

Mechanism of the solvent-mediated transformation of taltirelin polymorphs promoted by methanol

Shoji Maruyama^{a,b}, Hiroshi Ooshima^{a,*}

^a Department of Bioapplied Chemistry, Faculty of Engineering, Osaka City University,
3-3-138 Sugimoto, Sumiyoshi-ku, Osaka 558-8585, Japan

^b Product & Technology Development Laboratory, Tanabe Seiyaku, 16-89, Kashima 3-chome,
Yodogawa-ku, Osaka 532-8505, Japan

Received 10 November 1999; received in revised form 13 January 2000; accepted 13 January 2000

Abstract

Taltirelin, a central nervous system activating agent, has two crystal forms (α - and β -forms). Previously it has been reported that methanol (MeOH) as a co-solvent promoted the solvent-mediated phase transformation from α -form to β -form. In the present study, the mechanism of the promotion was investigated. Of the three processes in the solvent-mediated transformation, namely dissolution of the metastable form, nucleation of the stable form, crystal growth of the stable form, MeOH promoted the latter two processes. In particular, the nucleation was strongly promoted. The nOe (nuclear Overhauser effect) data suggested that MeOH promotes the conformational change from the α -form to the β -form. The solute–MeOH interaction resulted in the formation of pseudo-polymorphs of the β -form ($2\text{H}_2\text{O}\cdot\text{MeOH}$ or $\text{H}_2\text{O}\cdot 2\text{MeOH}$ solvate), although these pseudo-polymorphs were exchangeable to the β -form ($4\text{H}_2\text{O}$ solvate) under a humid atmosphere. Based on these findings, a possible mechanism of the transformation of taltirelin polymorphs in the presence of MeOH was discussed. © 2001 Elsevier Science B.V. All rights reserved.

Keywords: Crystallization; Polymorphism; Transformation; Taltirelin

1. Introduction

Controlling polymorphism of drugs is important in the pharmaceutical industry [1,2]. Differences in crystal forms cause several problems in bioavailability [3–5], stability [6], and industrial properties [7–9]. In our previous paper [10], taltirelin (TTL), which is a central nervous system activating agent [11], (–)-*N*-[(*S*)-hexahydro-1-methyl-2,6-dioxo-4-pyrimidinylcarbonyl]-1-histidyl-L-prolinamide tetrahydrate (Fig. 1), was studied in connection with good manufacturing practice (GMP). This compound has two crystal forms: α -form (triclinic, P1) and β -form (orthorhombic, P2₁2₁2₁). The α -form is chosen as a dosage form. However, this form is metastable in crystallization slurry, so that solvent-mediated phase transformation to β -form occurs. In order to suppress the transformation, critical parameters that affect the transformation were investigated, and methanol (MeOH) was revealed to strongly promote the transformation [10,12].

In the present study, the mechanism of the MeOH-promoted transformation was investigated. The solvent-mediated transformation proceeds through three processes, namely dissolution of the metastable form, nucleation of the stable form, and crystal growth of the stable form. First, we examined which process is promoted by MeOH. Then, the mechanism of the promotion was discussed through the X-ray structural analysis of the β -form precipitated in the MeOH–water mixed solvent.

2. Experimental

2.1. Materials and analysis of polymorph

TTL (supplied by Tanabe Seiyaku; molecular formula $\text{C}_{17}\text{H}_{23}\text{N}_7\text{O}_5\cdot 4\text{H}_2\text{O}$, Mw 477.47, Lot 301030) was used without further purification. X-ray powder diffraction (Mac Science, MXP3VA) and IR spectroscopy (Horiba, FT-IR 300) were used to identify the polymorphs and to estimate their composition in the precipitate. Aqueous solutions of MeOH of various concentrations were used as solvent in

* Corresponding author. Tel.: +81-6-6605-2700; fax: +81-6-6605-2701.
E-mail address: ooshima@bioa.eng.osaka-cu.ac.jp (H. Ooshima).

Nomenclature

X_{β}	mass fraction of β -form in precipitate
k_T	overall transformation rate constant in Eq. (1) (h^{-1})
t	elapsed time (h)
θ	induction period of transformation (h)
R_g	growth rate of the β -form ($\mu\text{m/h}$)
C_{α}	solubility of α -form (solute weight/solution weight %)
C_{β}	solubility of β -form (solute weight/solution weight %)
ΔC	degree of supersaturation expressed as $(C_{\alpha} - C_{\beta})$ (solute weight/solution weight %)

crystallization. In this paper, 50% MeOH means that the aqueous solvent contains 50 wt.% MeOH.

2.2. Transformation processes

2.2.1. Measurement of the dissolution rate of the metastable form (α -form)

To a 200 ml glass vessel equipped with an anchor paddle was charged 70 ml of solvent (0 and 10% MeOH). The inner temperature was controlled at 10°C using a thermostat and the solvent was agitated at 300 rpm. After 30 g of TTL was added, the supernatant was sampled at regular time intervals and the TTL concentration was determined by HPLC.

2.2.2. Influence of seed on nucleation of the stable form (β -form)

Experiments for the transformation were carried out in the same manner as described in the previous paper [10]. Seventeen grams of solvent was placed in a 200 ml cylindrical jacketed glass vessel and the inner temperature was controlled at 10°C. Thirty grams of the α -form crystal was then added. Sieved β -form crystals (105–149 μm) were used as seeds for the transformation in water containing 3% MeOH. The amount of seeds was 0.1% (30 mg) for the charged amount of TTL (30 g).

2.2.3. Influence of MeOH on crystal growth of the stable form (β -form)

After a crystal of the β -form was fixed on a needle in a cell, crystal growth in water containing 0–10% MeOH was

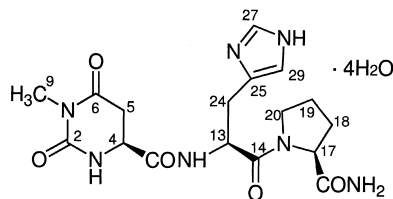


Fig. 1. Chemical structure of taltirelin and position number of carbon atom.

measured under a digital microscope (Keyence, VH-6300). The TTL concentration in the cell was adjusted at the concentration corresponding to the solubility of the α -form at the MeOH concentration.

2.3. Measurement of nuclear Overhauser effect (nOe)

The intensity of nOe between C₉-H and C₂₉-H was measured using a 500 MHz NMR spectrometer (Varian Unity 500) in D₂O–CD₃OD mixed solvent. Taltirelin recrystallized twice from D₂O was used for the measurement.

2.4. Recrystallization in a high concentration of MeOH

One gram of the α -form was placed in a 5 ml sample tube and was dissolved in 3 ml of solvent (50–100% MeOH) by heating (<45°C). The tubes were sealed and allowed to stand for several days at room temperature. Prism crystals were precipitated. X-ray diffraction data were obtained using a Rigaku AFC5R diffractometer (graphite monochromatized Cu K α radiation). The single crystal was placed in a capillary tube together with the mother solution in order to avoid disintegration of the crystal.

2.5. Exchange of solvate molecule

Exchange of solvate molecule from MeOH to water in the crystal was performed using a humidity cabinet (Tabai espec corp., LHL-112M). The MeOH solvated crystal was maintained at 80% relative humidity (RH) at 40°C for 16 h. The water content of crystals was measured using a Karl–Fischer (KF) moisture meter (Kyoto electronics, MKA-3).

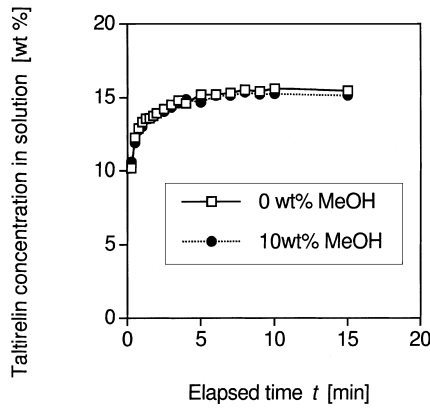
3. Results and discussion

3.1. Influence of MeOH on transformation processes

The solvent-mediated transformation proceeds through the following three processes: (1) dissolution of the metastable form; (2) nucleation of the stable form; and (3) growth of the stable form. In order to clarify the influence of MeOH on the transformation, each of the three processes was examined.

3.1.1. Influence of MeOH on dissolution of the metastable form

Fig. 2 shows the time-course of TTL concentration during the dissolution of the α -form in 0 and 10% MeOH. The dissolution rates were fast: the concentration reached the solubility after 5 min. No difference was observed in these rates. In conclusion, MeOH had little influence on the dissolution of the metastable form (the α -form).

Fig. 2. Influence of MeOH on dissolution rate of α -form.

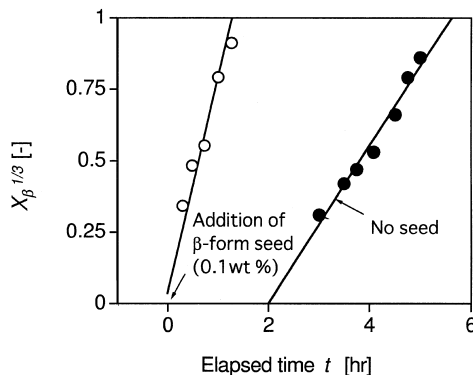
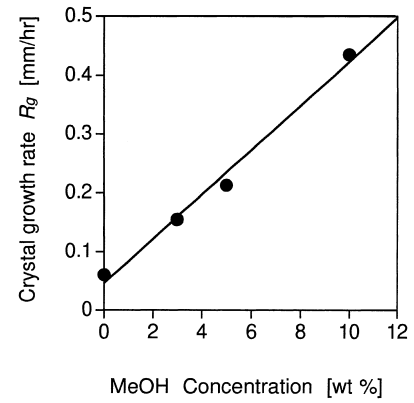
3.1.2. Influence of MeOH on nucleation of stable form

In our previous paper [10], we reported that the induction period for the solvent-mediated transformation became shorter as a result of an increase in MeOH concentration. The induction period includes the time required for the dissolution of the metastable form and the primary nucleation of the stable form. Since the dissolution was fast and not affected by MeOH as shown in Fig. 2, the decrease in the induction period can be explained by the phenomenon that the nucleation of the stable form (the β -form) is promoted. If this explanation is true, the addition of the β -form seed crystals should extremely reduce the induction period. In order to confirm this, the effect of β -form seed crystals on the induction period was examined. Fig. 3 shows the effect of the addition of β -form seed crystals to the suspension of the α -form crystals in 3% MeOH. In Fig. 3, the experimental data were arranged by the following transformation rate equation [10,13].

$$\frac{dX_{\beta}}{dt} = k_T X_{\beta}^{2/3} \quad (1)$$

$$X_{\beta}^{1/3} = \frac{k_T}{3}(t - \theta) \quad (0 \leq t \leq \theta : X_{\beta}^{1/3} = 0) \quad (2)$$

where X_{β} is the mass fraction of the β -form in the precipitate, the k_T is the overall transformation rate constant, and θ is the

Fig. 3. Additive effect of seed of β -form on transformation.Fig. 4. Influence of MeOH on crystal growth rate of β -form.

induction period for the transformation. The transformation began with no induction period just after the β -form seeds were added. The transformation rate also increased, probably due to the secondary nucleation. These results show that the nucleation is an important process for the transformation.

3.1.3. Influence of MeOH on crystal growth of the stable form

The presence of impurities often affects crystal growth [13,14]. Generally, impurity acts as an inhibitor of crystal growth by adsorbing onto the crystal surface. In order to examine the influence of MeOH on the crystal growth of the stable form (the β -form), the rate of crystal growth was measured at a TTL concentration equal to the solubility of the α -form in 0–10% MeOH. The crystal morphology of the β -form was little affected by MeOH at these concentrations. Fig. 4 shows the relationship between the crystal growth rate of the stable form at the solubility of the α -form, R_g , and the MeOH concentration. The growth rate increased with an increase in MeOH concentration.

Based on these results, MeOH was concluded to simultaneously affects nucleation and crystal growth. In our previous paper [10], we reported that the overall transformation rate constant, k_T , increased exponentially with an increase in MeOH concentration. However, as shown in Fig. 4, the growth rate was proportional to MeOH concentration. The difference in the dependencies of k_T and R_g on MeOH concentration means the difference in their sensitivities to MeOH, although the absolute values cannot be directly compared. The high sensitivity of k_T to MeOH is probably originated from the higher sensitivity of the nucleation rather than the crystal growth.

3.2. Considerations for the promotion effect of MeOH in nucleation

3.2.1. Effect of MeOH on the solubility

If the solubility of the β -form is decreased by MeOH more than that of the α -form, the nucleation of β -form would be promoted. In order to examine this possibility, the solubility

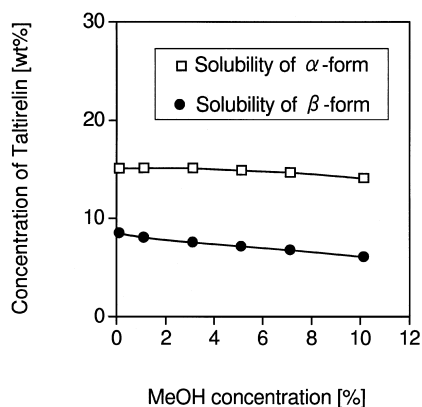


Fig. 5. Solubilities of taltirelin polymorphs in various concentration of MeOH.

of the polymorphs was measured in 0–10% MeOH at 10°C. Fig. 5 shows MeOH dependencies on the solubility of the polymorphs. The solubility of each polymorph was slightly decreased with an increase in MeOH concentration. However, the differences between the solubility of the two, ΔC , were little changed by MeOH, indicating that the large effect of MeOH on the transformation cannot be explained by ΔC .

3.2.2. Effect of MeOH on the conformation of TTL

The conformation of solute molecules in solution must be influenced by solute–solvent interaction [12,14–18]. In the case of TTL, β -conformer might become predominant with an increase in the concentration of MeOH. Here, we discuss the conformational change through nOe experiment, which is one of NMR spectroscopies. If the different hydrogen atoms exist within 4–5 Å in a solution, nOe can be observed. Fig. 6 shows the structures of TTL polymorphs determined from the X-ray structural analysis [10]. The distance between the two protons, C₉-H and C₂₉-H, in the α -form is 2.913 Å. If the conformation of the solute molecules in the solution is similar to that of the α -form, nOe should be observed. Fig. 7 presents the change in the intensity of nOe between C₉-H and C₂₉-H. It shows that the conformation of the solute is similar to the α -conformer in water and that the distance between C₉-H and C₂₉-H increased with

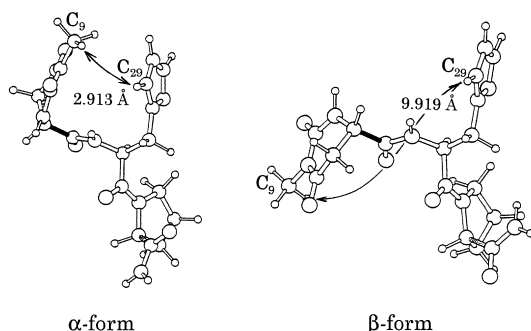


Fig. 6. Conformation of polymorphs [10].

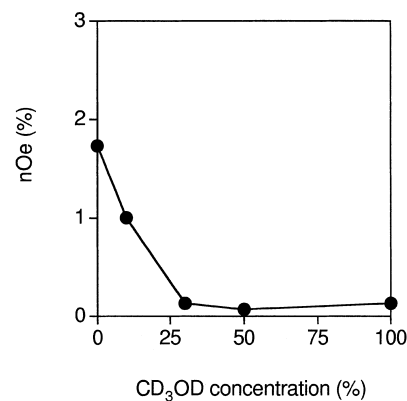


Fig. 7. Intensity of nOe between C₉-H and C₂₉-H at various concentration of CD₃OD in D₂O.

an increase in MeOH concentration. This result suggests that MeOH causes the conformational change from the α -conformer to β -conformer through the solute–MeOH interaction. In other words, it is implied that TTL molecules tend to take the β -conformer in the presence of MeOH. The increase in β -conformer should be advantageous for the nucleation and crystal growth of the β -form.

3.2.3. Solute–MeOH interaction

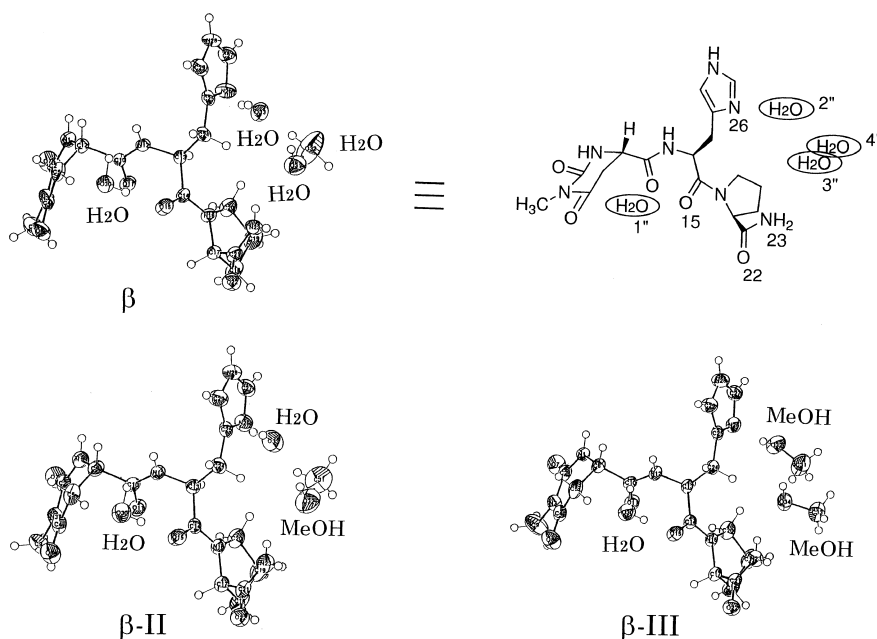
In order to examine the solute–MeOH interaction, X-ray structure analysis was carried out for the crystals that were precipitated in a high concentration of MeOH. As mentioned in Section 2, none of the crystals obtained were stable on exposure to the air, upon which the crystals disintegrated. However, in the case of the crystals that were precipitated in 50% MeOH, the disintegration was not severe. Care was taken to avoid drying during the X-ray analysis.

X-ray structure analysis showed that all crystals are of the β -form. However, the solvating molecules were different from those in the β -form (tetrahydrate) that were precipitated in water, as presented in Table 1 and Fig. 8. The crystals that were precipitated in 70% MeOH were monomethanolate-dihydrate (MeOH–2H₂O; β -II-form) and the crystals that were precipitated in 90 and 100% MeOH were dimethanolate-monohydrate (2MeOH–H₂O; β -III-form). The hydrating water in the crystals (β -III-form) that were precipitated in 100% MeOH must have origi-

Table 1
Relation of MeOH concentration and crystal form

MeOH concentration (wt.%)	Crystal form	Solvation
0	β	4H ₂ O
50	β -I	Not determined ^a
70	β -II	2H ₂ O–MeOH
90	β -III	H ₂ O–2MeOH
100	β -III	H ₂ O–2MeOH

^a Solvation of crystal could not be determined from X-ray structural analysis because of disorder of the solvating MeOH and water molecule.

Fig. 8. Crystal structures of pseudo-polymorphs of β -form.

nated from the parent tetrahydrate crystals (α -form) that were dissolved to prepare TTL solution. The solvation of the crystals that were precipitated in 50% MeOH could not be determined, although the structure of TTL was determined. This implied that the solvent coordinated in the crystal was not fixed in MeOH or water in the whole crystal. For convenience, we refer to this as the β -I-form. The details of the crystallographic data for the β -, β -II-, and β -III-forms are presented in Table 2, along with the α -form. The three β -form crystals belong to the orthorhombic crystal system and the $P2_12_12_1$ space group, whereas the α -form belongs to the triclinic crystal system and $P1$ space group. Therefore, the β -II- and β -III-forms are regarded as the pseudo-polymorph of the β -form. As the number of solvating MeOH molecules increased, the unit

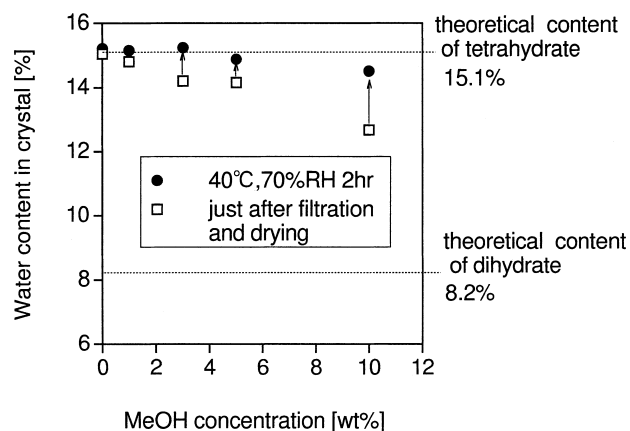


Fig. 9. Change of water content after filtration.

Table 2
Crystal data of polymorphs and pseudo-polymorphs at 293 K

	α -form	β -form	Pseudo-polymorphs of β -form	
	(4H ₂ O)	(4H ₂ O)	β -II (2H ₂ O–MeOH)	β -III (2H ₂ O–MeOH)
<i>a</i>	12.345 Å	11.981 Å	12.103 Å	12.499 Å
<i>b</i>	12.835 Å	21.809 Å	21.716 Å	21.875 Å
<i>c</i>	7.938 Å	8.942 Å	8.918 Å	8.911 Å
α	103.67°	90°	90°	90°
β	106.89°	90°	90°	90°
γ	91.18°	90°	90°	90°
Crystal system	Triclinic	Orthorhombic	Orthorhombic	Orthorhombic
Space group	$P1$	$P2_12_12_1$	$P2_12_12_1$	$P2_12_12_1$
<i>Z</i>	2	4	4	4
Density (calculated)	1.362 g/cm ³	1.357 g/cm ³	1.342 g/cm ³	1.329 g/cm ³

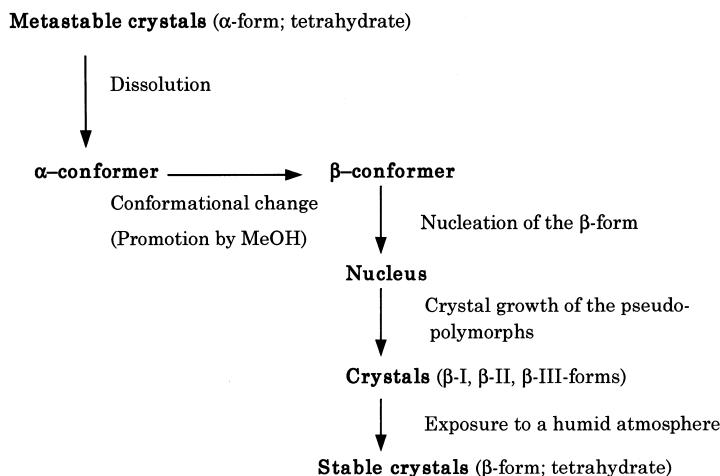


Fig. 10. Possible mechanism of the solvent mediated-transformation of taltirelin promoted by MeOH.

cell length of the a -axis elongated and that of the c -axis shortened slightly, probably due to the volume and position of the MeOH molecule.

The solvation of crystal with MeOH was also observed for the crystals obtained in a low concentration of MeOH (less than 10% MeOH). Fig. 9 shows the water content of crystals after the transformation in the mixed solvent of water and MeOH. The water content decreased from the theoretical value of tetrahydrate (15.1%) with an increase in MeOH concentration. The content, however, did not decrease down to a theoretical value of dihydrate (8.2%) even in 10% MeOH. When the crystals were incubated at 70% RH at 40°C for 2 h, the water content recovered to the theoretical value of tetrahydrate. The exchange of solvate molecule from MeOH to water in the crystal was possible for the β -II- and β -III-forms by immediately exposing them at 70% RH at 40°C for 16 h after separation from the solution.

These findings suggest that significant interaction exists between TTL and MeOH in solution.

3.3. Overview of the promotion effect of MeOH in the transformation of the α -form to the β -form

Fig. 10 shows a model of the transformation of the α -form to the β -form in the presence of MeOH. The dissolution of the metastable crystals (α -form) is not influenced by MeOH. TTL molecules that have just been released from the crystal should be α -conformers. In the solution, TTL molecules tend to take β -conformers through TTL–MeOH interaction. The increase in β -conformer is advantageous for the nucleation and crystal growth of the β -form. Consequently, the presence of MeOH promotes the solvent-mediated transformation of TTL. The crystals produced in this manner contain MeOH molecules as solvate. Depending on the concentration of MeOH in solution, two pseudo-polymorphs (β -II-, and β -III-forms) or an intermediate (β -I-form) are formed. MeOH in these pseudo-polymorphs can be

exchanged with water by exposing them to a humid atmosphere.

4. Conclusion

1. The transformation process that was most strongly affected by MeOH was the nucleation of the β -form (stable form). An increase in MeOH concentration causes the induction period of the transformation, namely the waiting time for the nucleation of the β -form, to become shorter.
2. As the solubility of polymorphs was little affected by MeOH, the solute–solvent interaction was thought to be a dominant factor of the transformation. The nOe measurement suggested that MeOH promotes the conformational change of the solute from the α -form to the β -form.
3. Unstable crystals were obtained by recrystallization of the α -form in a high concentration of MeOH. These crystals were the pseudo-polymorphs of the β -form with solvated MeOH. The solvate exchange from MeOH to water occurred easily under a humid atmosphere while maintaining the crystal form, so that these crystals were regarded as an intermediate of the transformation.
4. A possible mechanism of the solvent mediated transformation of taltirelin polymorphs promoted by MeOH is proposed. The nucleation and crystal growth of the β -form crystals are promoted. The conformational change of the α -form to β -form is promoted by MeOH through TTL–MeOH interaction. Pseudo-polymorphs also act as an intermediate.

Acknowledgements

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

References

- [1] M. Bavin, *Chem. Ind.* 21 (1989) 527–529.
- [2] K. Kagara, *Pharm. Tech. Jpn.* 13 (1997) 1311–1319.
- [3] A.J. Aguiar, J. Krc Jr., A.W. Kinkel, J.C. Samyn, *J. Pharm. Sci.* 56 (1967) 847–853.
- [4] H. Kiwada, H. Kojima, Y. Kato, *Chem. Pharm. Bull.* 31 (1983) 1345–1349.
- [5] M. Audran, Y. Lasserre, J. Rambaud, B. Pauver, G. Berge, L. Maury, *Pharm. Acta Helv.* 63 (1988) 343–346.
- [6] H. Nakamachi, T. Yamaoka, Y. Wada, K. Miyata, *Chem. Pharm. Bull.* 30 (1982) 3685–3694.
- [7] J. Pirttimaki, E. Laine, *Eur. J. Pharm. Sci.* 1 (1994) 203–208.
- [8] M. Otsuka, Y. Matsuda, *Drug Dev. Ind. Pharm.* 19 (1993) 2241–2269.
- [9] A. Bauer-Brandi, *Int. J. Pharm.* 140 (1996) 195–206.
- [10] S. Maruyama, H. Ooshima, J. Kato, *Chem. Eng. J.* 75 (1999) 193–200.
- [11] M. Suzuki, H. Sugano, K. Matumoto, M. Yamamura, R. Ishida, *J. Med. Chem.* 33 (1990) 2130–2137.
- [12] S. Maruyama, H. Ooshima, Crystallization behavior of taltirelin polymorphs in a mixture of water and methanol, *J. Crystal Growth* 212 (2000) 239–245.
- [13] K. Kagara, K. Machiya, K. Takasuka, N. Kawai, *Kagaku Kougaku Ronbunshu* 21 (1995) 437–443.
- [14] R.J. Davey, N. Blagden, G.D. Potts, R. Docherty, *J. Am. Chem. Soc.* 119 (1997) 1767–1772.
- [15] M. Kitamura, *J. Crystal Growth* 96 (1989) 541–546.
- [16] M. Kitamura, *J. Chem. Eng. Jpn.* 26 (1993) 303–307.
- [17] M. Kitamura, H. Furukawa, M. Asaeda, *J. Crystal Growth* 141 (1994) 193–199.
- [18] J. Starbuck, R. Docherty, M.H. Chaiton, D. Buttar, *J. Chem. Soc., Perkin Trans. 2* (1999) 677–691.