Study of the Pseudo-Crystalline Transformation from Form I to Form II of Thiamine Hydrochloride (Vitamin B_1)

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The purpose of the current study was to evaluate the rate of the pseudo-crystalline transformation of thiamine hydrochloride form I to form II depending on the temperature and humidity. Changes in the appearance and weight of form I crystals were observed under humidified conditions using a water vapor sorption system equipped with a CCD camera. The form I crystals and tablets were stored under various temperature and humidity conditions (the saturation salt desiccator method), and the pseudo-crystalline transformation was observed using X-ray powder diffractometry with our laboratory equipment for drug substances and using synchrotron X-ray powder diffractometry for tablets. It was confirmed that the activation energy in the pseudo-crystalline transformation rate from form I to form II was dependent on humidity, and the calculated values were 121—155 kJ/mol at 70—90% relative humidity. Moreover, when thiamine hydrochloride was formulated as tablets, it was confirmed that the transformation rate was slower compared with the drug substances alone. The pseudo-crystalline transformation rate therefore declined after formulation in tablet form, perhaps due to the shielding effects of thiamine hydrochloride crystal from contact with water molecules by excipients, compaction, *etc.*

Key words thiamine hydrochloride; pseudo-crystalline transformation; polymorphism; synchrotron X-ray powder diffractometry

There are three types of pseudo-polycrystalline forms of thiamine hydrochloride (vitamin B_1): form I (monohydrate); form II (hemihydrate); and form III (anhydrate). These crystal structures were elucidated using single-crystal X-ray structural analysis, form I by Kraut and Reed in 1962,[1] form II by Watanabe *et al.* in 1979,[2] and form III by Te *et al.* in 2003.[3] Since then, there have been various reports on the physiochemical properties of these pseudo-polycrystalline forms.[4,5]

The relationship between form I (monohydrate) and form III (anhydrate) is that of the enantiotropic transformation accompanying changes in the hydration state. The pseudo-crystalline transformation from form I to form III is achieved by drying, and form III returns to form I by rehydration. The reversibility of form I and form III as well as the dehydration behavior of form II were reported in detail based on each crystal structure.[6,7] It was reported that the pseudo-crystalline transformation from form I to form II occurs when form I is stored for a long time under usual laboratory conditions, in a wet granulation process, in a slurry wash with water, or under specific conditions of 40 °C and 75% relative humidity (RH).[2,8] However, the elucidation of the rate of the pseudo-crystalline transformation depending on storage conditions was lacking. The present study was therefore undertaken to examine the pseudo-crystalline transformation behavior from form I to form II in detail, including kinetic analysis by varying the storage conditions of temperature and humidity. In addition, we aimed to determine whether the rate was affected by formulation with establishing the most appropriate method to detect small amounts of the crystalline polymorph in tablets.

As part of the evaluation of the quality of tablets, which are the final product, the evaluation of crystalline forms of drug substances is important in the quality control of pharmaceutical products. Even if the crystalline form of drug substances in a tablet changes, it is not easy to confirm small amounts of crystalline change in drug substances when combined with various excipients. In this study, we observed changes in the crystalline forms of thiamine hydrochloride in tablets using the synchrotron X-ray powder diffraction (XRPD) method which allows sensitive measurement with parallel bright X-ray beams. We have optimized the methodology of synchrotron XRPD to detect small amount of the drug substance. The detection rate using this methodology is equivalent to about 0.02% of the amount of drug substances in a common size tablet. The details of this methodology will be published separately in the near future. In addition to the high sensitivity, it should be emphasized that the XRPD method is a non-destructive inspection, and the whole tablets can be used as specimen without grinding preparation by utilizing the advantage of highly brilliant and parallel synchrotron radiation.

Experimental

Materials Form I thiamine hydrochloride crystals were purchased from Sigma Chemical (St. Louis, MO, U.S.A.), and used after humidity conditioning at 25 °C and 75% RH (saturation NaCl desiccator) for several days. Form II crystals were purchased as the U.S. Pharmacopeia reference standard from Sigma Chemical and used without modification.

Form I crystals of thiamine hydrochloride 6.0 g, Tablettose-100 (lactose for direct compression) made by Meggle (Tokyo, Japan) 53.4 g, talc made by

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Hayashi Kasei Co., Ltd. (Osaka, Japan) 0.3 g, and magnesium stearate made by Merck KGaA. (Darmstadt, Germany) 0.3 g were measured precisely, then stirred and mixed for 5 min at 1000 rpm using a mixer. A desktop-type tableting machine (Minipress-MII, Stec, Kanagawa, Japan) was set up so that this mixed powder could be formed into tablets weighing about 110 mg, with tablet diameter of 6 mm and tablet thickness of 3 mm, using a tableting pressure of 6.5 kN.

XRPD XRPD data were obtained using a Rigaku (Tokyo, Japan) RINT2200 powder diffraction system, equipped with an Ultima$^+$ (Tokyo, Japan) goniometer I-type in $\theta/2\theta$ geometry. The X-ray generator was operated at 40 kV and 20 mA, using Cu$K\alpha$ radiation (wavelength 1.5418 Å). The scans were performed at room temperature in the range between 5° (2θ) and 25° (2θ), with a scan rate of 2°/min and step size of 0.02°. The slits used were: 0.5° (DS); 0.3 mm (RS); and 0.5° (SS). The number of measurement was once for each experiment.

Dynamic Vapor Sorption Dynamic vapor sorption (DVS) data were obtained using Surface Measurement Systems (SMS) Ltd. equipment (London, U.K.). Samples were stored at 25 °C and 87% RH for 10 d. While monitoring changes in weight during that time, changes in the appearance of the samples were photographed with a CCD camera at specific times.

Synchrotron XRPD Synchrotron XRPD data were recorded at 300 K on beamline BL19B2 at SPring-8 (high resolution type Debye-Scherrer camera equipped with a curved imaging plate detector) with wavelength 0.69817(5) Å. The exposure time was 20 min for one diffraction image, allowed to fade for 10 min, and then read with a scanner. A glass capillary was glued to the center of the tablets to serve as a rotating wheel axis, then it was fixed to the goniometer head with height adjustment. The measurements were performed while rotating the tablet at the rate of one revolution per second with the incident direction taken as the tablet radial direction (perpendicular to the rotating wheel axis) so that synchrotron radiation irradiated an entire tablet. The number of measurement was once for each experiment.

Calibration Curve In the combination of the intensity of XRPD peak at 10.27° (2θ) for the form II and 11.46° (2θ) for the form I, an approximation of a linear equation was performed employing the least squares method, and a calibration curve was prepared. On the other hand, in the combination of 8.11° (2θ) for the form II and 8.58° (2θ) for the form I, approximation to a secondary equation was performed employing the least squares method, and a calibration curve was prepared, because the peak intensity ratio differs greatly when an equimolar mix of the two is made (the peak intensity ratio of form II is about three-fold greater than that of form I).

Calculation of the Transition Kinetic Constant Using the Prout-Tompkins Equation Calculation of the kinetic constant of the crystalline transformation (form I to form II) in samples stored under each temperature and humidity condition was carried out using Origin ver. 6J software (Microcal Software, Inc., Northampton, MA, U.S.A.) using the following Prout-Tompkins equation (Eq. 1).[2] Curve fitting was performed employing the nonlinear least squares method with the Levenberg–Marquardt algorithm using over seven data points.

$$\log\left(\frac{y}{1-y}\right) = Kt + C \quad (1)$$

where y is the molar ratio of form II, t is time (d), K is the kinetic constant, and C is a constant term.

For calculation of the half-life (from form I to form II), curve fitting was performed as in the above kinetic constant calculation employing the following Boltzmann function (Eq. 2).

$$y = \frac{-1}{1 + e^{(t-t_{1/2})/dt}} + 1 \quad (2)$$

where y is the molar ratio of form II, t is time (d), $t_{1/2}$ is half-life (d), and dt is a time constant measured in the experiment.

Calculation of Activation Energy The logarithmic value of the kinetic constant of the crystalline transformation at each RH was plotted for each temperature. An approximation to a linear equation was performed employing the least squares method. Based on relational expressions obtained here, a simulation was performed of the temperature dependency of the crystalline transformation kinetic constant at RH of 70%, 75%, 80%, 85%, and 90%. Furthermore, at each RH, the kinetic constant of the crystalline transformation was plotted for each absolute temperature (Arrhenius plots). At each RH, fitting was carried out to a linear equation using the least squares method, and the activation energy for each RH was calculated based on the inclination of this linear equation.

Results and Discussion

Pseudo-Crystalline Transformation Behavior from Form I to Form II At the beginning of this experiment, the crystal forms of those materials were checked by XRPD for according to the XRPD patterns of form I and form II on the previous reports,[2—4] and solid state NMR for the purity of crystal forms. In this result, those materials were confirmed form I and form II, and had good crystal purity. Generally speaking, the detection limit of crystal impurity is a few percentages by solid state NMR[9] (data not shown).

While storing form I crystals at 87% RH using the DVS and monitoring the changes in weight during that time, changes in the crystalline appearance were observed with the CCD camera as shown in Fig. 1. From the start of measurement, a significant weight increase of form I due to moisture absorption was observed about 5 d afterward, and 2 d after that, *i.e.*, at 7 d of storage, a sharp decrease in weight (moisture desorption) was observed. Form I had good flowability in powder form at the time of preparation, and deliquescence in the crystal surface was seen when the rapid increase in weight occurred after about 5 d. After the subsequent weight reduction, it reverted to white crystals.

Based on the results observed when form I was stored at high RH, we assume that the following occurred gradually: 1) Moisture was adsorbed on the form I crystal surface. 2) Part of the form I crystals dissolved (deliquesced) in the adsorbed moisture. 3) Since form II has less solubility in water than form I, a recrystallization transformation to form II crystals occurred. 4) Since form II crystals are hemihydrates, they release moisture and their weight decreases. In addition, after examining the XRPD data of samples after this measurement period, complete transformation into form II (hemihydrate) was confirmed. Therefore, it was concluded that the pseudo-crystalline transformation rate from form I to form II was markedly affected by the temperature and RH during storage.

Then, the behavior of the pseudo-crystalline transformation to form II after form I was stored under various temperature and humidity conditions was examined. Form I crystals were placed in a desiccator at 25 °C, 30 °C, 40 °C, and 50 °C with saturated salt solutions of sodium chloride, potassium

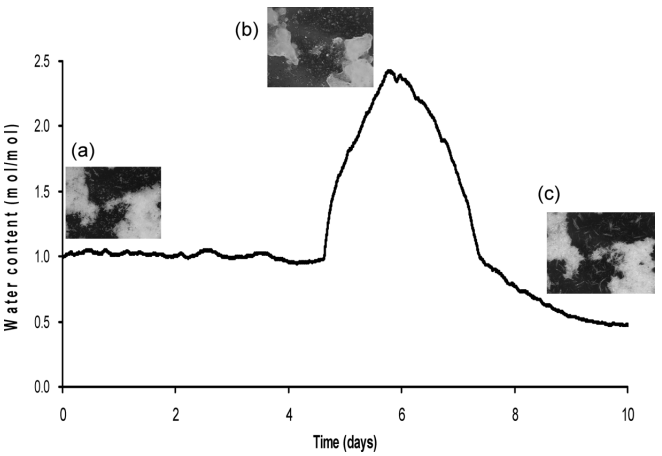

Fig. 1. Gravimetrics of Thiamine Hydrochloride Form I in Humid Conditions (25 °C, 87% RH): Time Course of Weight Change Shown in Photos Taken with a CCD Camera during Storage

(a), Initial status; (b), at 6 d of storage; (c), at 10 d of storage.

bromide, potassium chloride, and potassium nitrate (see Table 1). The samples were then removed at predetermined times, and XRPD measurement was performed. Using the two-dimensional detector of synchrotron XRPD, the diffraction patterns of polymorphs were initially determined as uniform intensities for quantitative analysis. Furthermore, the quantitation accuracy of this XRPD method was separately confirmed by solid-state NMR on several experimental points. Those data were almost agreed each other (data not shown).

Calculation of the molar ratio of the crystalline polymorph mixture of form I and form II was carried out employing the calibration curve created separately in advance (data not shown). XRPD measurements were performed for crystalline polymorph mixtures of various molar ratios of form I and form II (100 : 0, 75 : 25, 50 : 50, 25 : 75, and 0 : 100). Based on the molar ratio of the crystalline polymorphs, a calibration curve was prepared by plotting the ratio of the integrated intensity of the XRPD peak of form II to the sum of the integrated intensity of the XRPD peak of form I and form II. Calculation of the molar ratio of the crystalline polymorph of form I and form II was carried out using XRPD peaks at 8.58, 11.46° (2θ), and 8.11, 10.27° (2θ) for form I and form II, respectively.

The pseudo-crystalline transformation behavior from form I to form II in XRPD pattern is shown in Fig. 2a at 50 °C and 74% RH as a representative example. The behavior of the pseudo-crystalline transformation from form I to form II was gradually appeared, while the peak of form I was extinguished by gradation. This can be explained as an irreversible pseudo-crystalline transformation phenomenon in the water-containing environment. The behavior of the pseudo-crystalline transformations from form I to form II under various temperature and humidity conditions is shown in Fig. 3. It was confirmed that the pseudo-crystalline transformation progressed with an approximately sigmoidal curve under all temperature and humidity conditions (Fig. 2b). Prout and Tompkins proposed that this behavior of changes following a sigmoidal curve is represented by the Prout–Tompkins equation, Watanabe *et al.* reported that the pseudo-crystalline transformation to form II could be described by the Prout–Tompkins equation when form I was suspended with water, and results with good fit were obtained.[2] Since it was assumed that the crystalline transformation from form I to form II under various temperature and humidity conditions in the present experiments also followed the Prout–Tompkins equation, curve fitting of the Prout–Tompkins equation was performed as reported by Watanabe *et al.*,[2] and the kinetic constant of the crystalline transformation was calculated (Table 2).

Table 1. RH at Each Temperature and Saturated (Sat.) Salt Condition

Temperature (°C)	Sat. NaCl	Sat. KBr	Sat. KCl	Sat. KNO_3
25	75.3	80.9	84.3	93.6
30	75.1	80.3	83.6	92.3
40	74.7	79.4	82.3	89.0
50	74.4	79.0	81.2	84.8

A linear relationship was found at each temperature for the change in the logarithm of the crystalline transformation rate with RH. Then, when linear regression with linear expression was performed at 25 °C, 30 °C, 40 °C, and 50 °C, the correlation coefficients obtained of 0.940, 0.951, 0.913, and 0.914, respectively, were reasonable. It was thus confirmed in this experimental scope that linear regression analysis is possible. Using this result, the values of $\ln k$ were derived corresponding to the relative humidity values, such as exactly 70%, 75%, 80%, 85%, and 90% RH. Then, Arrhenius plots were drawn for each RH to show the reciprocal relationship of absolute temperature and the kinetic constant of the pseudo-crystalline transformation (Fig. 4).

The activation energy of the pseudo-crystalline transformation at each RH is shown in Fig. 5. The pseudo-crystalline transformation rate from form I to form II depended on both temperature and humidity, and it was calculated that the activation energy of the pseudo-crystalline transformation was 121 to 155 kJ/mol in the RH range of 70—90%.

The results indicate that a good approximation is given by the Prout–Tompkins equation expressed with a sigmoidal

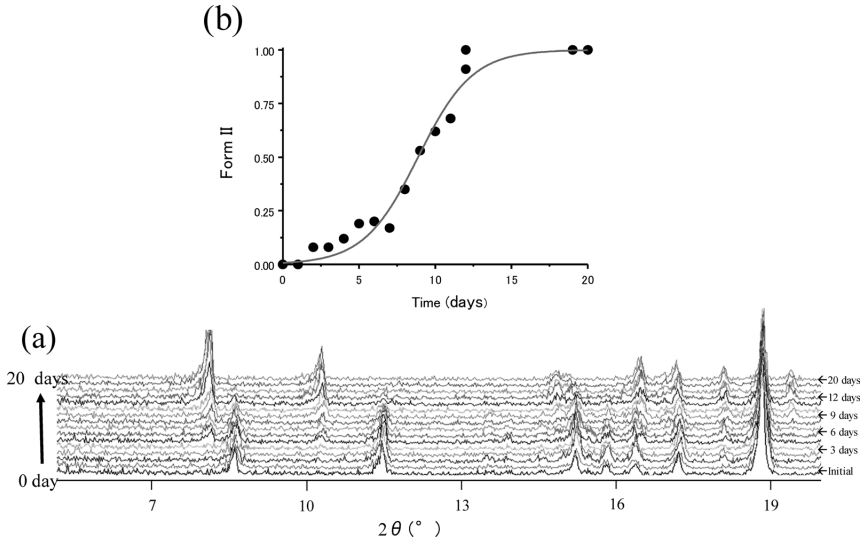

Fig. 2. (a) Pseudo-Crystalline Transformation Behavior of Thiamine Hydrochloride at 50 °C and 74% RH in XRPD. (b) Form II Ratio Obtained Using the Calibration Curves with Sigmoidal Curve Fitting

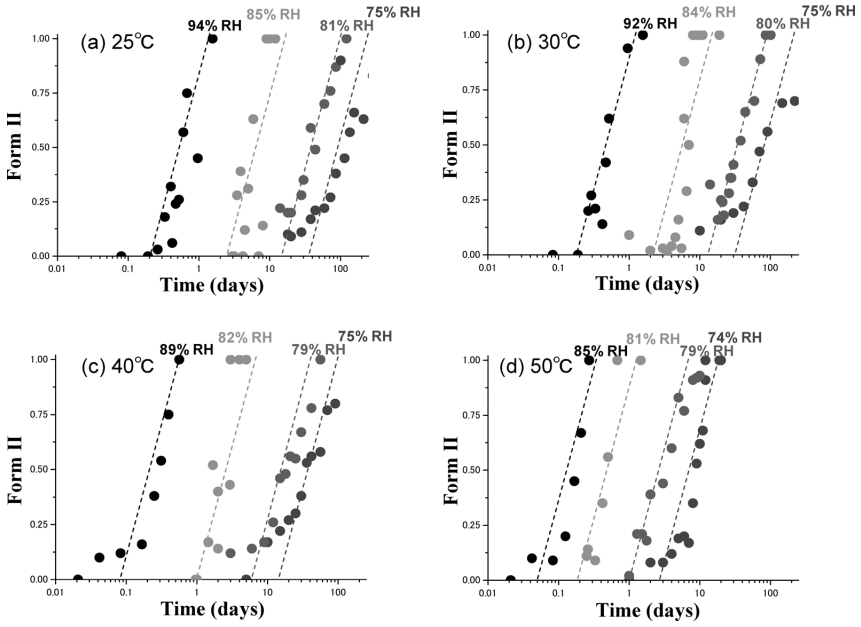

Fig. 3. Pseudo-Crystalline Transformation Behavior of Thiamine Hydrochloride at Various Temperature and Humidity Conditions

(a), 25 °C/75—94% RH; (b), 30 °C/75—92% RH; (c), 40 °C/75—89% RH; (d), 50 °C/74—85% RH.

Table 2. Rate Constants of the Pseudo-Crystalline Transformation of Thiamine Hydrochloride from Form I to Form II under Various Temperature and Humidity Conditions

	Sat. NaCl	Sat. KBr	Sat. KCl	Sat. KNO_3	Correlation coefficient for %RH *vs.* ln *k*
Temperature (°C)			25		
% RH	75.3	80.9	84.3	93.6	
Rate constant of polymorph transition *k* (1/d)	0.02	0.05	1.63	9.63	
ln *k*	−3.80	−3.08	0.49	2.27	0.940
Temperature (°C)			30		
% RH	75.1	80.3	83.6	92.3	
Rate constant of polymorph transition *k* (1/d)	0.02	0.07	1.51	7.11	
ln *k*	−4.04	−2.7	0.41	1.96	0.951
Temperature (°C)			40		
% RH	74.7	79.4	82.3	89	
Rate constant of polymorph transition *k* (1/d)	0.04	0.10	4.49	9.84	
ln *k*	−3.20	−2.31	1.50	2.29	0.913
Temperature (°C)			50		
% RH	74.4	79	81.2	84.8	
Rate constant of polymorph transition *k* (1/d)	0.48	0.77	11.20	20.72	
ln *k*	−0.74	−0.26	2.42	3.03	0.914

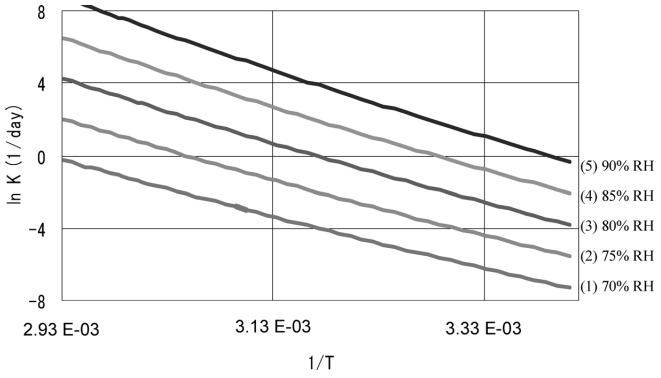

Fig. 4. Arrhenius Plot Simulation of the Pseudo-Crystalline Transformation of Thiamine Hydrochloride from Form I to Form II at Different Humidity Levels

(1), 70% RH; (2), 75% RH; (3), 80% RH; (4), 85% RH; (5), 90% RH.

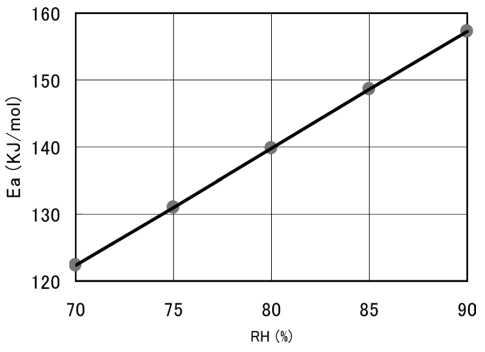

Fig. 5. Dependence of the Activation Energy of the Pseudo-Crystalline Transformation of Thiamine Hydrochloride on RH

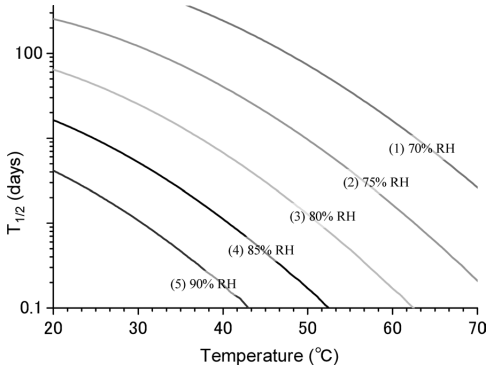

Fig. 6. Simulation of the Half-life of the Pseudo-Crystalline Transformation of Thiamine Hydrochloride from Form I to Form II under Different Temperature and Humidity Conditions

(1), 70% RH; (2), 75% RH; (3), 80% RH; (4), 85% RH; (5), 90% RH.

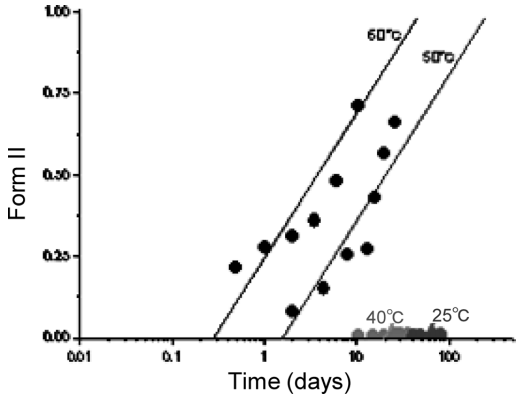

Fig. 8. Time Course of the Pseudo-Crystalline Transformation of Thiamine Hydrochloride in Tablet from Form I to Form II at Approximately 75% RH at Temperatures of 25 °C, 40 °C, 50 °C, and 60 °C

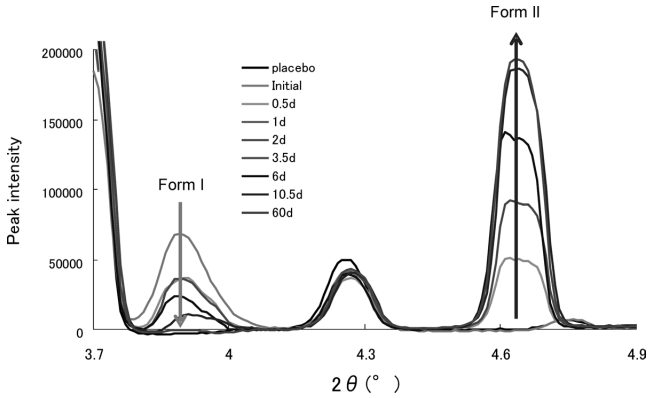

Fig. 7. Pseudo-Crystalline Transformation Behavior of Thiamine Hydrochloride Tablet at 60 °C and Approximately 75% RH in XRPD

curve for the pseudo-crystalline transformation from form I to form II under our RH conditions. We therefore estimated the half-life of this crystalline transformation as expressed by the inflection point of the sigmoidal curve. The Boltzmann function formula was used for each sigmoidal curve, which could express the molar ratio (y) of form II at any time (t), half-life ($t_{1/2}$), and time constant (dt); it was determined in advance that the initial molar ratio of form II was 0, and the final molar ratio of form II was 1. The inflection point of curve fitting was used as the half-life ($t_{1/2}$) (Eq. 2). The half-life in relation to the temperature at various RH values is shown in Fig. 6. The lower the RH during storage, the longer the half-life was extended in an exponential manner for the pseudo-crystalline transformation.

Pseudo-Crystalline Transformation Behavior of Form I in Thiamine Hydrochloride Tablets Using Synchrotron XRPD After tablets of form I crystals were stored for a predetermined period at 25 °C, 40 °C, 50 °C, and 60 °C in NaCl saturation desiccators (about 75% RH), they were removed, subjected to synchrotron XRPD, and the pseudo-crystalline transformation behavior from form I to form II in tablets was examined. The pseudo-crystalline transformation of thiamine hydrochloride tablets behavior from form I to form II in XRPD pattern is shown in Fig. 7 at 60 °C and 75% RH as a representative example. XRPD peaks of form I and form II showed at 3.89, 5.20° (2θ) and 3.67, 4.66° (2θ), respectively with wavelength 0.69817(5) Å. These 2θ values correspond to the 8.58 and 11.46°, and 8.11 and 10.27° (Cu$K\alpha$ X-ray) for form I and form II, respectively, which were used in the previous section and also in the previous reports.[2—4]

The pseudo-crystalline transformation to form II was confirmed at 50 °C and 60 °C, while form II was not detected at 25 °C and 40 °C (Fig. 8) even they were stored in high humidity desiccators in 18 d in tablet form. It is enough time considering the half-life of the pseudo-crystalline transformation from form I to form II was about 10 d when drug substances alone were stored at 50 °C and about 75% RH. The pseudo-crystalline transformation rate therefore declined after formulation, perhaps due to the shielding effects of contact with water molecules by excipients, compaction, *etc.*

Conclusion

The behavior of the pseudo-crystalline transformation from form I to form II of thiamine hydrochloride was investigated based on changes in appearance and weight. Partial deliquescence occurred, and subsequent loss of moisture (from monohydrate to hemihydrate) under humidified conditions was also seen. Furthermore, the pseudo-crystalline transformation rate under different temperature and humidity conditions was elucidated.

Using the synchrotron XRPD method, the pseudo-crystalline transformation rate from form I to form II of thiamine hydrochloride in tablet form was determined, and it was found that the crystalline transformation rate was declined after formulation into tablets.

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