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RESEARCH PAPER

Solid-State Variation of Troglitazone Drug Substance by Using a Different Recrystallization Method

Nobuyuki Suzuki,[1,*] Ko Kasahara,[1] Yoshiaki Watanabe,[1] Suzue Kinoshita,[1] Hirokazu Hasegawa,[2] and Takao Kawasaki[1]

[1]Product Development Laboratories, Sankyo Co., Ltd., Shinagawa-ku, Tokyo, Japan
[2]Process Development Laboratories, Sankyo Co., Ltd., Hiratsuka-shi, Kanagawa, Japan

ABSTRACT

Rapid and slow crystallization methods (A and B) were applied for troglitazone, an equal mixture of four stereoisomers. Differences in the powder x-ray diffractometry patterns and hygroscopic patterns were observed among the samples crystallized by these methods, suggesting that troglitazone has solid-state variation. In this article, troglitazone recrystallized by method A was evaluated to clarify its structural characteristics and physical property. The crystal structure of predried troglitazone recrystallized by method A was proved to be a dihydrate. By drying, it changed reversibly to an anhydrate, which is the same structure as the RS/SR form, keeping the same enantiomer ratio. The solubility of the troglitazone by method A higher than that by method B at all enantiomer levels. But making the troglitazone amorphous equalized the enantiomeric solubilities of the substances by both methods as well as increased the intrinsic solubilities. Troglitazone by both methods was proved to be stable and retained the ratio of the stereoisomers.

Key Words: Troglitazone; Powder x-ray diffractometry; Physical property; Hygroscopicity; Hydration.

INTRODUCTION

In general, solid-state variation of a drug substance has the possibility of display pharmaceutically different properties such as solubility, stability, and bioavailability.[1–4] Some pharmaceutical compounds exist in various solid states such as polymorphs or hydrates on changing the processing conditions.

*Correspondence: Nobuyuki Suzuki, Product Development Laboratories, Sankyo Co., Ltd., 2-58 Hiromachi 1-chome, Shinagawa-ku, Tokyo 140-8710, Japan; Fax: +81-3-5436-8571; E-mail: nobuyu@shina.sankyo.co.jp.

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The investigation of the physical property of a drug substance is very important for drug development, especially for the selection of the conditions for crystallization in the final stage of manufacture.[5]

Troglitazone, an oral antidiabetic agent, has four stereoisomers due to two asymmetric carbons in its molecule, and it is developed as a mixture of equal amounts of the four isomers.[6,7] The chemical structures of the troglitazone stereoisomers are shown in Fig. 1. The diastereomers of troglitazone, which are named the RS/SR form and the RR/SS form in this report, have different physicochemical properties from each other.[8] Therefore, the stereoisomer ratios of such compounds have to be considered for the evaluation of their physicochemical properties.[9]

There are two production methods for troglitazone: one is a rapid crystallization method called method A and the other is a slow crystallization method called method B. Occasionally, changing the process of a drug substance results in the creation of different solid states with a different physical property. The physical property of troglitazone crystallized by method B (troglitazone by method B) has been reported and its solid state is characterized as a simple physical mixture of the two diastereomeric crystals.[8] In this report, we clarified the structural characteristics and physical property of troglitazone drug substance crystallized by method A (troglitazone by method A) by evaluating the stereoisomer ratio, which is one of the factors that varies in solid states.

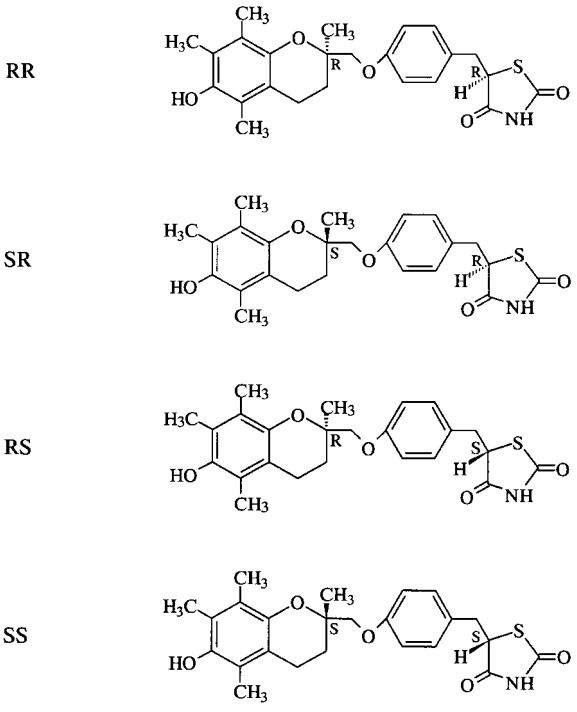

Figure 1. Chemical structures of troglitazone stereoisomers.

MATERIALS AND METHODS

Materials

Troglitazone drug substance, the RS/SR and RR/SS diastereomers, were synthesized as described in a previous article.[10] A chiral high-performance liquid chromatography (HPLC) column, Chiralcel OJ-R (4.6 mm i.d. × 150 mm, particle size: 5 μm), was purchased from Daicel Chemical Industries (Osaka, Japan). Water was purified by a Milli-Q SP TOC system (Millipore Co., Ltd., Bedford, MA, USA). Hydranal Aqualyte RS and Hydranal Coulomat CG for Karl Fischer titration reagent were purchased from Riedel-de Haën GmbH (Seelze, Germany). All other reagents and solvents were commercially available and of analytical grade.

Sample Preparation of Troglitazone

Two different methods, method A and method B, were used for the recrystallization of troglitazone. Method A is a rapid crystallization method and method B is a slow and mild crystallization method. The details of these methods are described as follows.

Method A Recrystallization

One hundred g of troglitazone (or diastereomer) was dissolved into 1 L of acetone, and this solution was added dropwise to 5 L of water at a constant rate for 60 min while keeping the crystallization temperature at 25–30°C. The mixture was stirred for an additional 120 min at this temperature to allow complete crystallization. The crystallized substance was filtered. One portion of the residue was used as the predried troglitazone, and the other was dried with heating at 60°C in vacuo for 15 h.

Method B Recrystallization

One hundred g of troglitazone was dissolved into 300 mL of acetone with heating at 55°C, and 130 mL water heated above 55°C was added to this solution, and the mixture was kept above 55°C so as not to

generate a drug precipitate. After confirming its dissolution, the solution was cooled gradually for slow crystallization. The mixture was cooled to 25°C during a period of 130 min, and then the substance was crystallized as much as possible. At around 25°C, 230 mL of water was dropped at a constant rate into the mixture for 120 min, and then stirred for an additional 60 min to allow complete crystallization. The residue was dried with heating at 60°C in vacuo for 15 h.

The amorphous forms of the troglitazone by methods A and B were made by rapid cooling of the completely melted troglitazone (melt-quenching). Samples were heated to melt on the furnace of a differential scanning calorimeter (DSC) (Thermoplus 2, Rigaku Corp., Akishima, Tokyo, Japan) and then taken out of the furnace and immediately cooled by placing the sample onto a steel plate. To confirm the amorphous forms, the crystallinities were checked with a polarized light microscope (Nikon Optiphoto II, Chiyoda, Tokyo, Japan).

Powder X-Ray Diffractometry

The powder x-ray diffractometry (PXRD patterns of troglitazone were determined at ambient temperature using a diffractometer (RINT 2200, Rigaku Corp., Japan) with Cu Ka radiation at 40 mA and 45 kV. Each sample was packed into an aluminum holder and scanned with a diffraction angle of 2θ, increasing from 4° to 40° with a scanning rate of 4°/min. The simulated PXRD pattern from the single crystallographic data was calculated by using the PLATON (Ver. 70302) and the TEXSAN (Ver. 1.11) programs provided by the Cambridge Crystallographic Data Centre (Cambridge, UK).

Water Content and Adsorption/Desorption Isotherm Measurement

The water content, expressed as % w/w, was determined by Karl Fischer titration using a Moisture Meter (Model AQ-5, Hiranuma Sangyo Co., Ltd., Ibaraki, Japan). Hydranal Aqualyte RS and Hydranal Coulomat CG were used as an anolyte and a catholyte, respectively. Samples (0.1 g) were accurately weighed, quickly transferred to the titration vessel, and dissolved in the anolyte.

Troglitazone and diastereomers were used for the determination of the adsorption/desorption isotherm measurements in the same way as follows. Samples of about 300 mg of these substances were each put into an open clear glass bottle, which was placed into humidity-controlled desiccators for 20 days until the water adsorption/desorption reached equilibrium. The relative humidities (RH) were controlled using saturated salt solutions with known RH values in the desiccators at 25°C. The samples before and after placing in the desiccators were weighed and the increase (% w/w) due to the water adsorption/desorption was calculated.

Evaluation of the Four Troglitazone Stereoisomers with High-Performance Liquid Chromatography (HPLC)

The concentrations of the troglitazone stereoisomers were determined by HPLC (Model HP1090, Agilent Technologies, USA), using the following conditions: The reversed-phase chromatography on a Chiralcel OJ-R was performed using a mobile phase composed of methanol/acetic acid (1000:1) at a flow rate of 0.5 mL/min. The column temperature was controlled at 25°C, the injection volume was 5 μL, and detection was achieved at UV 285 nm. All of the stereoisomers were completely separated.[10]

Solid-State NMR (SSNMR) Spectroscopy

^{13}C cross polarization/magic angle spinning (CP/MAS) NMR spectra were obtained using a Varian Unity INOVA 400 MHz spectrometer operating at a carbon frequency of 100.556 MHz and equipped with a complete solids accessory and Varian Jacobsen CP/MAS probe. Samples were filled in 7-mm zirconium rotors and tightly closed with ZrO endcaps. Measurement conditions were as follows: 90° proton rf pulse, 3.1 μs; contact time, 4 ms; pulse repetition time, 10 s; MAS frequency, 6.0 kHz; spectral width, 50 kHz; and acquisition time, 40 ms. The chemical shifts were referenced to the CH_3 of hexamethylbenzene ($d = 17.3$ ppm) by sample replacement. Spectral assignments were made by comparing chemical shifts observed in solution spectra[11] with those in the ^{13}C CP/MAS and interrupted decoupling spectra.

Solubility

A sample of about 10 mg of troglitazone was put into a glass tube. Ten milliliters of 0.01 mol/L borate

buffer, pH 9, was added and the suspensions were incubated at 37°C. After 20 and 360 min (melt-quenched sample: 5, 20, and 360 min), 1 mL of each suspension was filtered with a Mini-Uniprep™ with polyvinylidene difluoride (PVDF) filter (5 mm in inside diameter and pore size of 0.45 μm, Whatman Inc., Springfield Mill, UK). The filtrate was immediately measured by the chiral HPLC method described above to obtain the concentration of each troglitazone stereoisomer. The concentrations of troglitazone were calculated by the peak area method using the three points of the calibration curve (2 to 200 μg/mL).

Solid-State Stability

The stability test was carried out under conditions of 40°C/75% RH. Samples of approximately 5 g of the troglitazone drug substances were each placed in a high-density polyethylene (HDPE) sack, which was placed in a 110 mL steel can. They were kept in a room controlled at 40°C/75% RH for 6 months. The water content was determined by the Karl Fischer titration method, the assay was determined by HPLC, and the stereoisomer ratio was determined by the same chiral HPLC method as the solubility study.

Micromeritics

The specific surface areas of the troglitazone were measured by two methods. One was calculation of the surface area from the particle size determined using the laser light scattering method. Suspended troglitazone in water was supplied to measure the particle size using a laser light scattering system (Microtrac MKII, Nikkiso Co. Ltd., Shibuya, Tokyo, Japan). The other method used was the N_2 gas adsorption method using a BET surface area measurement instrument (QUANTASORP OS-8, Yuasa Ionics Co., Ltd., Takatski, Osaka, Japan). The particle shapes of the troglitazone drug substances were investigated using a scanning electron microscope (SEM) (JSM6301F, JEOL Corp., Akishima, Tokyo, Japan).

RESULTS AND DISCUSSION

Structural Characteristics and Hydration Mechanism

The predried troglitazone from method A contained 7.7% water, which is equivalent to stoichiometrically 2 mol (theory = 7.58%) for troglitazone. It has been reported that troglitazone could be a dihydrate crystal structure with the diastereomer molecules.[12] We compared the PXRD pattern of the predried troglitazone with the simulated pattern from the crystallographic data of the dihydrate structure, as shown in Fig. 2. These patterns are nearly identical especially at the diffraction angles, revealing that the pre-dried troglitazone has a dihydrate crystal structure. As shown in Table 1, the stereoisomer ratio of troglitazone by method A was about 1:1:1:1. From the dihydrate crystal structure report, the single crystals used for structural analysis consist of the RS/SR and RR/SS isomers in a ratio of about 2:1, but the asymmetric carbon on the thiazolidine ring is disordered. Method A, which is intended to be used for larger scale manufacturing, is quite different from the method for making single crystals of the most stable stereoisomer ratio. Thus, it is likely that a disordered form consisting of equal amounts of the four stereoisomers could be made using method A.

As shown in Fig. 3, the PXRD pattern of troglitazone by method A, containing 0.1% of water after drying, was almost the same as the pattern of the

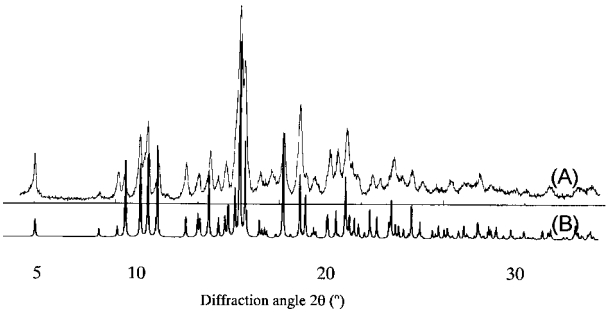

Figure 2. Comparison of the PXRD patterns of troglitazzone. (A) Predried troglitazone by method A, (B) simulated pattern of troglitazone dihydrate.

Table 1. Evaluation of stereoisomer ratios of troglitazone and diastereomers by method A using the Chiral HPLC method.

	Stereoisomer ratio (%)			
Substance	RR	SR	RS	SS
Troglitazone	25	26	25	24
Predried troglitazone	24	26	26	24
RS/SR Form	0	50	50	0
RR/SS Form	51	0	0	49

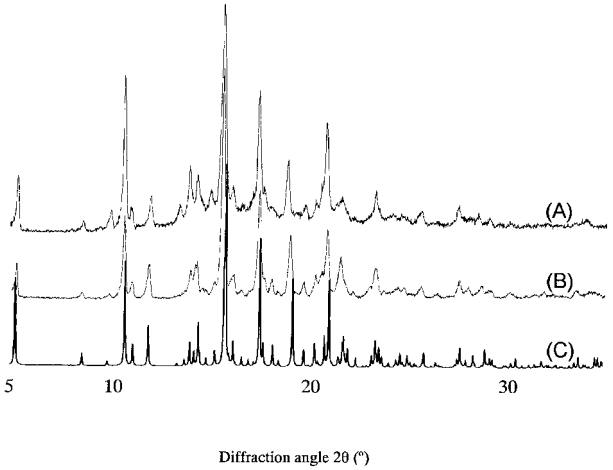

Figure 3. Comparison of the PXRD pattrens of troglitazone. (A) Troglitazone by method A, (B) the RS/SR form of troglitazone, (C) simulated pattern of the RS/SR (anhydrate).

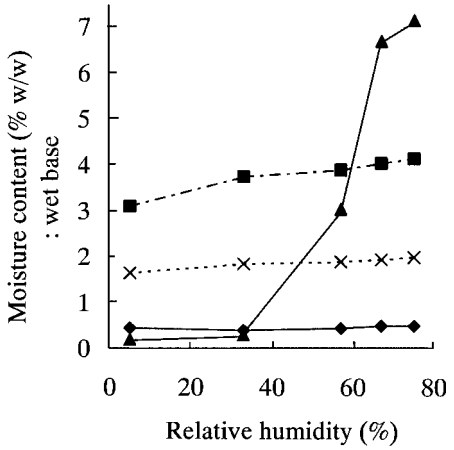

Figure 4. Water adsorption isotherms of troglitazone in terms of effect by recrystallization method. (◆) The RS/SR form, (■) the RR/SS form, (x) troglitazone by method B, and (▲) troglitazone by method A.

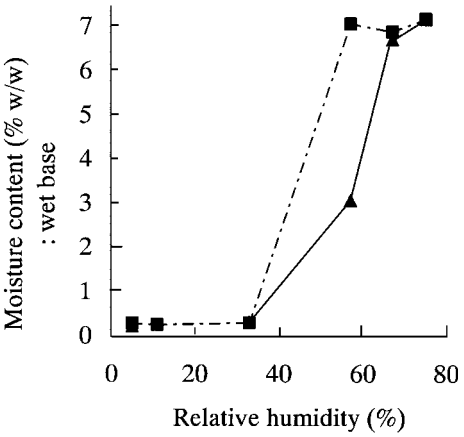

Figure 5. Water adsorption/desorption isotherm of troglitazone by method A at 25°C. (▲) Adsorption curve, and (■) desorption curve after storage for 20 days.

RS/SR form. And these patterns were identical to the simulated pattern from the crystallographic data of the anhydrous RS/SR form.[13] But troglitazone by method A still included the RR and SS isomers as shown in Table 1. Unfortunately, the same structures of single crystals as the RS/SR form, including the RR and SS isomers, have not yet been obtained because it has been relatively easy to crystallize separately to the RS/SR and RR/SS forms like in troglitazone by method B.[8] What can be said at present is that troglitazone by method A could be an anhydrate with crystal structure similar to the RS/SR form.

Troglitazone and the diastereomers were used to investigate water adsorption. Figure 4 shows the water adsorption isotherm in various humidity conditions at 25°C. The adsorption patterns of troglitazone by methods A and B were quite different from each other. The RS/SR form does not show any hygroscopicity, and the RR/SS form shows 3–4% water uptake in the same range. The recrystallized substance by method B shows the intermediate pattern of the RS/SR and RR/SS form hygroscopic patterns because they are present as a simple physical mixture.[8] On the contrary, about a 7% moisture uptake jump, which is close to 2 mol for troglitazone, was clearly observed between 33% and 67% RH in the troglitazone by method A. Figure 5 shows the water adsorption/desorption isotherm of troglitazone by method A. Hysteresis, which suggests hydration, was observed as shown by the jump in the curve of the water adsorption while keeping the stereoisomer ratio. To make sure of the crystal structure, the PXRD of these samples was measured before and after the water adsorption isotherm experiment. The PXRD pattern of the troglitazone after about 7% water adsorption was identical to the predried troglitazone by method A, and the pattern was strictly different from the pattern of troglitazone by method A. These results indicated that the troglitazone by method A, an anhydrate, changes reversibly into a dihydrate, even when the similar structural RS/SR anhydrate had no chance to be hydrated under the same conditions.

To represent clearly the solid-state structural change of troglitazone by method A, these samples

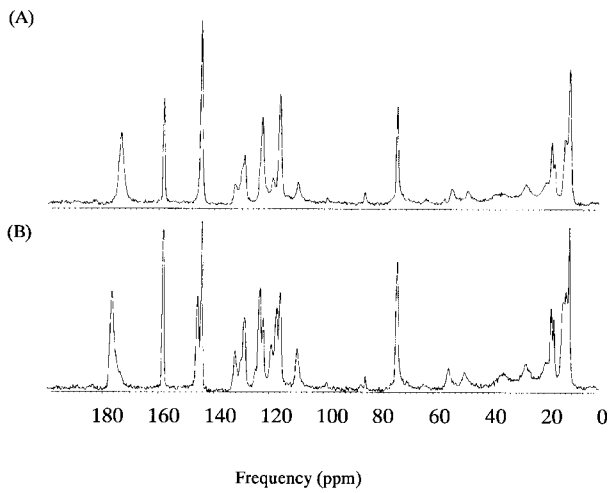

Figure 6. Solid-state NMR spectra of (A) troglitazone by method A, and (B) predried troglitazone by method A.

were provided to measure the SSNMR spectrometry, which is a powerful technique for the physical characterization of pharmaceutical solids.[14–17] Since these samples for SSNMR are filled in rotors tightly closed with endcaps, they would be rarely exposed to undue humidity during the measurement. Figure 6 shows the SSNMR patterns of troglitazone and predried troglitazone by method A. The chemical shift of 176 ppm, which was assigned to the 6-position of the chroman ring, was completely moved to 173 ppm after drying. At the same time, the chemical shifts of 146 ppm and 144 ppm, which were assigned to the two carbonyl carbons of the thiazolidine ring, were gathered. It was surmised that water desorption caused the rebuilding of weaker hydrogen-bonding interactions, changed shielding, and shifted these carbonyl resonances to a higher magnetic field. In this way, the SSNMR result supported that by PXRD and revealed that they were pseudopolymorphs with high polymorphic purities.

Physical Property

It is a well-known and important issue that different physical properties of a substance can be obtained due to different manufacturing processes.[5] Hygroscopic profile, specific surface area, and individual crystal particle shapes are important factors for drug development. The specific surface area, in particular, has the possibility of affecting the dissolution profile. Table 2 shows the specific surface areas of troglitazone by the N_2 gas adsorption and laser light scattering methods. Differences in the surface areas were observed between the two substances, especially the results by the N_2 gas adsorption method. The N_2 gas adsorption method can be used to determine the surface area of opened narrow spaces in a solid mass, such as in aggregated or porous materials. But all particles are regarded as spherical-shaped by the laser light scattering method, so the surface area derived from that narrow space is ignored. Figure 7 shows the electron microscope photographs of troglitazone by both methods. Smaller individual crystal particles were observed in troglitazone by method A than by method B. Most of them were flaky-shaped and aggregated into large composite structures. It was revealed from the result of the electron scanning microscope of the troglitazone by method A that the aggregation of the smaller particles made the narrow spaces that caused the differences in the specific surface areas. It is possible to consider that the rapid recrystallization of method A allows for the making of flaky-shaped smaller particles. This difference should be taken into account in the formulation study if troglitazone by both methods is used as it is.

Table 2. Specific surface area of troglitazone by methods A and B: comparison of N_2 gas adsorption and laser light scattering methods.

	Method A (m^2/g)	Method B (m^2/g)
N_2 gas adsorption	2.98	1.05
Laser light scattering	0.72	0.58

The most decisive factors of the physical property of a substance for the active pharmaceutical ingredient, especially for the development of the oral dosage form, are stability and solubility which can sometimes affect the bioavailability. Figure 8 shows the stereoisomeric solubility, of troglitazone by method A, method B, and these samples melt-quenched, whose stereoisomer ratios were about 1:1:1:1. The solubility of troglitazone by method A was higher than that by method B for the enantiomers at all levels. But the solubility of troglitazone by both methods was significantly increased by melt-quenching, and they had about the same enantiomeric solubility profile as each other. It was revealed practically that making troglitazone amorphous could equalize the enantiomeric solubilities of troglitazone by both method A and method B, as well as increase their intrinsic solubilities. This was

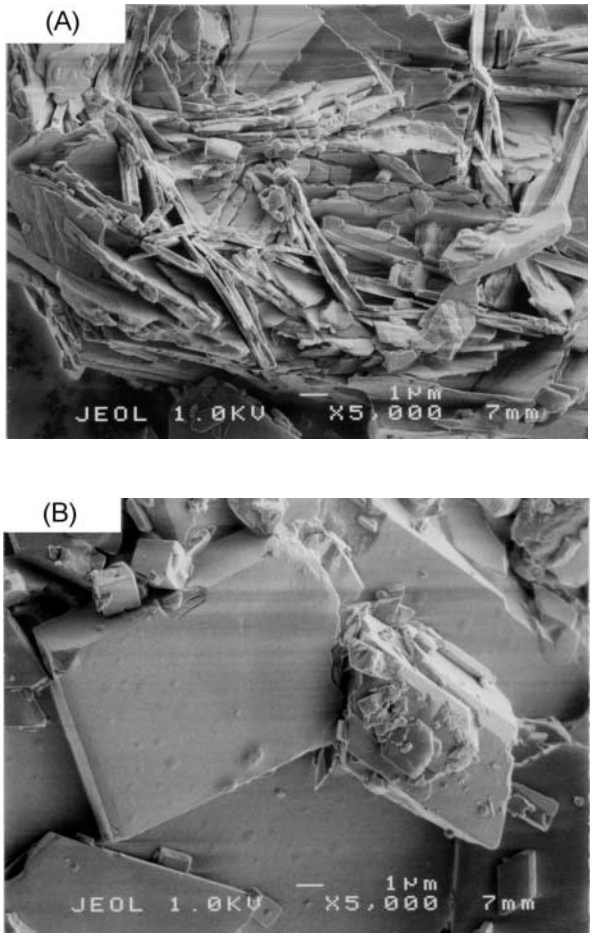

Figure 7. Electron microscope photographs of troglitazone. (A) Troglitazone by method A, (B) troglitazone by method B.

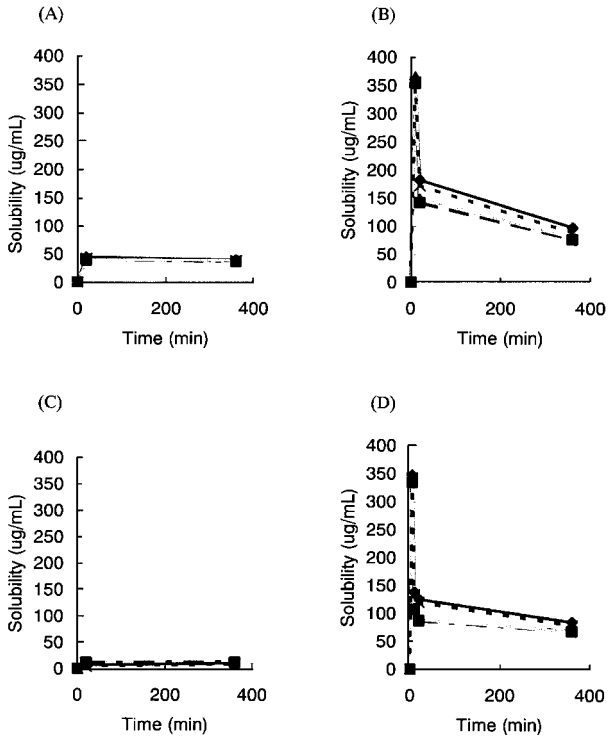

Figure 8. Stereoisomeric solubility profile of troglitazone by method A, method B, and these samples melt-quenched. (A) Troglitazone by method A, (B) melt-quenched troglitazone (method A), (C) troglitazone by method B, and (D) melt-quenched troglitazone (method B). Enantiomers: (◆) RR, (■) SR, (▲) RS, (×) SS.

important information for the solid formulation research of the troglitazone drug product. Regarding the stability of troglitazone by both methods, the purity levels, PXRD patterns, and the stereoisomer ratios (data not shown) were not changed during the accelerated conditions under storage mimicking the actual factory style. Stability of troglitazone by both methods proved to be stable chemically and physically, retaining the stereoisomer ratio.

Selection of the Solid States

From the results of the solubility and surface area, troglitazone by both methods will be allowed to be used for manufacturing tablets with further conversion (i.e., by metting) of troglitazone to an amorphous form. In fact, 100-mg and 200-mg troglitazone tablets are produced by a spray-drying method with dissolving in ethanol or a extrusion method with melting, and converting the substance to a solid dispersion to make it amorphous. Thus, the solid-state variation of troglitazone as described herein will not present a significant problem from a regulatory aspect,[18] but this characteristic of hygroscopicity must be taken into consideration for the development of this compound, as well as for material handling for the formulation and consistency of production, including crystallinity.

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