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The Effects of Grinding and Drying on the Solid State Stability of Sodium Prasterone Sulfate

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The effects of grinding and drying (removal of water of crystallization) processes on the solid state stability of sodium prasterone sulfate (DHA·SO_3Na·$2H_2O$) were studied. As the grinding time was increased, the sample became more unstable. From the study of the thermal behavior of the water of crystallization, it was suggested that the bonding force between the water of crystallization and DHA·SO_3Na molecule within the hydrate crystal was weakened by grinding, and water molecules participated in the hydrolysis of DHA·SO_3Na more easily. Although DHA·SO_3Na was found to be hydrolyzed, the ground DHA·SO_3Na·$2H_2O$ was more stable under conditions of high humidity than low humidity. This result is very interesting, and it was considered that the bonding force between the water of crystallization and DHA·SO_3Na molecule was strengthened in conditions of high humidity.

The dehydration of DHA·SO_3Na·$2H_2O$ resulted in a higher disorder of the crystal structure and water molecules were sufficiently free to participate in a solid state hydrolytic reaction. Therefore, partially dehydrated DHA·SO_3Na·$2H_2O$ decomposed easily. After almost complete removal of the water of crystallization, the sample was relatively stable, since it contained few water molecules which could participate in hydrolysis. The dihydrate form was the most stable.

Keywords——sodium prasterone sulfate; solid state stability; grinding; drying; water of crystallization

Introduction

In the previous paper, we reported on the stability of sodium prasterone sulfate (sodium dehydroepiandrosterone sulfate, DHA·SO_3Na·$2H_2O$) in aqueous solution and in the solid state, as well as on the properties of the water of crystallization.[1-3] It was found that DHA·SO_3Na·$2H_2O$ was in a stable phase under ordinary storage conditions, and underwent hydrolysis in aqueous solution and in the solid state. Various operations, *e.g.*, grinding, drying, compressing, mixing and so on are performed on medicinal agents during pharmaceutical processing. Such procedures may affect the physicochemical properties and stability. In many instances, therefore, it is important to understand what factors might influence the stability. In the case of medicinal agents which have water of crystallization, the above processes might also affect the bonding strength of water molecules within the hydrate crystal, and this in turn might influence the pharmaceutical formulation. In this report, the effects of grinding and drying (removal of water of crystallization) processes on the solid state stability of DHA·SO_3Na·$2H_2O$ were studied.

Experimental

Materials——DHA·SO_3Na·$2H_2O$ was prepared as described previously.[1] DHA·SO_3Na·$2H_2O$ was recrystallized twice from H_2O-EtOH (1: 9) and a 42—80 mesh fraction was used. Water content (Calcd; 8.45%) was found to be 8.71% and 8.61% by the Karl-Fischer method and by the loss on drying method, respectively.

Grinding——DHA·SO_3Na·$2H_2O$ (70 g) was ground in a automated mortar (Type No. 16, Ishikawa Kojyo Co., Ltd.). Samples were taken out at appropriate intervals (15 min, 30 min, 1 h, 2 h). The samples (0.5 g each) were transferred into a series of 10 ml ampules and the ampules were sealed. In the humidity effect study, the ampules were not sealed. In the study of changes in the X-ray diffraction patterns of the ground DHA·SO_3Na·$2H_2O$ after storage under definite humidities at 40°C, 30 g of DHA·SO_3Na·$2H_2O$ was ground for 3 h.

Dehydration——DHA·SO_3Na·$2H_2O$ (0.5 g) was weighed into a 10 ml ampule. The ampules were placed in a drying oven (HIVAC OVEN HV-3, Tabai Mfg. Co., Ltd.) and dehydrated under reduced pressure for an appropriate period. P_2O_5 (phosphorus pentoxide) was used as a desiccant. After dehydration, the ampules were sealed immediately.

Kinetic Procedure——The ampules were kept at 40°C in a thermostated chamber (Type PR3A, Tabai Mfg. Co., Ltd.), and sampled at appropriate intervals. In the humidity effect study, samples were kept in a desiccator adjusted to the appropriate humidity by means of a saturated salt solution at 40°C (P_2O_5 was used for 0% R.H.).

Analytical Procedure——Determination of DHA·SO_3Na: DHA·SO_3Na was determined by the method described previously.[1]

Determination of DHA: The formed DHA was determined by the modified gas chromatographic method described previously.[4] The reaction sample (0.1 g) was weighed accurately into a centrifuge tube, then 10 ml water and 10 ml chloroform were added. The tubes were shaken for 20 min and centrifuged. The chloroform layer (5 ml) was withdrawn, dried over anhydrous sodium sulfate and freed of solvent *in vacuo*. The residue was dissolved in an internal standard solution and 1.0 μl of the solution was injected into a gas chromatograph equipped with a flame-ionization detector (Hitachi 063 gas chromatograph, Hitachi Ltd.).

Internal standard solution; pregnenolone (E. Merck, Darmstadt) chloroform solution.

TLC——The operating conditions were the same as reported previously.[1]

Water Content——Water content assays were performed by the loss on drying method (*in vacuo*, P_2O_5, 60°C, 3 h) and the Karl–Fischer method (AQUACOUNTER AQ-1, Hiranuma Sangyo Co., Ltd.).

X-Ray Diffraction——X-Ray diffraction patterns were obtained with an X-ray diffractometer (Rigakudenki Geigerflex 2027, Rigakudenki Co., Ltd.). The measurement conditions were as follows: target Cu, filter Ni, voltage 25 kV and current 5 mA. In the dehydration effect study, DHA·SO_3Na·$2H_2O$ was held in a sample holder which was designed to maintain the powder bed at a relatively constant temperature, and the sample was dehydrated *in vacuo* at 60°C for a definite time. Then dry N_2 gas was introduced into the sample chamber. The measurement was done at 40°C when the pressure in the sample chamber reached atmospheric pressure.

Thermal Measurement——Thermal behavior of the water of crystallization was measured by DTA and TG (Shimadzu DT-20B and TG-20, Shimadzu Seisakusho Ltd.) at a temperature increase rate of 5°C/min. In TG measurement, water vapor pressure in the atmosphere was maintained constant by introducing an N_2 gas flow at 70 ml/min, which was adjusted to 14.9 mmHg water vapor pressure (P_{H_2O}) by being passed over an NaCl-saturated solution.

Specific Surface Area——Specific surface area was determined by the air permeability method (Shimadzu SS-100 specific surface area meter, Shimadzu Seisakusho Ltd.).

Results and Discussion

Effects of Grinding on the Physicochemical Properties of DHA·SO_3Na·$2H_2O$

Figure 1 shows the X-ray diffraction patterns of DHA·SO_3Na·$2H_2O$ and ground DHA·SO_3Na·$2H_2O$. Relative ratios of the peak heights were changed by grinding, but no change of the peak position was observed. The changes in relative ratios of the peak heights were thought to be due to a preferred orientation of crystals, since the intact DHA·SO_3Na·$2H_2O$ was a thin plate crystal. Thus, the crystal structure was not thought to be changed by grinding. Crystallinity of the ground DHA·SO_3Na·$2H_2O$ relative to the intact DHA·SO_3Na·$2H_2O$ as 100%, was determined by Hermans' method[5] using the diffraction patterns reported by Nakai *et al.*[6] Figure 2 shows a good linear relationship between $\int I_c(\theta)d\theta$ and $\int I_a(\theta)d\theta$, where $\int I_c(\theta)d\theta$ and $\int I_a(\theta)d\theta$ are the integrated intensities of crystalline peaks and of the amorphous region, respectively. Diffraction angles were confined to the region between 2 and 40°. The determined values of crystallinity of the ground samples are shown in Table I. Values of crystallinity decreased with grinding time, and these results showed a higher disorder of the crystal structure after grinding. Table I also shows the water content and specific surface area of the ground sample. Only the 2 h-ground sample was dehydrated a little in the grinding process. Specific surface area (S_w) was increased by grinding. S_w of 2 h-ground sample was about 9 times larger than S_w of the intact sample.

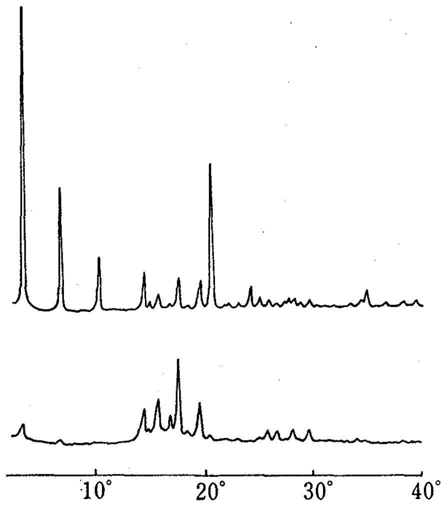

Fig. 1. X-Ray Diffraction Patterns of Intact and Ground $DHA \cdot SO_3Na \cdot 2H_2O$

a, intact $DHA \cdot SO_3Na \cdot 2H_2O$; b, 2 h-ground $DHA \cdot SO_3Na \cdot 2H_2O$.

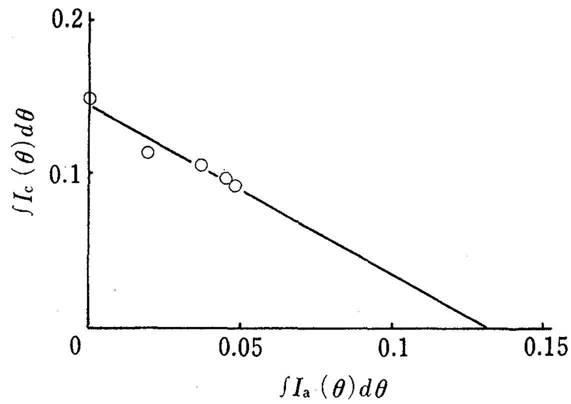

Fig. 2. Relationship between $\int I_c(\theta)d\theta$ and $\int I_a(\theta)d\theta$

TABLE I. Effect of Grinding on the Water Content, Specific Surface Area and Crystallinity

Grinding time	Water content (%)	S_w (m^2/g)	Crystallinity (%)
Intact	8.61	0.17	100.0
15 min	8.64	0.54	84.1
30 min	8.58	0.82	72.2
1 h	8.42	1.10	66.1
2 h	7.60	1.52	63.5

Effect of Grinding on the Stability of $DHA \cdot SO_3Na \cdot 2H_2O$

The solid state stability of ground $DHA \cdot SO_3Na \cdot 2H_2O$ was studied at 40°C in sealed ampules. Figure 3 shows the time course of decomposition of $DHA \cdot SO_3Na \cdot 2H_2O$. As the grinding time was increased, the samples became more unstable. After 6 weeks, 87.5% of the 2 h-ground sample had decomposed, but the intact sample had decomposed to the extent of only 1.2%. In the previous paper,[1] it was reported that the main decomposition product of $DHA \cdot SO_3Na$ was DHA. Figure 3 (B) shows plots of the percentage of formed DHA *versus* time. It was clear that DHA was the major product. This was also confirmed by the results of thin-layer chromatography (TLC). Figure 3 shows a sigmoidal curve. It was suggested that the decomposition proceeded by an autocatalytic reaction, that is, the $NaHSO_4$ formed by hydrolysis of $DHA \cdot SO_3Na$ acted as a catalyst.

Thermal Behavior of the Water of Crystallization of $DHA \cdot SO_3Na \cdot 2H_2O$

Figure 4 shows the effect of grinding on DTA curves of $DHA \cdot SO_3Na \cdot 2H_2O$. The endothermic dehydration reaction of the intact $DHA \cdot SO_3Na \cdot 2H_2O$ proceeded gradually, but that of the ground $DHA \cdot SO_3Na \cdot 2H_2O$ proceeded more quickly, and the dehydration reaction was initiated at a slightly lower temperature. From these results, it was suggested that the bonding force between the water of crystallization and $DHA \cdot SO_3Na$ molecule was weakened by grinding. The dehydration curves of 2 h-ground sample at a constant temperature and $P_{H_2O}=14.9$ mmHg were also studied. Jander's equation was applied to the dehydration curves, as reported previously[3] (Fig. 5). From the slopes, dehydration

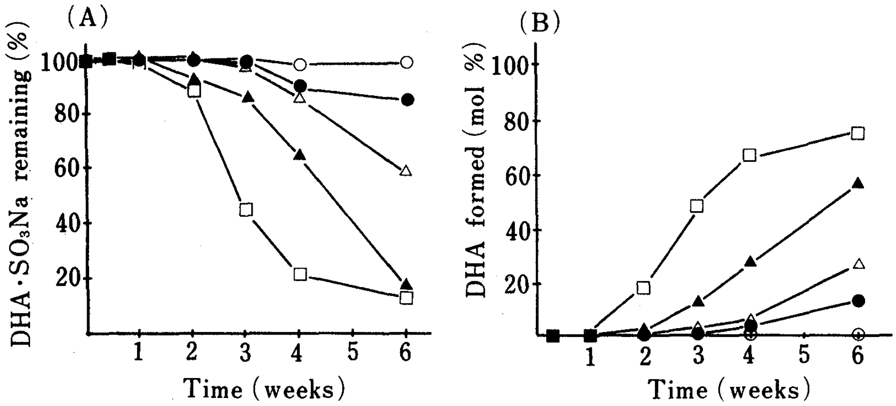

Fig. 3. Effect of Grinding on the Stability of $DHA \cdot SO_3Na \cdot 2H_2O$

(A): decomposition of $DHA \cdot SO_3Na \cdot 2H_2O$. (B): formation of DHA.
Grinding time: ○, intact; ●, 15 min; △, 30 min; ▲, 1 h; □, 2 h.

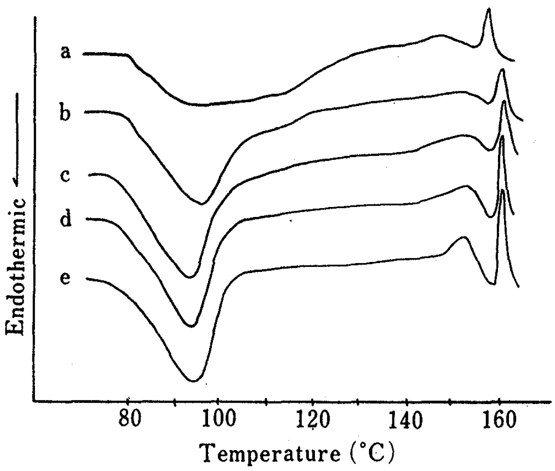

Fig. 4. Effect of Grinding on DTA Curves of $DHA \cdot SO_3Na \cdot 2H_2O$

Grinding time: a, intact; b, 15 min; c, 30 min; d, 1 h; e, 2 h.

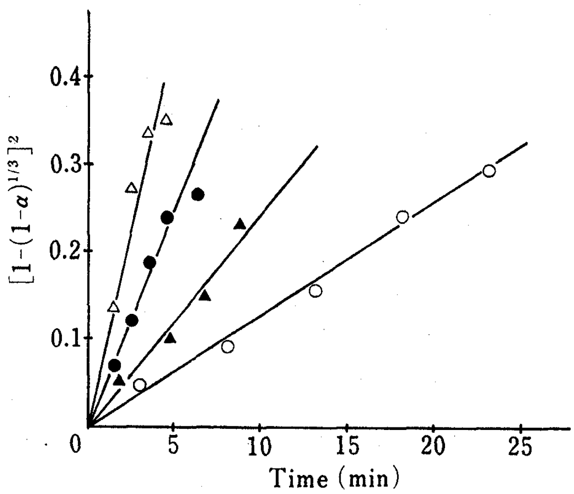

Fig. 5. Plots based on Jander's Equation

○, 85°C; ▲, 95°C; ●, 100°C; △, 109°C.

rate constants were determined, and Arrhenius plots were drawn (Fig. 6). As shown in Figure 6, a good linear relationship was obtained and the activation energy was determined to be 23.3 kcal/mol. This value is smaller than the activation energy for the intact sample, 31.5 kcal/mol.[3] The increase of dehydration rate constants at all temperatures, and the decrease of activation energy, suggested that the bonding force between the water of crystallization and $DHA \cdot SO_3Na$ molecule was weakened by grinding, and this might affect the stability of $DHA \cdot SO_3Na \cdot 2H_2O$.

Effect of Humidity on the Stability of Ground $DHA \cdot SO_3Na \cdot 2H_2O$

The effect of humidity in the atmosphere on the stability of ground $DHA \cdot SO_3Na \cdot 2H_2O$ at 40°C was studied. Figure 7 shows the results for a 2 h-ground sample. It is very interesting that the ground $DHA \cdot SO_3Na \cdot 2H_2O$ was most stabilized under conditions of higher humidity, although $DHA \cdot SO_3Na$ was found to be hydrolyzed.[2] A number of studies have been reported on the decomposition mechanisms of pharmaceuticals in the solid state. In the presence of moisture, a simple "sorbed moisture layer theory"[7,8] was proposed, and moisture accelerates the decomposition rate in such cases. In recent reports, the decompositions of

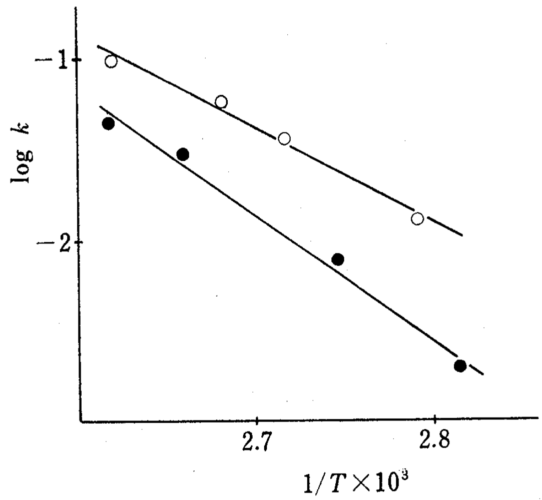

Fig. 6. Arrhenius Plots

○, 2 h-ground; ●, intact.

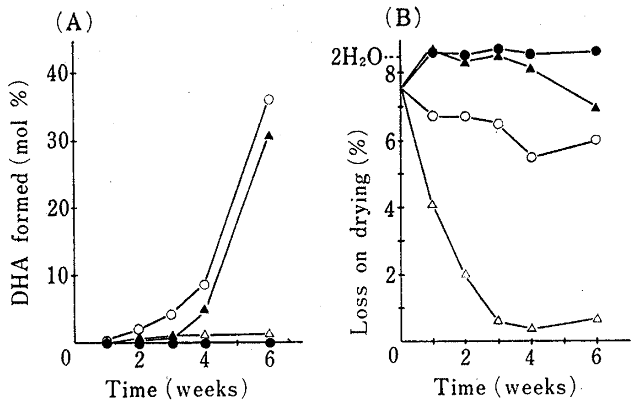

Fig. 7. Effect of Humidity on the Stability of Ground DHA·SO_3Na·$2H_2O$ (2 h)

(A): formation of DHA. (B): loss on drying.
R.H.: △, 0%; ○, 11%; ▲, 40%; ●, 80%.

5-nitroacetylsalicylic acid,[9] glutathione[10] and sulpyrine[11] in the solid state were accelerated under conditions of high humidity. However, in this study, such a moisture effect was not observed and the ground DHA·SO_3Na·$2H_2O$ was stabilized under conditions of high humidity. This result suggests that DHA·SO_3Na may not decompose in an adsorbed water layer on the crystal surface, but may be hydrolyzed by the water molecules within the hydrate crystal, which are sufficiently free to participate in a solid state hydrolytic reaction, and that the bonding force between the water molecule and DHA·SO_3Na molecule is strengthened at high humidity. This assumption was supported by the X-ray diffraction patterns. The ground DHA·SO_3Na·$2H_2O$, whose value of crystallinity as determined by Hermans' method was 51.6%, was stored at 40% R.H. or 80% R.H. at 40°C. After 3 days, the X-ray diffraction patterns of the stored samples were measured and the values of crystallinity were determined. Those of the samples stored at 40% R.H. and 80% R.H. at 40°C were 75.8 and 77.4%, respectively. From these results, it is suggested that this increase of crystallinity implies strengthening of the bonding force between the water molecule and DHA·SO_3Na molecule, and that this effect resulted in a stabilization of DHA·SO_3Na·$2H_2O$. As shown in Figure 7 (B), the 2 h-ground sample at 80% R.H. absorbed water quickly to give an amount of 8.67%, which is almost equal to that of the dihydrate, and was stabilized. At 40% R.H., although absorption of water was observed, the degree of strengthening of the bonding force between the water of crystallization and DHA·SO_3Na molecule was assumed to be lower than at 80% R.H. Thus, the sample stored at 40% R.H. was more unstable than the sample at 80% R.H. At 11% R.H., the sample did not absorb water, and water of crystallization, whose bonding force was weakened by grinding, might attack the DHA·SO_3Na molecule and cause rapid hydrolysis. At 0% R.H., since water molecules available for hydrolysis were scarce, decomposition did not proceed.

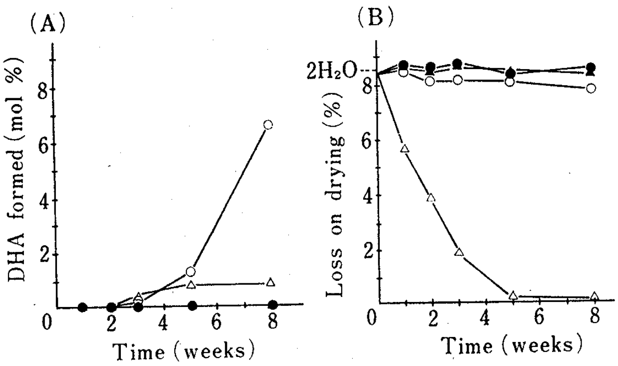

Fig. 8. Effect of Humidity on the Stability of Ground DHA·SO_3Na·$2H_2O$ (1 h)

(A): formation of DHA. (B): loss on drying.
R.H.: △, 0%; ○, 11%; ▲, 40%; ●, 80%.

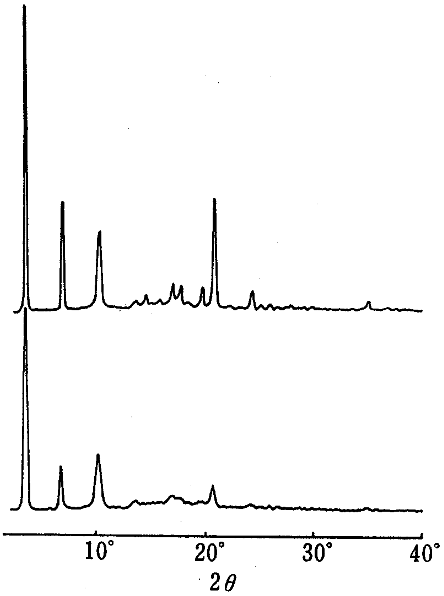

Fig. 9. X-Ray Diffraction Patterns of Dehydrated DHA·SO_3Na·$2H_2O$

Water content: a, 6.35%; b, 1.53%.

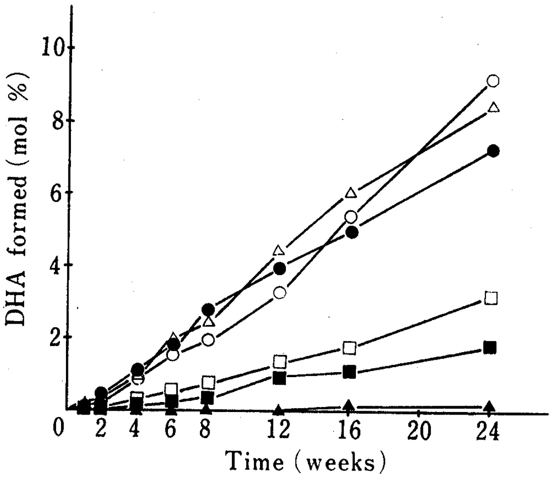

Fig. 10. Effect of Dehydration on the Stability of DHA·SO_3Na·$2H_2O$

Initial water content: ■, 0.12%; ●, 1.49%; △, 2.55%; ○, 5.26%; □, 7.53%; ▲, 8.71%.

Figure 8 shows the results for a 1 h-ground sample. The results are similar to those mentioned above for the 2 h-ground sample. In this case, the sample stored at 40% R.H. was still stable after 8 weeks at 40°C.

Effect of Dehydration on the Stability of DHA·SO_3Na·$2H_2O$

Figure 9 shows X-ray diffraction patterns of partially dehydrated DHA·SO_3Na·$2H_2O$. Crystalline peaks decreased upon dehydration, but no change of the peak position was observed. The values of crystallinity of dehydrated samples whose water contents were 8.62, 6.35, 4.74, 3.93, 1.53 and 0.61%, as determined by Hermans' method using the X-ray diffraction patterns, were 100, 82.7, 77.7, 67.6, 65.4 and 59.8%, respectively. The removal of the water of crystallization resulted in a higher disorder of the crystal structure. The solid state decomposition of partially dehydrated DHA·SO_3Na·$2H_2O$ was studied at 40°C. Figure 10 shows a plot of the percent DHA formation *versus* time. It was shown that the dihydrate form was the most stable. Material from which the water of crystallization had been almost completely removed was relatively stable, but the partially dehydrated product was unstable. Hüttenrauch *et al.*[12] reported that the lattice defects of lactose monohydrate were increased by the drying process. In the case of DHA·SO_3Na·$2H_2O$, it was considered that the disorder induced by drying resulted in weakening of the bonding force between the water molecule and DHA·SO_3Na molecule within the hydrate crystal. This is why the partially dehydrated sample was unstable. From this viewpoint, the sample which had a water content of 0.12% was expected to be the most unstable since it had the highest disorder. However, the sample was actually rather stable. This may be due to the absence of sufficient water molecules to participate in hydrolysis. Grant *et. al.*[13] reported on ampicillin stability and stated that the anhydrous form was more stable than the monohydrate form for the same reason.

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