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Chemical Engineering Journal 75 (1999) 193–200

Chemical Engineering Journal

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Crystal structures and solvent-mediated transformation of Taltireline polymorphs

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Accepted 30 June 1999

Abstract

Taltireline, a central nervous system activating agent, has two crystal forms (α-form and β-form). These crystal structures and the solvent-mediated transformation are described. The α-form was triclinic, P1, and the β-form was orthorhombic, $P2_1\,2_1\,2_1$. A conformational change associated with the transformation was also postulated. The solubility profile of polymorphs showed an enantiotropic system and the α-form chosen as a drug substance was found to be metastable at its crystallization temperature (10°C). In order to suppress the transformation from the α-form to the β-form, the critical parameters that influence the crystallization process were examined. Experiments for measuring the transformation rate were carried out under various conditions using XRD, and the overall transformation rate constant k_T and the waiting time θ for the nucleation of the β-form were estimated by fitting to an equation for the crystal growth rate. The results showed that the coexistence of MeOH, excess cooling during the crystallization, and fast stirring promoted the transformation. The desirable α-form can be consistently obtained by controlling these factors.

Keywords: Crystallization; Polymorphism; Transformation; Pharmaceuticals; Taltireline

Nomenclature

C	concentration of Taltireline (percentage of g g^{-1}solution: wt.%)
C_α	solubility of α-form (wt.%)
C_β	solubility of β-form (wt.%)
ΔC	degree of supersaturation expressed as $(C_\alpha - C_\beta)$ (wt.%)
X_β	mass fraction of β-form in precipitate (−)
k_0	transformation rate constant in Eq. (1) (h^{-1})
k_T	overall transformation rate constant in Eq. (2) (h^{-1})
t	elapsed time (h)
θ	waiting time (h)
T	suspending temperature (°C)
σ	relative supersaturation expressed as $\Delta C/C_\beta$ (−)

1. Introduction

Polymorphism in pharmaceuticals is very important from the viewpoints of bioavailability [1–3], stability and handling [4,5], because the difference in crystal form causes a change in physical properties. Although much investigation on polymorphism, e.g., chemical engineering approach [6], effects of additives [7–9] and solvents [10,11], and prediction of polymorphs using a computer [12], has been done, the polymorphic behavior is essentially not well understood [13].

In the present work, the polymorph of a drug, Taltireline (Fig. 1), which is a central nervous system activating agent [14], (−)-*N*-[(s)-hexahydro-1-methyl-2,6-dioxo-4-primidinyl]-*L*-histidyl-*L*-prolinamido tetrahydrate, was investigated. This compound is a peptide consisting of three components (1-methyl dihydroorotic acid, *L*-histidine, and *L*-prolinamido), and has two crystal forms. The form having the higher solubility at its crystallization temperature (10°C) was defined as the α-form and the other as the β-form. Both forms were crystallized from water as the tetrahydrate. The α-form, which has good characteristics in solid–liquid separation, was chosen as the drug substance. However, the α-form is metastable, because the solubility of the α-form is higher. The problem is that α-form crystals are forced to change to the β-form by a solvent-mediated transformation. In relation to the Good Manufacturing Practice (GMP), a constant quality of product must be

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1385-8947/99/$ – see front matter
PII: S1385-8947(99)00115-1

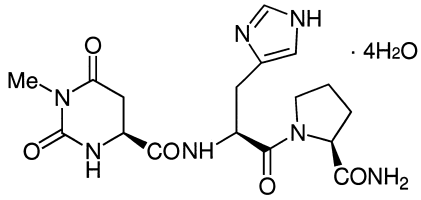


Fig. 1. Chemical structure of Taltireline.

assured. To suppress this transformation during the crystallization process of Taltireline, it is necessary to clarify the factors that influence this process.

In this work, crystal structures and physical properties of the two crystal forms were investigated. Furthermore, critical factors affecting the solvent-mediated transformation were discussed. In particular, we focused on the influence of methanol (MeOH) on the transformation, because the final step of the synthesis of Taltireline is performed in water containing MeOH and the crude Taltireline containing MeOH is used in the crystallization process.

2. Experimental

2.1. Materials and identification of polymorphic form

The α-form of Taltireline (lot No. 303030, supplied from Tanabe Seiyaku; molecular formula $C_{17}H_{23}N_7O_5{\cdot}H_2O$, Mw 477.47) was used without further purification. The β-form was prepared by recrystallization from an aqueous solution containing 30 wt.% MeOH. Each polymorph was identified by IR spectroscopy (Horiba FT-IR 300). X-ray powder diffraction (Mac Science, MXP3VA) was used to estimate the composition of the polymorphic forms. A microscopic observation of the crystals was carried out using an Olympus system. Single crystals (α-form, 0.4 mm × 0.4 mm × 0.3 mm; β-form, 0.4 mm × 0.3 mm × 0.3 mm) were used for the X-ray structural analysis with a Rigaku AFC5R diffractometer (graphite monochromatized Cu Kα radiation).

2.2. Solubility

The solubility of each polymorph was measured in the temperature range of 5–45°C, as follows. To a 50 ml glass vessel equipped with an agitator was placed 20 ml of solvent (water containing 0–10 wt.% MeOH), and controlled at a given temperature. A slight excess of Taltireline was suspended, and the supernatant was withdrawn using a sampling apparatus with a glass-filter (as shown in Fig. 2). The concentration of Taltireline in the supernatant was determined by HPLC. Using the residual crystals of Taltireline on the filter, it was confirmed by IR spectroscopy that the transformation did not occur while the solubility of the α-form was measured.

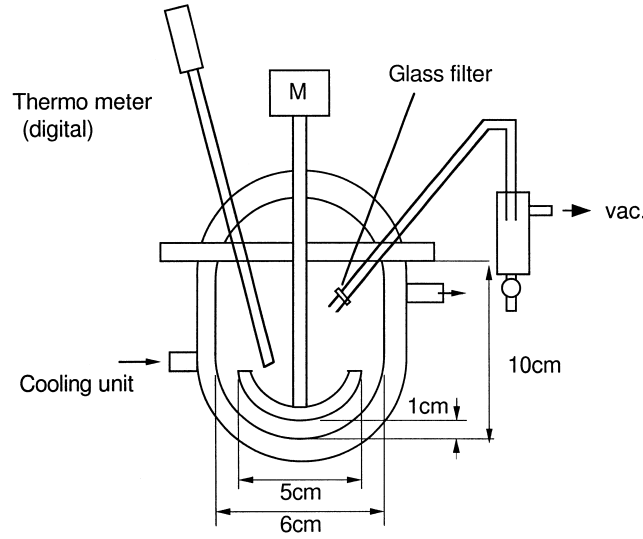


Fig. 2. Schematic diagram of experimental apparatus for the transform.

2.3. Measurement of transformation rate

The experimental apparatus is illustrated in Fig. 2. 70 g of solvent (water containing 0–10 wt.% MeOH) was placed in a 200 ml cylindrical jacketed glass vessel which was equipped with an anchor paddle (stirring speed: 300 rpm), and the inner temperature was controlled at 10°C. 30 g of the α-form crystal was then added. When the β-form crystals were observed with a microscope in the suspension, the sampling was started using the sampling apparatus with a glass-filter. The concentration of Taltireline in the supernatant was determined by HPLC and the composition of the polymorphic forms in the solid was estimated by X-ray powder diffraction after drying.

3. Results and discussion

3.1. Characterization of polymorphic form

IR-spectra: The IR spectra of the polymorphs using the nujol mull method are shown in Fig. 3. A difference in the absorption profile (peak shape and peak intensity) between both forms is observed. The absorption at 3630 cm^{-1}, which is the characteristic peak for the β-form, is absent in the α-form. The absorption at 1718 cm^{-1}, based on the C=O stretching vibration, of the α-form is weak, whereas that of the β-form is sharp and strong. Although the difference between the α-form and the β-form can be qualitatively distinguished, it is difficult to estimate the composition of each form from the IR spectra.

X-ray powder diffraction: The X-ray powder diffraction patterns of the polymorphs are shown in Fig. 4. In the β-form, its characteristic peaks (8.1°/2θ, 16.2°/2θ) are observed. Based on the results of data conversion of

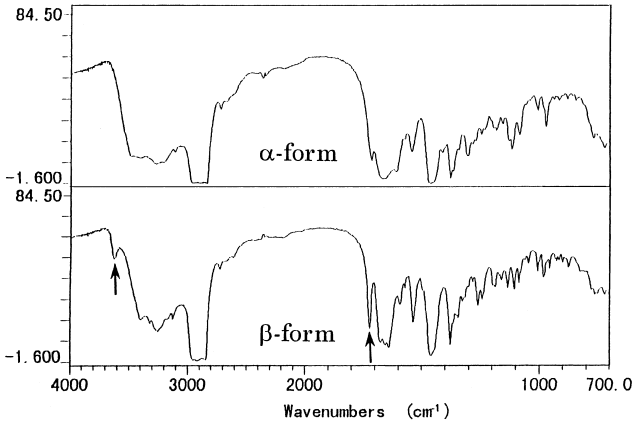


Fig. 3. IR spectra of polymorphs.

the single crystal analysis, it was found that the peaks of 8.1°/2θ and 16.2°/2θ correspond to 0 2 0 (reciprocal lattice point) and 0 4 0 respectively. As the peak of 16.2°/2θ in the β-form has high intensity without overlap of the α-form, the composition of the β-form could be estimated using the calibration curve obtained from the relationship between the mass fraction of the β-form and the intensity.

Microscopic observations: The difference in the crystal shape between the α-form and β-form is recognized. The shape of the α-form crystals is prismatic (Fig. 5a), whereas that of the β-form crystals is a thin plate with reduced crystal size (Fig. 5b). Therefore, the transformation from the α-form to the β-form could be followed using a microscope.

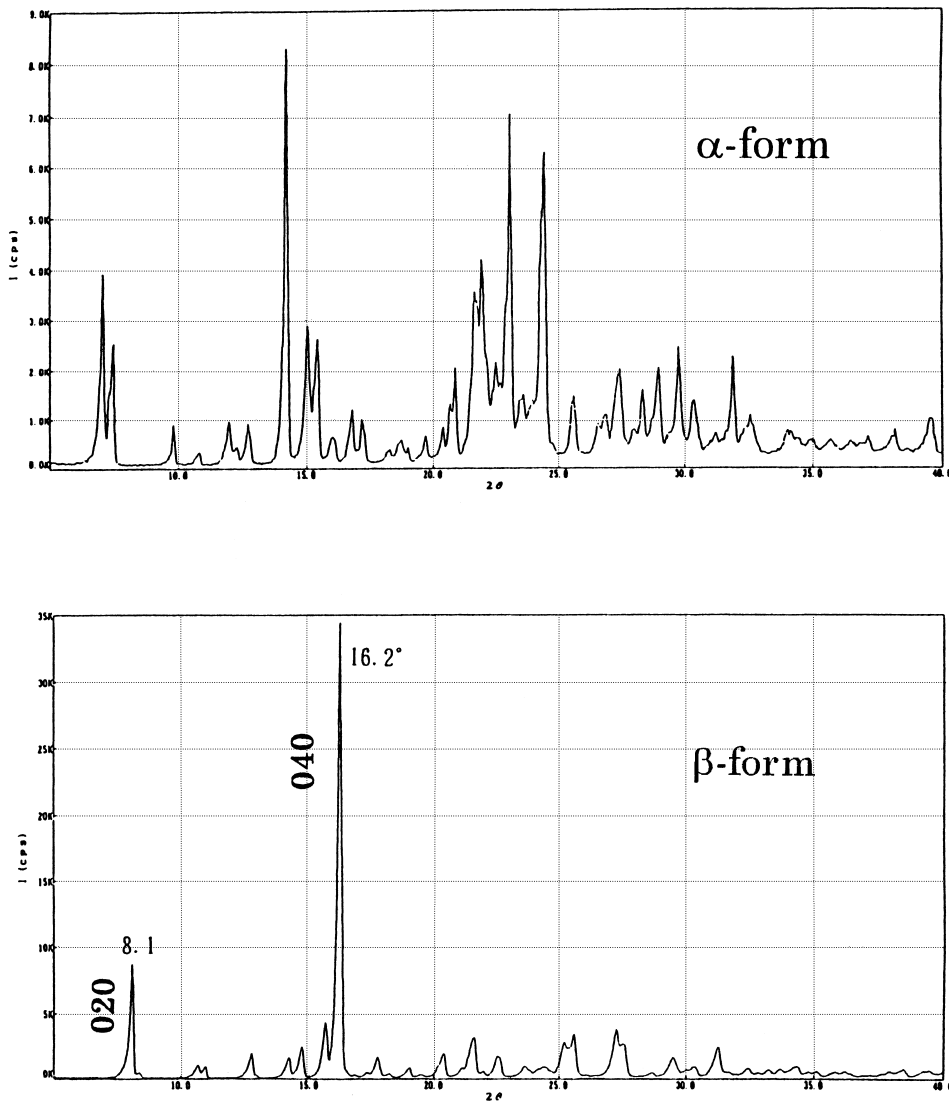
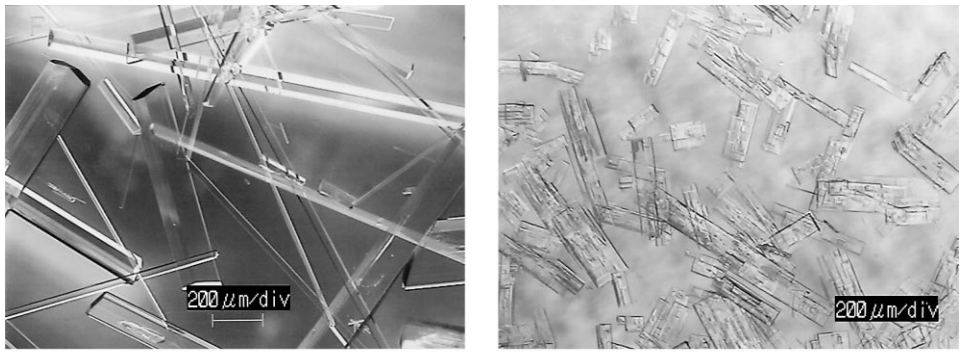


Fig. 4. X-ray powder diffraction patterns of α-form and β-form.



(a) (b)

Fig. 5. Microscope photographs of α-form (a) and β-form (b).

3.2. Crystal structures of polymorphs and the conformational change

The crystal data and molecular packing schemes are shown in Table 1 and Fig. 6. The crystal system and the space group of the α-form (triclinic, P1) are different from those of the β-form (orthorhombic, $P2_1\ 2_1\ 2_1$). Considering the cell angle, the β-form has good symmetry. Regarding the calculated crystal density, the α-form is more densely packed than the β-form. The space group of the α-form was P1 with $Z = 2$, which indicated that two molecules of Taltireline and eight molecules of water exist in a unit cell. Both molecules of Taltireline in a unit cell of the α-form have almost the same conformation, whereas the eight molecules of water are not equivalent. In both polymorphs, the water molecules are considered to play an important role as binders to form the hydrogen bond network in crystal, because the anhydrate crystal of Taltireline has not been obtained.

The conformational change from the α-form to the β-form is indicated in Fig. 7. The C_6–C_{10} bond in the neck of six members ring (1-methyl dihydroorotic acid) and the C_{17}–C_{21} bond of prolineamide characteristically rotate. In this case, the presence of the intermolecular hydrogen bond plays an interesting role. Through the transformation, the hydrogen bonds existing in the α-form, O_7–$HN_{23'}$ (3.190 Å) and $O_{15'}$–HN_1 (2.876 Å), are cut and new hydrogen bonds, O_8–$HN_{23'}$ (2.997 Å) and $O_{22'}$–HN_{12} (3.017 Å), are formed in the neighborhood of the old bonds.

Table 1
Crystal data of polymorphs at 293 K

	α-form	β-form
a	12.345 Å	11.981 Å
b	12.835 Å	21.809 Å
c	7.938 Å	8.942 Å
α	103.67°	90°
β	106.89°	90°
γ	91.18°	90°
Crystal system	Triclinic	Orthorhombic
Space group	P1	$P2_1\ 2_1\ 2_1$
Z	2	4
Density	1.362 g cm^{-3}	1.357 g cm^{-3}

3.3. Solubility

The solubility of the polymorphs of Taltireline in water and that in water containing 5 wt.% MeOH are shown in Fig. 8a and b. The solubility curves showed an enantiotropic system, namely, the solubility curves of both forms crossed at a certain temperature. The α-form is stable in solution above the cross point, whereas the β-form is stable in solution below the point. Since the crystallization was carried out at temperature near 10°C, the β-form is regarded as the stable-form (''stable'' means lower solubility).

3.4. Transformation rate

The transformation behavior: The transformation behavior in water is shown in Fig. 9 as a representative example. After an induction period (I), which should be the waiting time for nucleation, the transformation proceeded with keeping a constant concentration of Taltireline in the region (II). The constant concentration corresponds to the solubility of the α-form. It means that the decrease in the solution concentration caused by the crystallization of the β-form (stable-form) is supplemented with the dissolution of the α-form (metastable-form) without delay. This shows that the dissolution rate of α-form is faster than the growth rate of the β-form. Therefore, in the region (II), the growth rate of the stable form (β-form) is regarded as therate-determining step. In the next region (III), the solution concentration decreased and approached the solubility of the β-form. The decrease in the concentration is caused by the fact that the dissolution rate of the α-form becomes slower due to the decrease in the quantity of the α-form. A similar behavior was observed under other experimental conditions.

A solvent-mediated transformation consists of three processes: (1) the dissolution of the metastable form; (2) the nucleation of the stable form; (3) the crystal growth of the

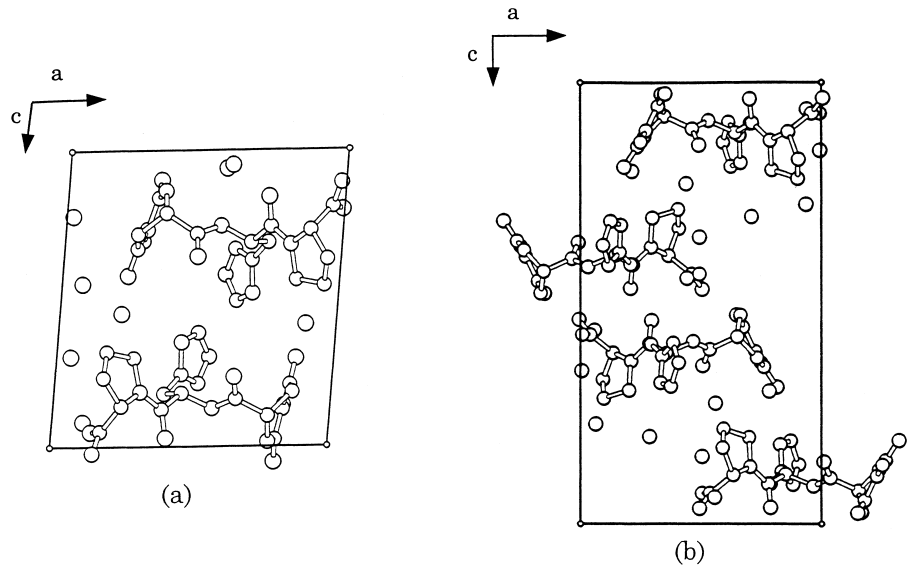


Fig. 6. Crystal structures of α-form (a) and β-form (b) viewed along *b*-axis.

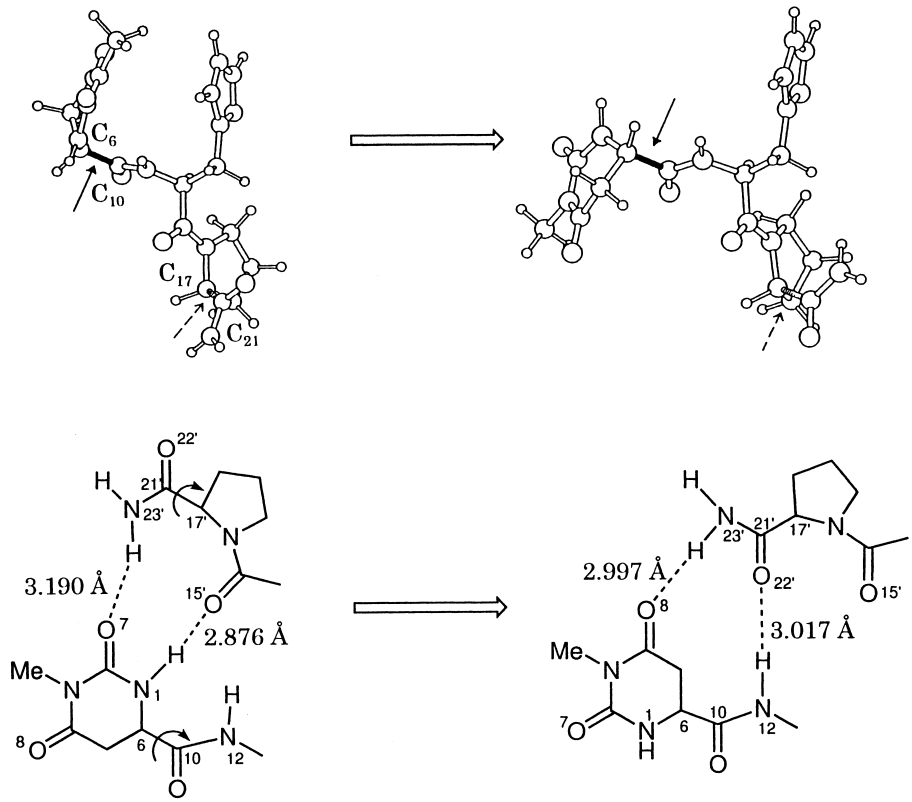


Fig. 7. Conformational change and the mode change of hydrogen bond associated with the transformation from α-form to β-form.

stable form. When the crystal growth of the stable form is the rate-determining step and the growth rate is proportional to the crystal surface area, the growth rate, namely the transformation rate, is expressed by

$$\frac{dX_\beta}{dt} = k_0 X_\beta^{2/3} f(x), \tag{1}$$

where X_β is the mass fraction of the β-form in precipitate, k_0 the rate constant and $f(x)$ the driving force for a crystal growth [6,15]. Since the difference in solubility between the α-form and the β-form, ΔC ($C_\alpha - C_\beta$), remained unchanged during the transformation in region (II), the driving force of the transformation could be regarded to be a constant. In this case, Eq. (1) can be expressed by

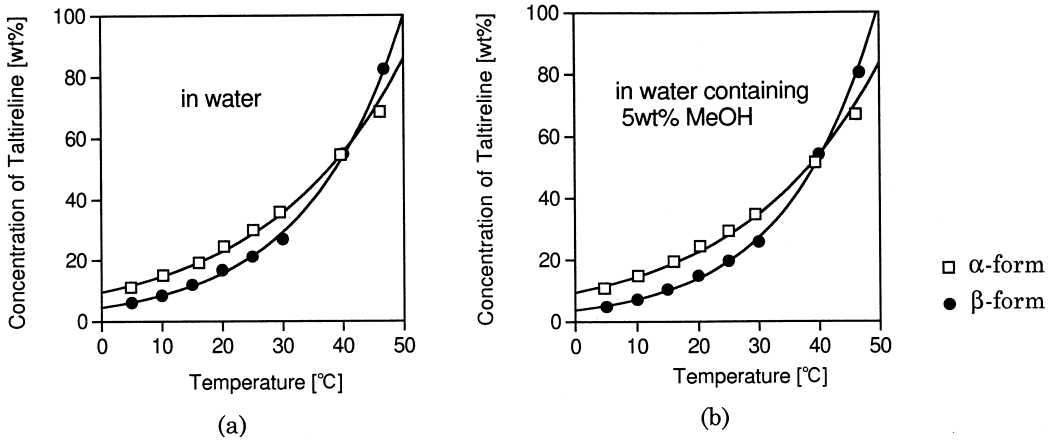


Fig. 8. Solubility of the polymorphs of Taltireline in water (a) and in water containing 5 wt.% MeOH (b).

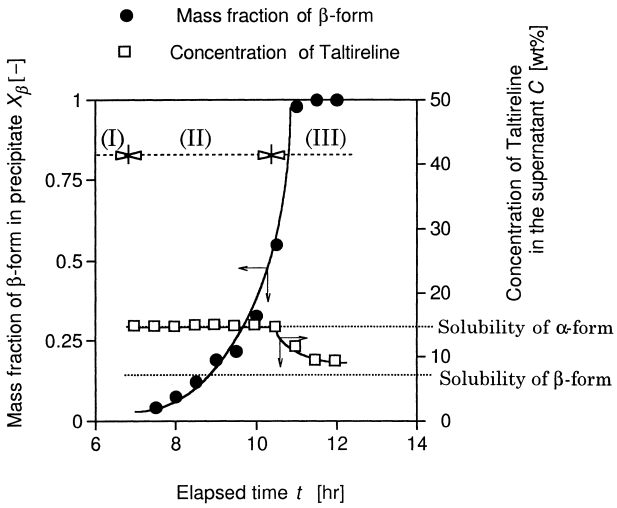


Fig. 9. Transformation behavior of Taltireline in water at 10°C.

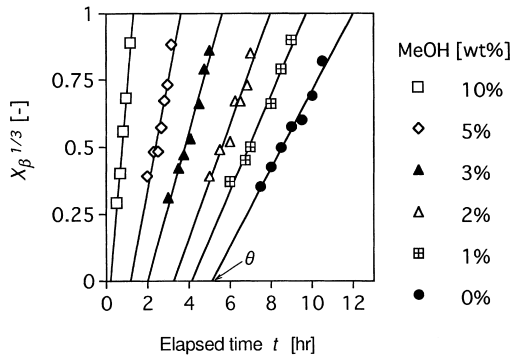


Fig. 10. Relationship between $X_\beta^{1/3}$ and elapsed time t.

$$\frac{dX_\beta}{dt} = k_T X_\beta^{2/3}, \quad (2)$$

or

$$X_\beta^{1/3} = \frac{k_T}{3}(t-\theta) \quad (0 \le t \le \theta:\ X_\beta^{1/3} = 0), \quad (3)$$

where k_T is the overall transformation rate constant and θ the waiting time for nucleation of the β-form.

As shown in Fig. 10, the relationship between $X_\beta^{1/3}$ and elapsed time t gave linear plots for water containing various concentrations of MeOH. The overall transformation rate constant k_T was determined from the slope and the waiting time θ was estimated from the intercept on the elapsed time axis.

Influence of MeOH on the transformation: With an increase in the concentration of MeOH, the transformation rate increased and the waiting time for nucleation of the β-form became shorter, as shown in Fig. 10. The relationship between the overall transformation rate constant k_T and the concentration of MeOH obtained from Eq. (3) was examined to clarify the degree of influence of MeOH on the transformation.

The logarithmic plots of k_T versus the concentration of MeOH gave a linear relation as shown in Fig. 11. Thus, MeOH plays a role in the exponential acceleration of the transformation.

Similarly, the relationship between the waiting time θ and the concentration of MeOH was examined. The plots of logarithm of the waiting time θ versus the concentration of MeOH also gave a linear relation with a negative slope in contrast to that of k_T as shown in Fig. 12. Namely, the waiting time became shorter with an increase in the concentration of MeOH. This fact shows that MeOH acts as a promoter of the nucleation of the β-form.

From these results, it is found that the coexistence of MeOH is a critical factor to cause the transformation from the desirable α-form to the undesirable β-form. Crude Taltireline is synthesized in a mixed solvent of MeOH and water. Therefore, it is necessary to remove MeOH from the crude precipitate of Taltireline in the drying process before the final purification step, which is recrystallization of the α-form from water, is performed.

Temperature dependence on the transformation: The overall transformation rate over the temperature range from 5°C to 20°C was examined to clarify the temperature

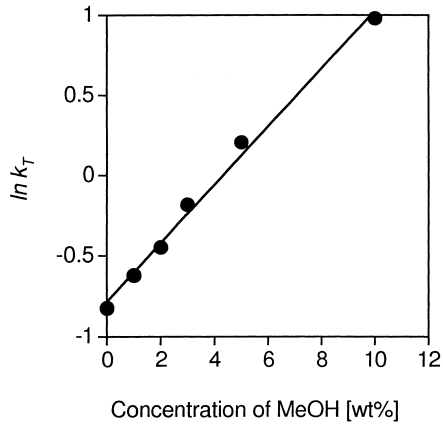


Fig. 11. Relationship between ln k_T and concentration of MeOH in water.

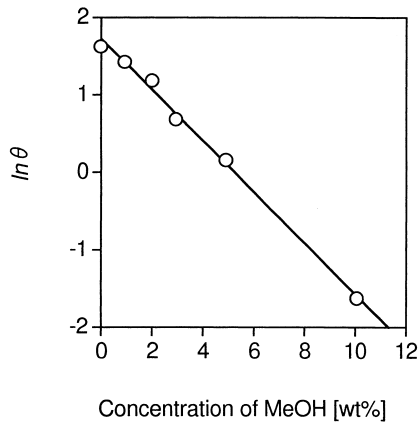


Fig. 12. Relationship between ln θ and concentration of MeOH in water.

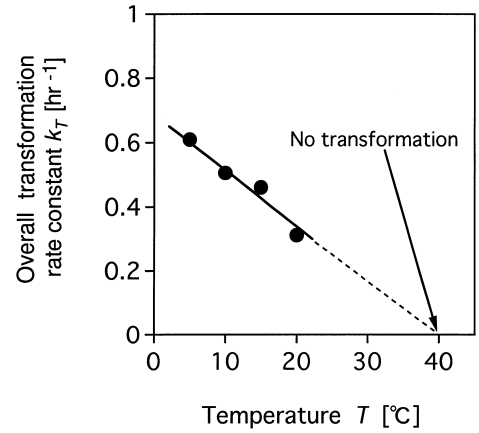


Fig. 13. Relationship between overall transformation rate k_T and temperature T.

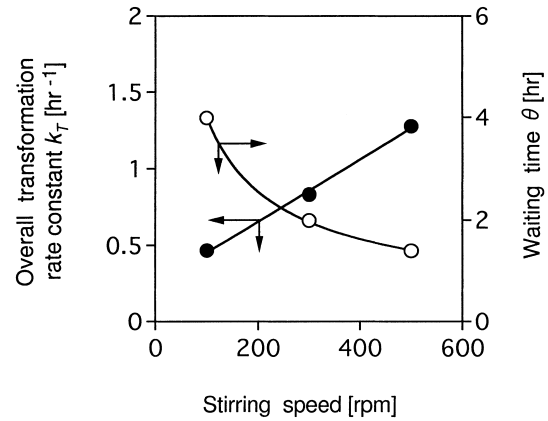


Fig. 14. Influence of stirring speed on the transformation.

dependence on the transformation under the same suspension density in water (20 g of α-form crystal was suspended in 80 g of saturated solution).

The relationship between the overall transformation rate k_T and the temperature T is shown in Fig. 13. Contrary to a normal chemical reaction, the transformation rate decreased with an increase in the temperature. This shows that the transformation is not so simple as an elementary reaction. Although collisions among the molecules and the conformational changes are thermally activated with an increase in temperature, the driving force for the transformation becomes smaller (relative supersaturation σ 0.78 at 10°C, 0.48 at 20°C) because the solubility behavior shows an enantiotropic system. When this experiment was carried out at 40°C (cross point temperature in solubility curves), no transformation occurred even after 3 days.

In order to suppress the transformation, it is important to control the crystallization temperature, which should not be kept low.

Influence of stirring speed on the transformation: The influence of stirring on the transformation was examined in water containing 3 wt.% MeOH. With an increase in stirring speed, the overall transformation rate constant k_T increased linearly and the waiting time θ became shorter as shown in Fig. 14. These results show that the stirring gives a serious influence to the nucleation of the stable form (β-form). As the transformation is promoted with an increase in stirring speed, the excess stirring should be avoided in designing the crystallization process of Taltireline.

4. Conclusion

The crystal structures and the transformation behavior of the polymorphs of Taltireline (α-form, β-form) were investigated and the following results were obtained.

1. The crystal structures of each polymorph were determined by a single crystal X-ray analysis; the α-form is triclinic, P1, and the β-form is orthorhombic, $P2_1\,2_1\,2_1$.
2. The conformational change associated with the transformation was postulated. After the intermolecular hydrogen bonds of the α-form were cut, new hydrogen bonds for the β-form were formed.

3. The solubility behavior shows an enantiotropic system and the α-form is metastable at its crystallization temperature (10°C).
4. The transformation rate from the α-form to the β-form can be expressed by Eq. (2). The overall transformation rate constant k_T and waiting time θ were estimated in order to clarify the critical factors that influence the transformation. As a result, it was found that the transformation is promoted by the co-existence of MeOH, excess cooling during the crystallization, and fast stirring. Now, Taltireline with the desired α-form can be consistently manufactured in an industrial process by controlling these factors.

Acknowledgements

The authors wish to thank Dr. K. Okamura and Mr. H. Hiramatsu for the X-ray crystal structural analyses and useful suggestions.

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