in physical properties of bulk powder in solid states.

MATERIALS AND METHODS

Materials

Homosulfamine hydrate (unpulverized homosulfamine hydrate; UHH) was purchased from Hamari Yakuhin Ind. Co., Ltd. (Tokyo, Japan). Pulverized homosulfamine hydrate (PHH) was made by pulverizing homosulfamine hydrate with an atomizer (pulverizing machine; Hitachi Co., Ltd., Tokyo, Japan). The grinding conditions were sample weight, 500 g; grinding speed, 200 rpm; temperature and humidity, about 20–30% RH (relative humidity) at 25 ± 3°C. The mean particle sizes of UHH and PHH were 86 and 56 μm, respectively. The unpulverized homosulafamine anhydrate (UHA) was dried at 100°C on a fluid bed dryer (MP-01M type, Powrex Inc., Osaka, Japan). The material was confirmed to be the anhydrate as reported previously (Kato & Kido, 1977) through physical characterization using powder X-ray diffraction (PXRD), differential scanning calorimetry (DSC), and thermogravimetry-differential thermal analysis (TG-DTA). Urea was purchased from Takasugi Pharmaceutical Co., Ltd. (Fukuoka, Japan). The chemical structures of homosulfamine hydrate (I) and urea (II) are shown in Figure 1.



Homosulfamine (I) Urea (II) 4-{[(aminocarbonyl)amino]methyl} benzenesulfonamide; 4-AAMB (III)

FIGURE 1. Chemical structure of homosulfamine and urea.

High-Performance Liquid Chromatography Analysis

An LC-10AVP system (Shimadzu, Kyoto, Japan) equipped with an SIL-10ADVP auto-injector, SPD-10AVVP detector, LC-10ADVP pump, DGU-14A degasser, CTO-10ACVP column oven, and C-R6A data processor was used to determine homosulfamine and the reaction product. The high-performance liquid chromatography (HPLC) conditions were as follows: column, TSK gel ODS-80TsQA (150 mm × 4.6 mm i.d., 5 μm, Tosoh, Tokyo, Japan); column temperature, 25°C; detection wavelength, 260 nm; mobile phase, acetonitrile–50 mM sodium acetate–glacial acetic acid (400:600:1.2, by vol) containing 0.2% SDS; flow rate, 1.0 mL/min; injection volume, 5 μL. An aliquot of the homosulfamine and urea solution from the accelerated reaction study was separated by reverse-phase HPLC to give two peaks. The resulting HPLC chromatogram is shown in Figure 2. Using this method, homosulfamine was eluted at 3.2 min and remained the peak in the chromatogram (% of total peak area). In contrast, urea was no detectable in homosulfamine and urea solution after HPLC separation. The peak eluted at 1.8 min in the chromatogram, suggesting the formation of the reaction product due to the interaction of homosulfamine and urea.

Isolation of Reaction Product

The powder mixtures comprising homosulfamine and urea (1:1, wt/wt) were stored at 60 ± 1°C for 45 days as described below. The mixture was extracted with water, and the extract was subjected to semipreparative HPLC to isolate the reaction product under the following conditions: column, Discovery C18 (250 mm × 10 mm i.d., 5 μm, Supelco, Bellefonte PA, USA); column temperature, 25°C; detection wavelength, 220 nm; mobile phase, water; flow rate, 2.0 mL/min; and injection volume, 100 μL. The fractions of the reaction product were collected and lyophilized. To elucidate their chemical structure, the NMR spectra of this compound were measured and the signals were assigned. All samples were run on a JNM-ECP500 instrument (JEOL, Tokyo, Japan) operating at 500 MHz for ^{1}H and 126 MHz for ^{13}C at 25°C, using a 5-mm (diameter) sample tube. We used DMSO-d_6 as a solvent, the water signal as an internal

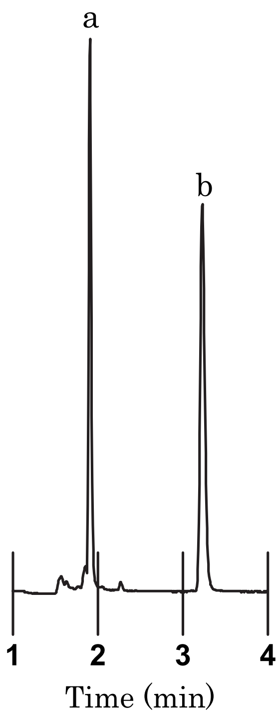


FIGURE 2. Typical HPLC chromatogram. Peak a: 4-AAMB (4-{[(aminocarbonyl) amino]methyl} benzenesulfonamide); peak b: homosulfamine.

reference for ^{1}H-NMR, and dioxane (66.5 ppm [parts per million] from tetramethylsilane [TMS]) as an external reference for ^{13}C-NMR. Chemical shifts were expressed in ppm. The ^{1}H- and ^{13}C-NMR signals of homosulfamine and urea were assigned according to the integral spectral database system (SDBS) for organic compounds. The ^{1}H- and ^{13}C-NMR signals for the reaction product were assigned by the ^{1}H-detected multiple quantum coherence (HMQC) and ^{1}H-detected multiple-bond heteronuclear multiple quantum coherence (HMBC) techniques. The ^{1}H-^{13}C HMQC data set for the protonated ^{13}C region of the reaction product was determined by in sensitive nuclei enhanced by polarization transfer (INEPT). The NMR data for this reaction product are as follows: (Figure 1—III): ^{1}H-NMR δ 7.82 (d, 1H, J = 7.8 Hz, H3), 7.47 (d, 1H, J = 7.8 Hz, H4), 7.36 (s, 2H, H1), 6.64 (t, 1H, J = 11.5 Hz, H7), 5.67 (s, 2H, H9), and 4.29 (d, 2H, J = 6.4 Hz, H6); ^{13}C-NMR δ 142.90 (C2), 159.26 (C8), 145.82 (C5), 127.76 (C4), 126.16 (C3), and 42.99 (C6). Mass spectrometry was performed using a Thermo Electron LCQ (San Jose, CA, USA) ion trap mass spectrometer fitted with an electrospray ionization (ESI) source running Xcalibur 2.0 SUR1 software (San Jose, CA, USA). The mass spectrum exhibited a base peak at m/z 230.1 that corresponded to the $[M + H]^+$ ion generated in the mass analysis of the reaction product; this peak was 43 Da larger than that of homosulfamine (m/z 187). The reaction product (4-{[(aminocarbonyl) amino]methyl} benzensulfonamide; 4-AAMB) was formed via the amide group of both homosulfamine and urea as shown in Figure 1—III.

Liquid Chromatography-Mass Spectrometry Analysis

Chromatographic separation for LC-MS (liquid chromatography-mass spectrometry) was performed on a Develosil RPAQUEOUS (100 mm × 2.0 mm i.d., 3 μm, Nomura Chemical, Aichi, Japan). The flow rate was 0.2 mL/min and injection volume was 2 μL. The mobile phase was 10 mM ammonium acetate with the pH adjusted to 4.0 with glacial acetic acid. The mass spectrometric detector was an ion trap mass spectrometer LCQ (Thermo Electron, San Jose, CA, USA) equipped with an electrospray ionization (ESI) source. Operating conditions for the ESI source were as follows: sheath gas flow rate, 70 arb (arbitrary unit defined by the software); aux gas flow rate, 20 arb; spray voltage, 4.5 kV; capillary temperature, 250°C; capillary voltage, 3 V; scanning mode, positive; scan range, 50–500 amu. Analytical data were acquired using Xcalibur software (version 1.0 SR1).

Preparation and Storage Conditions of Sample

Physical mixtures consisting of UHH/urea, PHH/urea, and UHA/urea (1:1 [wt/wt], 2 g) were prepared manually with an agate mortar and pestle, and then preserved in a sealed glass bottle. These physical mixtures were heated in an oven at 35 ± 1 and 50 ± 1°C, removed at predetermined intervals, and then dissolved in purified water to prepare sample solutions. To determined the stability of UHH, PHH, and UHA in their physical mixtures, the sample solutions were subjected to HPLC analysis.

Dehydration of the Hydrate Powders

The degree of dehydration was evaluated using a calibration curve generated from the relationship between the heat due to dehydration and the water content, which were calculated based on the DSC and TG curve of the PHH powder heated in an oven at 60°C, removed at predetermined intervals (0, 3, 6, and 9 h). The calibration curve showed a good linear correlation, and the linear regression equation was as follows:

$$Y = 11.2X + 0.50\ (r = .9991), \tag{1}$$

where Y (mJ/mg) is the latent heat due to dehydration of PHH, X is the water content (%), and r is the correlation coefficient. The powder samples (2 g) were heated in an oven at 35 ± 1 and 50 ± 1°C, removed at predetermined intervals, and then subjected to DSC, PXRD, and TG-DTA.

Dehydration Studied by DSC

Thermal analysis of the samples was performed with a DSC instrument (EXSTRA 6000 with measuring cell DSC 30E; Seiko Instruments Inc, Chiba–Shi, Chiba, Japan). Approximately 10 mg of sample was weighed into the DSC pan. The pan was not sealed and placed in the sample side of the instrument.

An identical reference pan was placed in the reference side. Scans between 0 and 300°C were carried out at a rate of 10°C/min, using a nitrogen gas purge at 50 mL/min.

TG-DTA Measurements

The TG-DTA curves were obtained by TG-DTA (EXSTRA 6000 with measuring cell DSC 30E; Seiko Co. Ltd.). Approximately 10 mg of sample was weighed into the DSC pan. The pan was not sealed and placed in the sample side of the instrument. An identical reference pan was placed in the reference side. Scans between 0 and 300°C were carried out at a rate of 3°C/min, using a nitrogen gas purge at 50 mL/min.

Powder X-Ray Diffraction Analysis

PXRD was performed at room temperature with a type Rint2550VHF diffractometer (Rigaku, Inc., Tokyo, Japan). Measurement conditions were as follows: target, Cu; filter, Kα; voltage, 40 kV; current, 450 mA; time constant, 1 s; step slit, 1.0°; counting time, 1.0 s; measurement range, $2\theta = 2.5°$ to $2\theta = 60°$. To avoid particle orientation during sample preparation, the loosely packed sample was prepared by pouring the powder into the holder without compressing it.

Scanning Electron Microscopy

Samples were mounted onto aluminum stubs and gold-coated in a sputter coater to a thickness of about 10 μm. The coated samples were then viewed at 400 × magnification in the FE-SEM (field emission-scanning electron microscopy) (JEOL, Tokyo, Japan). The beam accelerator voltage was set to 20 kV and the current was set to 12 μA.

Fourier Transform Infrared Spectroscopy

A dispersion (about 1%) of the sample in potassium bromide (KBr) was prepared by mixing the mass with KBr. Fourier transform infrared (FTIR) spectra of the prepared mixing with KBr was obtained on a Nicolet Magna-IR 760 spectrometer (Thermo Fisher Scientific K. K., Yokohama–Shi, Kanagawa, Japan) over the 4,000–400 cm^{-1} region. The number of scans were 16 and the resolution was 4 cm^{-1}.

RESULTS AND DISCUSSION

Effects of Urea on the Stability of Homosulfamine

Recently, we discovered that the stability of homosulfamine hydrate in ointment was significantly affected by its degree of mixing with urea powder. It is well known that homosulfamine exhibits polymorphism with four crystalline forms (hydrate: phase I, anhydrate: phases II-IV), as reported by Kato and Kido (1977). To confirm the stability of UHH, PHH, and UHA in a solid state, we prepared physical mixtures comprising UHH and urea, PHH and urea, and UHA and urea. The homosulfamine contents in these physical mixtures, which were preserved in sealed glass bottles, were determined by HPLC analysis. The contents of UHH and PHH decreased gradually during storage as they were dependent on the storage temperature, whereas that of UHA did not decrease (Figure 3A). In contrast, the stability of UHH was slightly higher than that of PHH (Figure 3A). Therefore, the stability of the hydrate form of homosulfamine in a physical mixture was affected by mechanical forces such as pulverization and by the presence of crystal water in homosulfamine. Moreover, we studied the extent of the formation of the reaction product 4-AAMB in physical mixtures comprising homosulfamine and urea during storage (Figure 3B). 4-AAMB was observed in the physical mixtures comprising UHH and urea and PHH and urea, but not in the physical mixture comprising UHA and urea. These results suggest that a solid-phase synthesis could occur between the hydrate form of homosulfamine and urea powder. On the contrary, the amount of 4-AAMB formed was less than the decrease in the homosulfamine content. The amount of 4-AAMB formed was approximately 1.6–1.8 and 9.4–12.1% at 35 ± 1 and 50 ± 1°C after storage, respectively. In contrast, the homosulfamine content decreased by approximately 3.2–5.6 and 12.6–17.1%, respectively, at the same temperatures. These results suggest that other reaction products appearing in a physical mixture comprising the hydrate form of homosulfamine and urea is formed through some reaction process.

Effects of Pulverization on the Dehydration Behavior of Homosulfamine Hydrate

In the above section, we clarified that the stability of homosulamine in a physical mixture comprising homosulfamine and urea is affected by the presence of crystal water in homosulfamine. To confirm the role of crystal water on the stability of the hydrate form of homosulfamine, we studied the thermal analysis of homosulfamine and dehydration behavior during

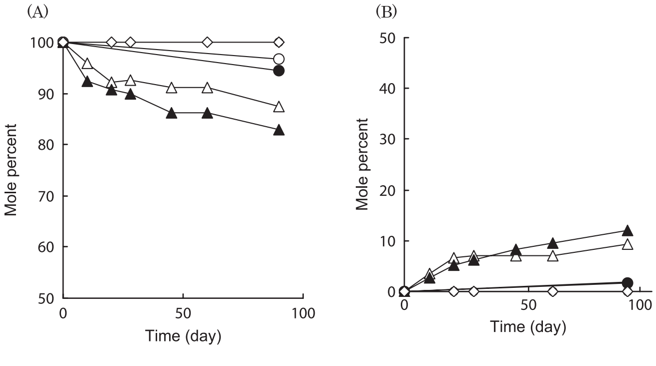


FIGURE 3. Mole percentage of remaining unpulverized homosulfamine hydrate (UHH) (○, △), pulverized homosulfamine hydrate (PHH) (●, ▲), and unpulverized homosulfamine anhydrate (UHA) (◇, ◆) (A), and that of formation of the 4-AAMB (B) at various storage temperatures. (○, ●, ◇), 35 ± 1°C; (△, ▲, ◆), 50 ± 1°C.

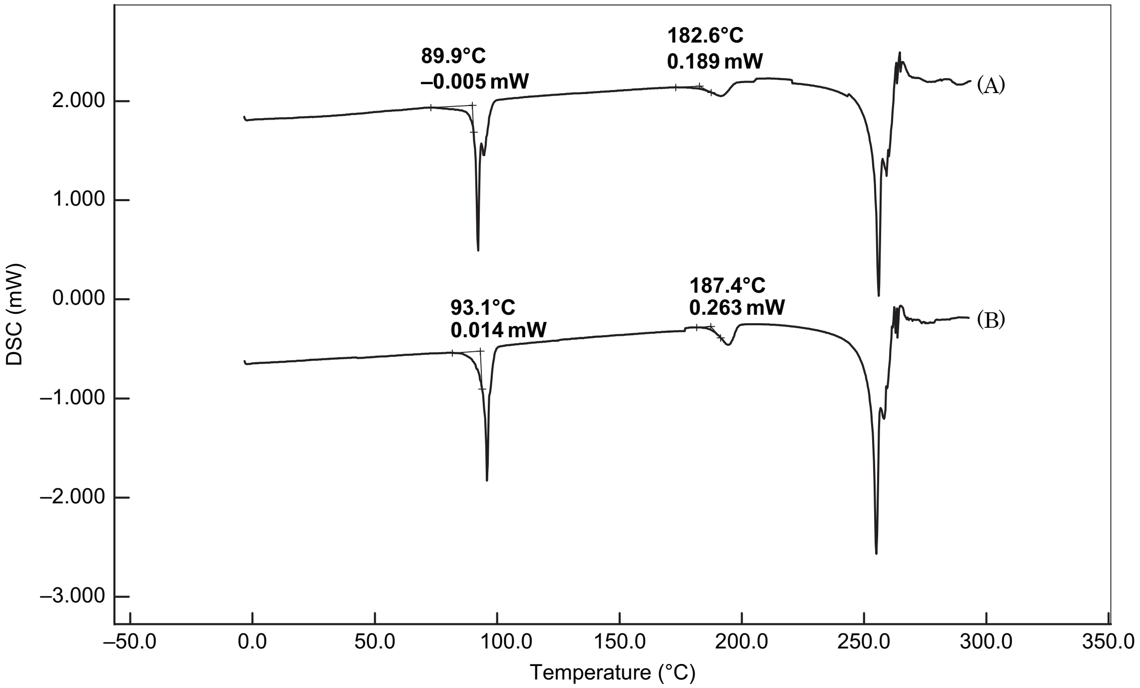


FIGURE 4. Differential scanning calorimetry (DSC) thermograph of unpulverized homosulfamine hydrate (UHH) (A) and pulverized homosulfamine hydrate (PHH) (B).

storage. Figure 4 shows the DSC profiles of UHH and PHH. The DSC curves of UHH and PHH exhibited an endothermic peak at 89.4 and 91.8°C due to the dehydration of crystal water, a broad endothermic peak at 184.4 and 185.1°C, and an endothermic peak at 257.7 and 257.0°C due to melting and decomposition, respectively. The heats of fusion of UHH and PHH were 50.92 and 50.33 mJ/mg, respectively. Therefore, the change in the DSC curve due to the dehydration of crystal water was slightly affected by pulverization treatment. In contrast, UHA exhibited a broad endothermic peak at 191.5°C and an endothermic peak at 264.3°C due to melting and decomposition (data not shown).

Figure 5 shows the typical dehydration plots of UHH and PHH powders. The dehydrated fraction of the sample powder at 35 ± 1 and 50 ± 1°C was obtained by calculating the heat of dehydration from the homosulfamine hydrate DSC curves. The degree of dehydration increased with storage time, which increased with increasing water activity (data not shown). At all temperatures tested, the dehydration rate increased with the storage temperature. Kato and Kido (1977) reported that the homosulfamine hydrate was easily transformed into the anhydrate form at temperatures such as 20 and 37°C, and lower relative humidities such as 20% RH, 30.5% RH, and 52% RH. Moreover, the dehydration of UHH was also significantly accelerated by pulverization treatment. The dehydration of homosulfamine hydrate was significantly affected by mechanical forces such as pulverization and by the storage conditions. Therefore, it is suggested that the stabilities of UHH and PHH are related to the free water formed by the dehydration of crystal water.

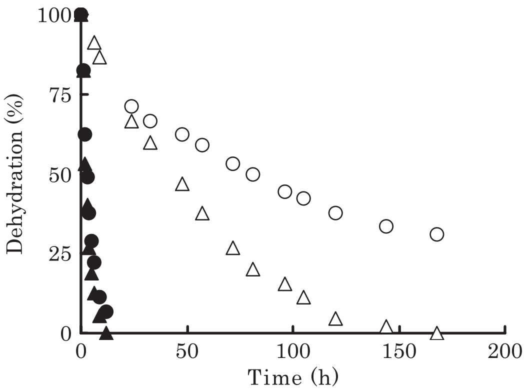


FIGURE 5. Dehydration curves of unpulverized homosulfamine hydrate (UHH) (clear symbols) and pulverized homosulfamine hydrate (PHH) (solid symbols) homosulfamine hydrate. (○, ●), 35 ± 1°C; (△, ▲) 50 ± 1°C.

Interaction Between Homosulfamine and Urea

We proposed that the stability of the hydrate form of homosulfamine would be dependent on the free water formed by the dehydration of homosulfamine in the presence of urea in solid states. Moreover, pulverization treatment strongly affected the dehydration behavior and led to an increase in the dehydration rate, whereas the stability of UHH in a physical mixture was slightly dependent on the particle size. To clarify the effect of the dehydration rate of crystal water on the stabilities of UHH and PHH in the presence of urea, we studied the interaction between homosulfamine and urea using SEM, PXRD, and FTIR spectroscopy.

Figure 6 shows the SEM photographs of typical crystals of UHH, PHH, UHA, and urea and their physical mixtures comprising UHH and urea, PHH and urea, and UHA and urea at 1:1 (wt/wt) before and after storage. The crystalline particles of UHH and PHH both appeared as monoclinic crystals (Figure 6A and F) that were different from those of UHA (Figure 6J). In contrast, the crystalline particles of urea had a tetragonal form (Figure 6E). Urea significantly affected the particle states of hydrated homosulfamine and its physical mixtures (Figure 6B and G), but not that of UHA (Figure 6K). Moreover, the appearance of physical mixtures comprising UHH and urea and PHH and urea were converted into a wet state in glass bottles at 35 ± 1°C (Figure 6C and H) and 50 ± 1°C (Figure 6D and I) after 90 days, and the extent of aggregation between homosulfamine and urea gradually increased during storage, whereas the physical mixture of UHA and urea exhibited no change (Figure 6K–M). El-Banna et al. (1978) reported that urea powder has hydrogen binding sites for the retention of water molecules. These results suggest that the solid state of a physical mixture comprising the hydrate form of homosulfamine and urea would be affected by the dehydration of the crystal water of homosulfamine hydrate before and after storage. In contrast, the appearance of PHH crystals differed significantly from that of UHH crystals, which exhibited a rugged particle surface due to the effect of pulverization. As shown in the DSC profiles, the heats of fusion of UHH and PHH did not differ (Figure 4). Therefore, it can be concluded that some form of mechanical force, such as pulverization changes the crystalline particles of UHH. Therefore, the stability of homosulfamine crystals appears to be strongly related to their surface states in addition to the free water formed by the dehydration of homosulfamine hydrate in the presence of urea powder.

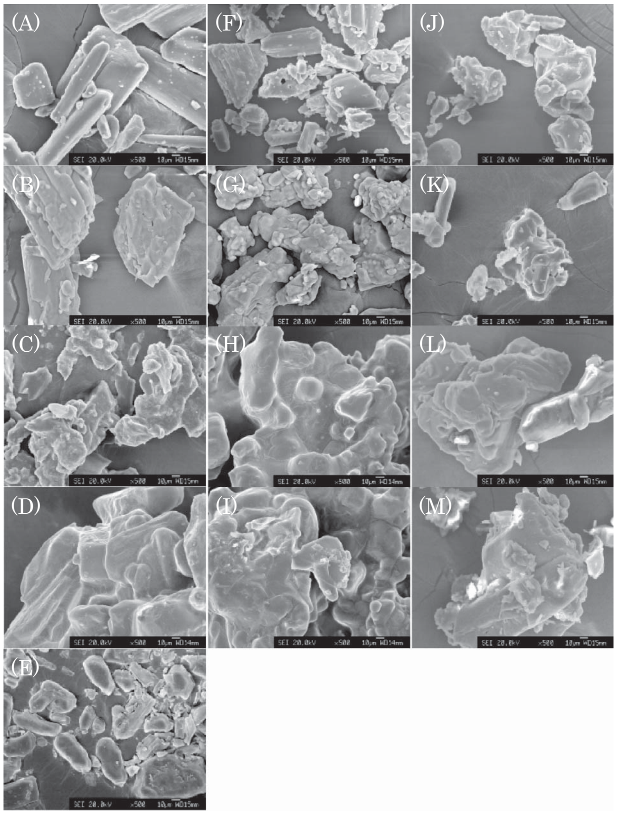


FIGURE 6. Scanning electron micrographs (SEMs) of unpulverized homosulfamine hydrate (UHH) (A), pulverized homosulfamine hydrate (PHH) (F), unpulverized homosulfamine anhydrate (UHA) (J), urea (E) and their physical mixtures consisting of UHH and urea (B, C, D), PHH and urea (G, H, I), and UHA and urea (K, L, M) before and after storage. (B, G, K) Physical mixture before storage; (C, H, L) physical mixture after storage at 35 ± 1°C; (D, I, M) physical mixture after storage at 50 ± 1°C.

Figure 7 shows the PXRD profiles of UHH, PHH, UHA, and urea and their physical mixtures comprising UHH and urea, PHH and urea, and UHA and urea at 1:1 (wt/wt). The PXRD patterns and the main diffraction angles of UHH and PHH agreed with the data for homosulfamine hydrate (phase I) obtained in a previous study. The diffraction pattern and the dehydration points (Figure 4) and heat of dehydration of UHH were not significantly different from those of PHH, indicating that the two forms have the same crystal structure and crystallinity (Figure 7A and C). Because the appearance of UHH crystals was significantly altered as a result of pulverization (Figure 6A and F), the stability of the hydrate form of homosulfamine in a physical mixture appeared to be dependent on both particle size and surface state. Moreover, the PXRD profiles of physical mixtures comprising UHH and urea and PHH and urea indicated the same diffraction pattern as that of UHA and urea following preparation (Figure 7B, D, and F). The diffraction peak of native UHH and PHH at $2\theta = 7.4°$ and 14.9° disappeared in physical mixtures comprising UHH and urea, and PHH and urea after at least 24 h at 25°C. In contrast, the PXRD pattern of UHA was not changed by the addition of urea. From these findings, we suggest that the hydrate form of homosulfamine in physical mixtures is transformed into the anhydrate form of homosulfamine upon mixing with urea. Kato and Kido (1977) reported that UHH was transformed into UHA after 3 months at room temperature. Therefore, it is reasonable to assume that the dehydration rate of UHH and PHH was accelerated by the presence of urea powder.

Figure 8 shows the PXRD profiles of physical mixtures comprising UHH and urea and PHH and urea at 1:1 (wt/wt) before and after storage. In a physical mixture comprising UHH and urea, and PHH and urea, the peak intensity at $2\theta = 15.5°$ and that at $2\theta = 22.3°$ because of native urea decreased significantly at 35 ± 1°C (Figure 8B and E) and 50 ± 1°C (Figure 8C and F) during storage. Moreover, the peaks at $2\theta = 11.6°$ and 26.7°

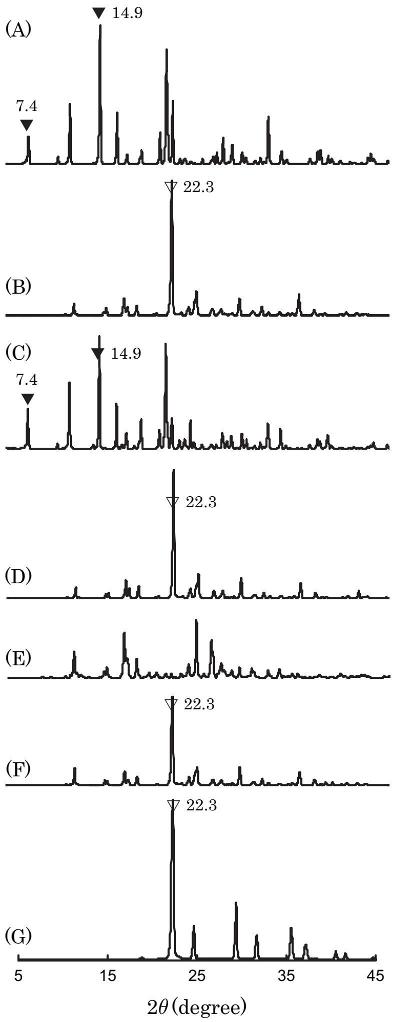


FIGURE 7. Powder X-ray diffraction (PXRD) analysis of unpulverized homosulfamine hydrate (UHH) (A), pulverized homosulfamine hydrate (PHH) (C), unpulverized homosulfamine anhydrate (UHA) (E), urea (G), and their physical mixtures consisting of UHH and urea (B), PHH and urea (D), and UHA and urea (F).

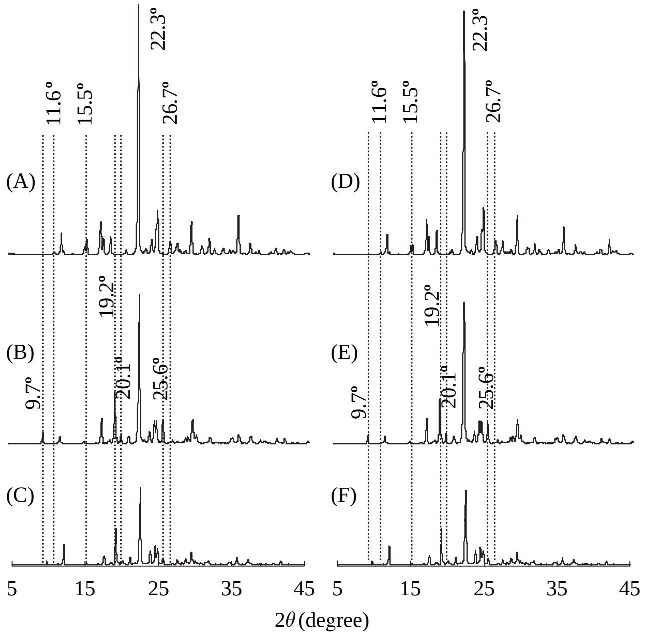


FIGURE 8. Powder X-ray diffraction (PXRD) analysis of physical mixtures consisting of unpulverized homosulfamine hydrate (UHH) and urea (A, B, C), and pulverized homosulfamine hydrate (PHH) and urea (D, E, F) before and after storage. (A, D) Physical mixture before storage; (B, E) physical mixture after storage at 35 ± 1°C; (C, F) physical mixture after storage at 50 ± 1°C.

disappeared, whereas those at $2\theta = 19.2°$, 20.1°, and 25.6° were observed after storage. In addition, the peak at $2\theta = 9.7°$ that was observed at 35 ± 1°C was not observed at 50 ± 1°C after storage. Therefore, the PXRD pattern and the diffraction intensities of physical mixtures comprising UHH and urea and PHH and urea were significantly changed before and after storage (Figure 8A–C and D–F, respectively), whereas those of UHA and urea did not change (data not shown). These results suggest that the crystal structure of UHH and PHH are strongly affected by mixing with urea. From these findings, it is suggested that the structural change would be related to the interaction between homosulfamine and urea due to the free water formed by the dehydration of homosulfamine hydrate during storage. As shown in the stability tests, the content of homosulfamine in physical mixtures comprising the hydrate form of homosulfamine and urea decreased, whereas that of the anhydrate form did not decrease (Figure 3). Therefore, these results suggest that the stability of homosulfamine in a physical mixture comprising homosulfamine and urea appears to be strongly related to the solid states of the physical mixture during storage.

Figure 9 shows the FTIR spectra of UHH, PHH, UHA, and urea and their physical mixtures comprising UHH and urea, PHH and urea, and UHA and urea at 1:1 (wt/wt). Kato and Kido (1977) reported that the FTIR spectra of the four crystal forms differ at approximately 3,200–3,400 cm^{-1}, 2,600–2,800 cm^{-1}, and 1,500–1,600 cm^{-1}. The FTIR spectrum of native UHH has the same profile as that of PHH, but is different from that of native UHA at approximately 3,200–3400 cm^{-1}, 3000–3100 cm^{-1}, 2500–2800 cm^{-1}, and 650–750 cm^{-1} (Figure 9A, D, and F). In contrast, the representative FTIR spectra of physical mixtures comprising UHH and urea, and PHH and urea exhibited peaks that were identical to those of a physical mixture of UHA and urea (Figure 9B, E, and G). The FTIR spectra of physical mixtures were also measured after at least 24 h at 25°C. The results indicated that urea accelerated the transformation of homosulfamine from the hydrate to the anhydrate form, which was in agreement with the SEM and PXRD results.

Figure 10 shows the FTIR spectra of physical mixtures comprising UHH and urea and PHH and urea at 1:1 (wt/wt)

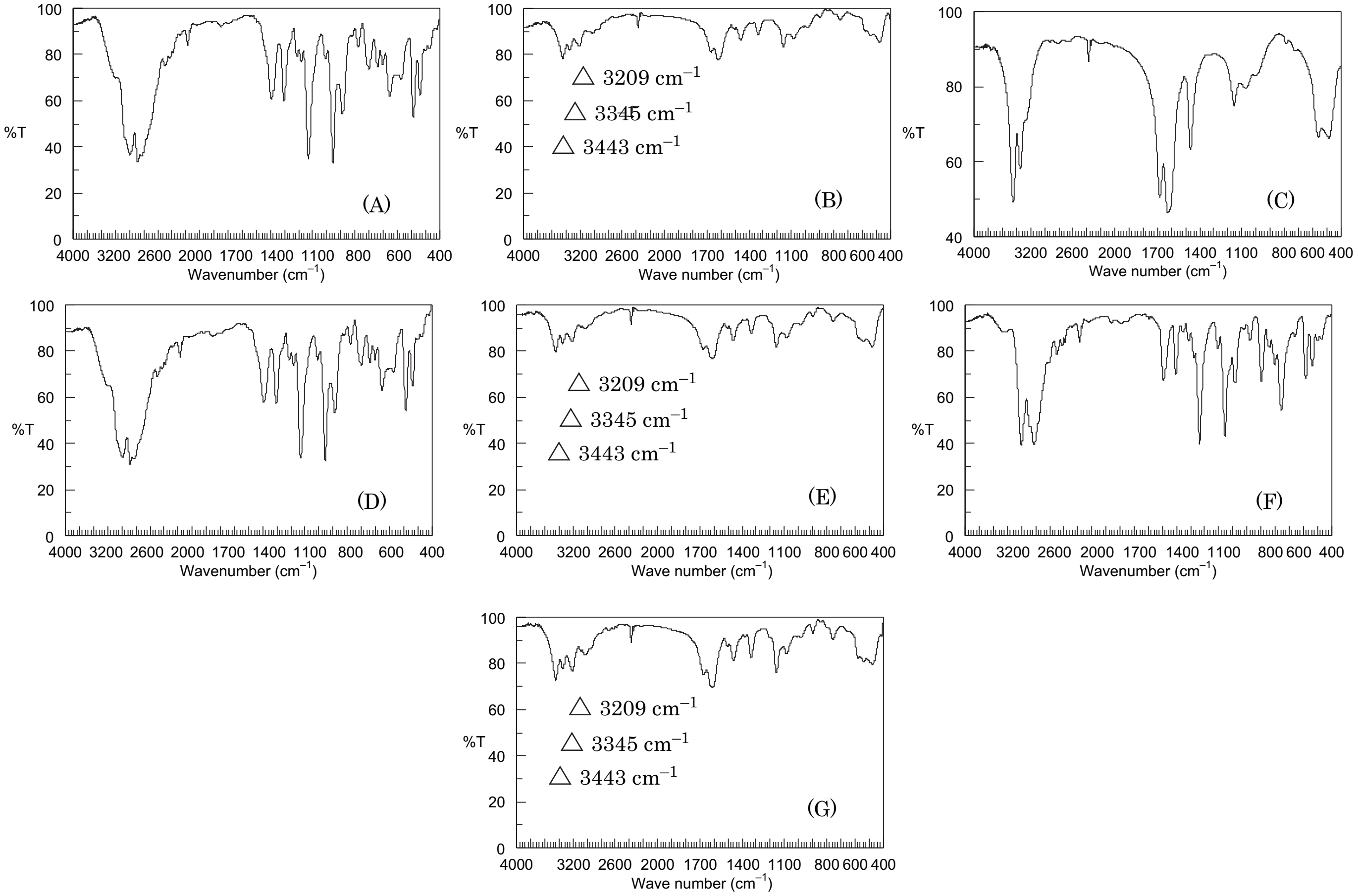


FIGURE 9. FTIR spectra of unpulverized homosulfamine hydrate (UHH) (A), pulverized homosulfamine hydrate (PHH) (D), unpulverized homosulfamine anhydrate (UHA) (F), urea (C) and their physical mixtures consisting of UHH and urea (B), PHH and urea (E), and UHA and urea (G).

before and after storage. The frequencies and the peak intensities of the FTIR spectra of physical mixtures of UHH and urea and PHH and urea changed significantly and increased after storage (Figure 10A–C and D–F, respectively), whereas that of UHA and urea did not change (data not shown). In the physical mixture comprising UHH and urea, the peaks at 3,443, 3,345, 1,677, and 1,608 cm^{-1} (Figure 10A) shifted to 3,441, 3,334, 1,667, and 1,617 cm^{-1} at 35 ± 1°C (Figure 10b), and 3,437, 3,334, 1,667, and 1,617 cm^{-1} at 50 ± 1°C (Figure 10c). Moreover, in the physical mixture comprising PHH and urea, the peaks at 3,443, 3,345, 1,677, and 1,608 cm^{-1} (Figure 10D) shifted to 3,432, 3,333, 1,661, and 1,617 cm^{1} at 35 ± 1°C (Figure 10E) and 3,437, 3,334, 1,661, and 1,617 cm^{-1} at 50 ± 1°C (Figure 10F). Moreover, the intensity of peaks at 3,443, 3,345, 1,677, and 1,608 cm^{-1} increased in the physical mixtures comprising UHH and urea and PHH and urea after storage. As shown in Figure 1, 4-AAMB was formed via the amide group of both homosulfamine and urea. The N–H stretching and N–H bending absorption bands are observed at approximately 3300 and 1600 cm^{-1}, respectively. The peaks at 3,443, 3,345, 1,677 and 1,608 cm^{-1} correspond to N–H stretching and N–H bending, respectively. In contrast, new peaks due to the formation of 4-AAMB are not observed in the FTIR spectra of physical mixtures comprising homosulfamine hydrate and urea. Therefore, the shift of their peaks and the increase of their peak intensity would correspond to the formation of 4-AAMB and/or the interaction between homosulfamine hydrate and urea.

El-Banna et al. (1978) reported that the stability of aspirin reduced in its solid dispersion with urea, because urea has hydrogen binding sites with water molecules. As shown in the PXRD and FTIR results, the hydrate form was easily transformed into the anhydrate form in the presence of urea, and free water appeared to form on the surface of the urea particles. Because the formation of 4-AAMB was observed in a physical mixture comprising the hydrate form of homosulfamine and urea powder, we concluded that the formation of free water is strongly related to the solid-phase synthesis between homosulfamine hydrate and urea.

CONCLUSIONS

The stability of the hydrate form of homosulfamine decreased significantly in the presence of urea powder and led to the formation of 4-AAMB, whereas that of the anhydrate

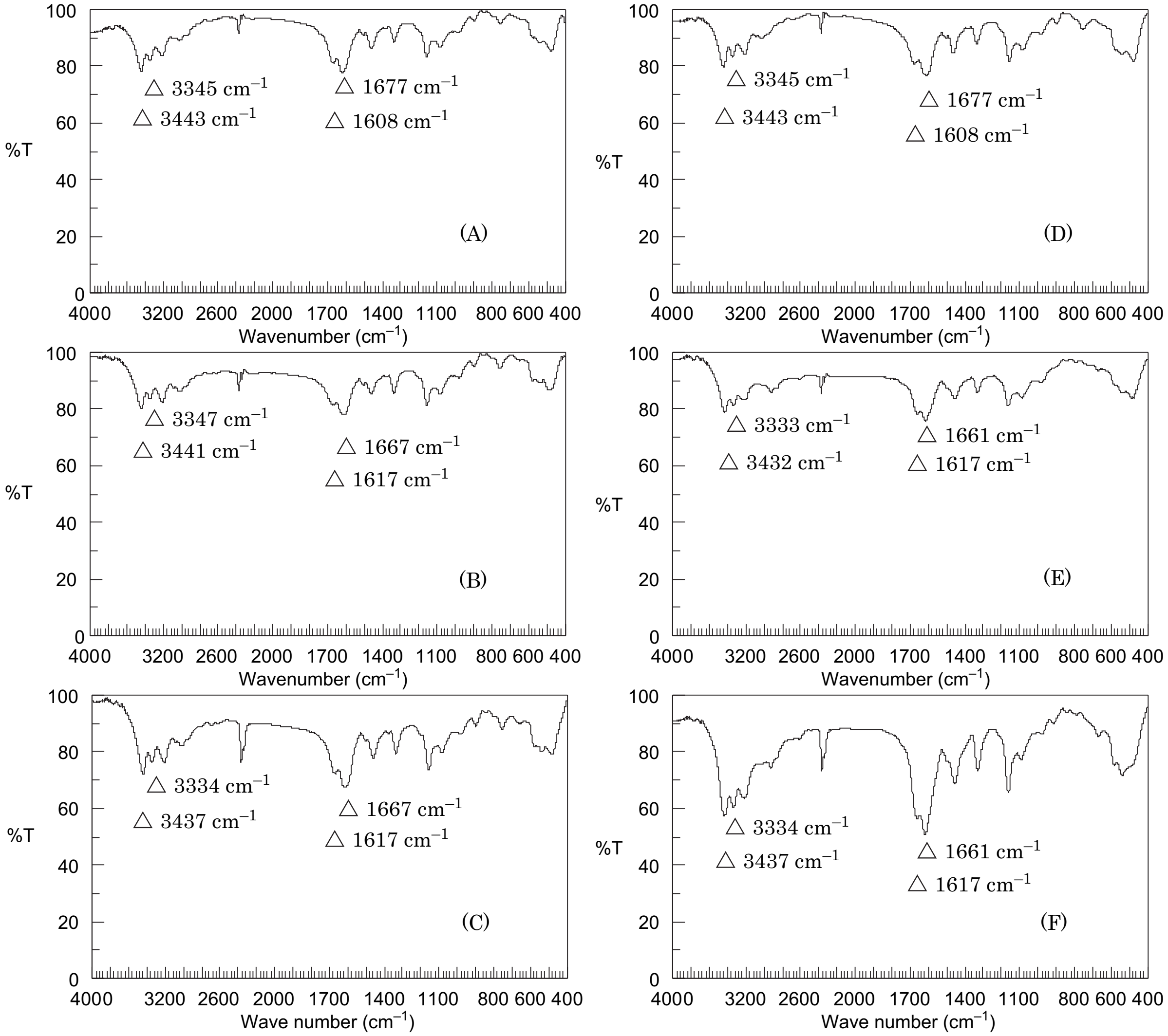


FIGURE 10. Fourier transform infrared (FTIR) spectra of physical mixtures consisting of unpulverized homosulfamine hydrate (UHH) and urea (A, B, C), and pulverized homosulfamine hydrate (PHH) and urea (D, E, F) before and after storage. (A, D) Physical mixture before storage; (B, E) physical mixture after storage at 35 ± 1°C; (C, F) physical mixture after storage at 50 ± 1°C.

form was not affected. The hydrate was transformed into the anhydrate by urea due to the possible formation of free water, which would be tightly retained by the urea. The free water formed would play an important role in the solid-phase synthesis between homosulfamine and urea. Therefore, the stability of homosulfamine would be strongly affected by the formation of free water. In contrast, the stability of the hydrate form was also slightly affected by mechanical forces such as pulverization. Pulverization treatment strongly affected the dehydration behavior, leading to an increase in the dehydration rate. Moreover, the surface states of homosulfamine hydrate exhibited considerable ruggedness as a result of pulverization. Therefore, the stability of homosulfamine hydrates was affected not only by the presence of urea and the dehydration of crystal water but also by the surface state and particle size of the bulk powder. In contrast, the stability of homosulfamine in ointment formulations was improved by using UHA instead of UHH and PHH.

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