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International Journal of Pharmaceutics 280 (2004) 39–46
international journal of pharmaceutics
www.elsevier.com/locate/ijpharm

Uniformity and physical states of troglitazone in solid dispersions determined by electron probe microanalysis and microthermal analysis

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Received 29 January 2004; received in revised form 10 April 2004; accepted 24 April 2004

Abstract

Solid dispersions of troglitazone with PVP K30 in a weight ratio of 1:2 were prepared by the closed melting method. Solid dispersions that exhibit different degrees of crystallinity (0 and 36%) were prepared by changing the charged amount of water, which functions as a plasticizer for PVP K30. Electron probe microanalysis (EPMA) and microthermal analysis (μTA) of the solid dispersions were performed to investigate the uniformity and physical state of troglitazone in the solid dispersions. The EPMA study confirmed that troglitazone was dispersed homogeneously in the sample whose apparent crystallinity was 0%. However, the sample with a 36% crystallinity was heterogeneous. The μTA study showed that the sample with a 36% crystallinity was present in two states, crystal and amorphous. EPMA and μTA would be useful tools to confirm the uniformity and physical states, respectively, of solid dispersions.

Keywords: Troglitazone; Solid dispersion; Uniformity; Physical state; Electron probe microanalysis; Microthermal analysis

1. Introduction

Troglitazone is a novel oral anti-diabetic drug (Yoshioka et al., 1989, 1991) that improves insulin sensitivity and responsiveness. Troglitazone also lowers hepatic glucose production. This compound is not only effective in insulin-dependent diabetes mellitus patients, but also in non-insulin-dependent diabetes mellitus patients (Fujiwara et al., 1988; Suter et al., 1992). Troglitazone has two asymmetric carbons, as shown in Fig. 1 , and is present as four isomers in equal amount. The solubility of each isomer is ca. 10 μg/mL in water, and this low solubility results in a low bioavailability of troglitazone (Suzuki et al., 2002).

Solid dispersions have traditionally been used as an effective method to improve the dissolution properties and bioavailability of poorly water-soluble drugs (Chiou and Riegalman, 1971; Serajuddin, 1999; Leuner and Dressman, 2000). The melting technique is one of the most widely used methods to prepare amorphous solid dispersions, but drug degradation must be taken into account as high temperature con-

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doi:10.1016/j.ijpharm.2004.04.024

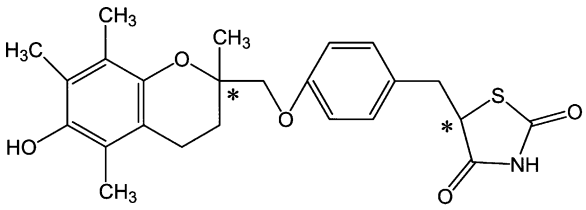

Fig. 1. Chemical structure of troglitazone. Asterisks represent asymmetric carbons.

ditions are used. Lowering the glass transition temperature of polymers allows for the preparation of amorphous solid dispersions by heating below the melting temperature of the drug. Therefore, using a plasticizer that will act with the polymer is thought to be effective to avoid drug degradation. Various compounds such as water and polyethylene glycol (PEG) are known to be used as plasticizers. It was also revealed that the glass transition temperature decreases significantly with increasing weight fractions of water or PEG (Hamaura and Newton, 1999). However, adding the plasticizer lowers the glass transition temperature of solid dispersions, and this can thus easily induce drug crystallization. Volatile plasticizers can be used to prevent drug crystallization as they can be evaporated after preparing the solid dispersions. In particular, water has the advantage of being a non-organic solvent.

Recently, samples can be evaluated at the micro level due to the increase in the sensitivity of analytical equipment. Electron probe microanalysis (EPMA) is the traditional method used and relies on the fact that when high-energy primary electrons of the scanning electron microscope (SEM) beam collide with atoms on the surface of solid samples, characteristic X-rays reflecting the atomic number of elements on the specimen are generated. If such characteristic X-rays can be detected, the distribution of the elements can be mapped. EPMA has been applied in some studies in the pharmaceutical field (Brown, 1986; Takeshima et al., 1992) but with some difficulty as organic materials degrade easily. Then, microthermal analysis (μTA) was introduced as a technique that combined the principles of thermal analysis and scanning probe microscopy. This allowed the sample to be scanned in terms of both topography and thermal conductivity. By placing the probe on a specific region of a sample and applying heat, it was possible to perform localized thermal analysis experiments in those regions. The technique has been applied to some studies in the pharmaceutical sciences recently (Craig et al., 2001; Bond et al., 2002).

In this investigation, solid dispersions of troglitazone were prepared by the closed melting technique, and solid dispersions having different apparent crystallinities were prepared by changing the content of water, which functioned as a plasticizer for the polymer, PVP K30. The homogeneity of troglitazone and its physical states in solid dispersions was evaluated using EPMA and μTA techniques.

2. Materials and methods

2.1. Materials

Troglitazone whose structure is shown in Fig. 1 was manufactured by Sankyo Co. Ltd. Lot T003 was used in this study. PVP K30 was purchased from BASF Japan Ltd. (Tokyo, Japan). All other reagents employed were of analytical reagent grade.

2.2. Sample preparation

2.2.1. Physical mixtures

Physical mixtures were prepared by mixing troglitazone with PVP K30 in a weight ratio of 1:2 using NMG-1 (Nara machinery, Tokyo, Japan), a high-shear mixer.

2.2.2. Solid dispersions prepared by melting method

From physical mixtures, 1 g was placed into vials and stored at 25 °C in desiccators containing saturated inorganic salt solutions giving equilibrium relative humidity (RH) until a constant weight was obtained. The salts used in this study were potassium acetate (23% RH) and sodium chloride (75% RH). The water content of the samples was determined by the Karl–Fischer method.

The vials were sealed and heated for 90 min at 130 °C. Then, the vials were opened and the samples were dried for 10 min at each temperature. The samples were collected from the vials and the smooth surface of samples was used for electron probe microanalysis (EPMA) and microthermal analysis (μTA).

2.2.3. Solid dispersion prepared by the solvent method

About 5 g of physical mixture was dissolved in 20 mL of a l:2 mixture of ethanol and acetone. This solution was dried in a Rotavapor RE-111, rotary evaporator (Shibata, Tokyo, Japan).

2.3. Dissolution

The dissolution rate of troglitazone from solid dispersions was determined using a JP14 type 2 apparatus (Toyama, Osaka, Japan). Samples equivalent to 100 mg of troglitazone was added to 500 mL of dissolution medium in a 1000 mL cylindrical beaker. The dissolution medium consisted of phosphate buffer (pH 9) maintained at 37 ± 0.5 °C. The paddle rotation speed was set to 250 rpm. The test medium was withdrawn at 60 min. The concentration of troglitazone in the medium was determined using a UV-1600, UV spectrophotometer (Shimadzu, Kyoto, Japan).

2.4. Differential scanning calorimeter (DSC)

Differential scanning calorimeter (DSC) measurement of troglitazone was carried out in hermetically sealed aluminum pans using Thermo plus 8230L (Rigaku, Tokyo, Japan) calibrated with indium. Samples were heated under a dry nitrogen gas purge between 40 and 200 °C at a rate of 10 °C/min.

2.5. X-ray powder diffraction (XRPD)

X-ray powder diffraction (XRPD) patterns were measured by a Geiger Flex Rint-2200 (Rigaku Co., Japan) diffractometer with Cu Kα radiation at 40 KV/40 mA. The sample was step-scanned at 0.02° intervals from 5.00° to 40.00° (2θ) at the rate of 4.00°/min.

2.6. Hot-stage microscopy (HSM)

Troglitazone was heated on a Stanton Redcroft hot-stage unit at a controlled rate of 5 °C/min by using a universal temperature programmer (Stanton Redcroft, London, UK). Photographs of thermal events were taken at several temperatures (100, 125, 130 and 175 °C) using a differential interference contrast (DIG) microscope, Olympus BX50, with U-DICT filter (Olympus, Tokyo, Japan).

2.7. Electron probe microanalysis

Electron probe microanalysis was carried out using JXA-8800R (JEOL, Tokyo, Japan). The samples were coated with thin gold film. The thickness of the gold film was about 30 nm. The coated samples were scanned for 50 cycles under the following operating conditions: accelerated voltage of 15 kV, probe current of about 10^{-8} A, and irradiation time of 3 ms.

2.8. Microthermal analysis

A μTA 2990 micro thermal analyzer (TA Instruments, Leatherhead, UK) equipped with a topometrix-scanning probe was used for microthermal analysis. Topography and conductivity images of the sample were obtained in the contact mode with the probe set at a temperature of 90 °C. These images were taken with a scan speed of 200 μm/s × 200 lines. Then, local thermal analysis at two points showing the different conductivity was performed by heating from 50 to 250 °C at a rate of 20 °C/s under ambient conditions. In this study, micro thermomechanical analysis (μTMA) and micro modulated differential thermal analysis (μMDTA) were conducted as a local thermal analysis. μTMA can measure the expansion or softening of the samples by determining the displacement on the *z*-axis with the photodetector when the probe heats the samples. μMDTA can measure the power required to maintain temperature modulation. The thermal response was calibrated using benzoic acid which had a melting point of 122 °C.

2.9. Calculation of apparent crystallinity

The solid dispersion prepared by the solvent method was confirmed to be completely amorphous by DSC and XRPD. This solid dispersion was used for the calculation of apparent crystallinity. The apparent crystallinity for the physical mixture and solid dispersion prepared by the solvent method was specified to be 100 and 0%, respectively. The solid dispersion and physical mixture were mixed in a certain ratio. A dissolution test of the resultant mixtures was conducted. The regression curve in a graph plotting the mixture

ratio and percentage dissolution at 60 min was obtained. There was a good relationship between each other. The apparent crystallinity of solid dispersions prepared by the melting method was calculated using the equation of this regression curve.

3. Results and discussion

The DSC thermogram of the troglitazone drug substance (Lot T003) is shown in Fig. 2. There were two endothermic peaks at about ca. 120 °C and ca. 175 °C. In the hot-stage microscopic images of the drug substance, shown in Fig. 3b and d, the endothermic peaks were attributed to the melting of troglitazone. As shown in Fig. 3c, crystallization was observed at about ca. 130 °C although this was difficult

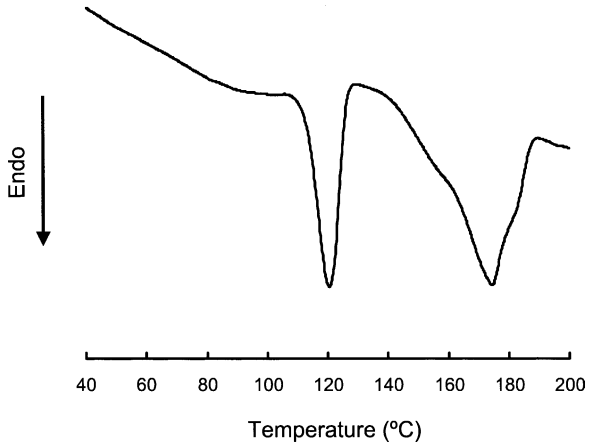

Fig. 2. DSC thermogram of troglitazone.

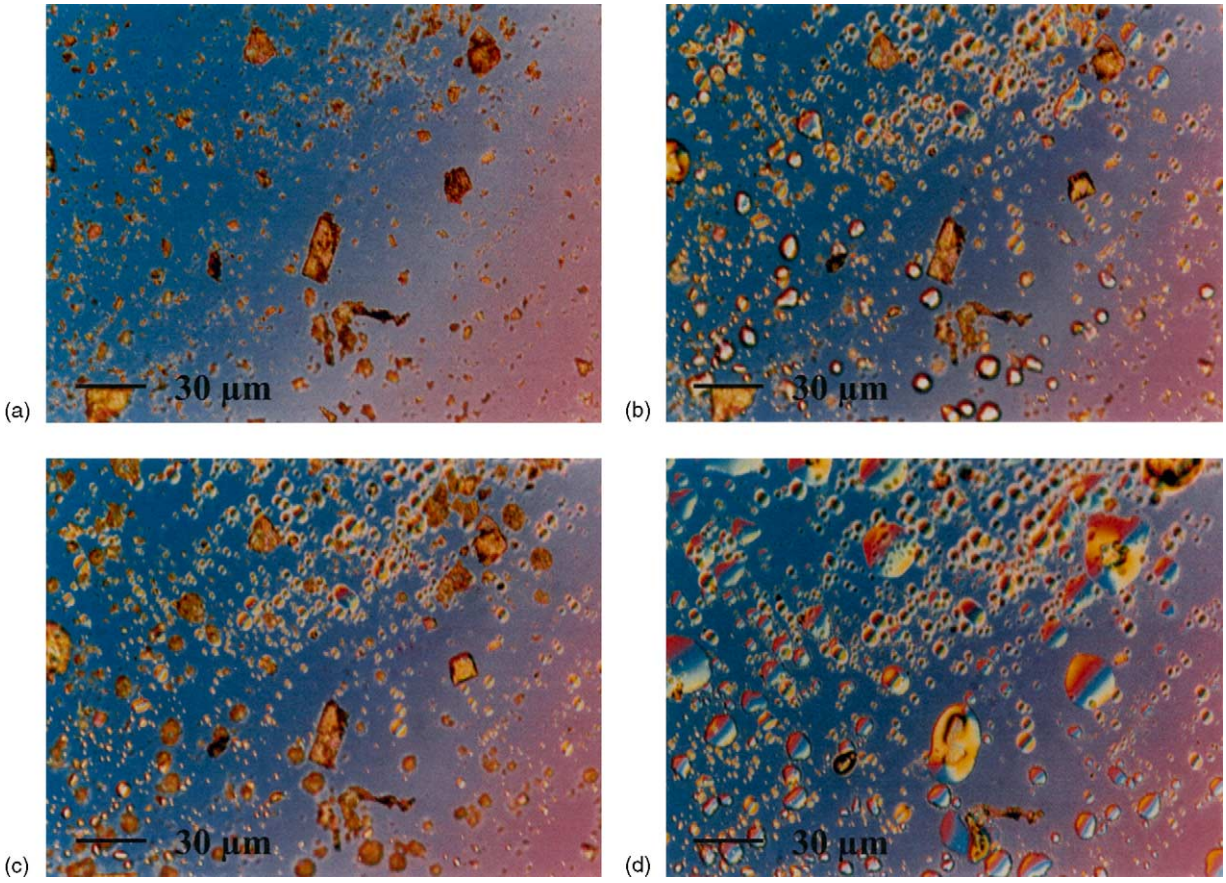

Fig. 3. Hot-stage microscopic images of troglitazone at (a) 100 °C (b) 125 °C (c) 130 °C and (d) 175 °C.

to determine in the DSC thermogram. Troglitazone has two asymmetric carbons as shown in Fig. 1, and is present as four isomers in equal amounts. It has been reported that the endothermic peak of the troglitazone drug substance at ca. 125 °C is due to the melting of the RR/SS form and the other peak at ca. 175 °C is due to the melting of the RS/SR form (Suzuki et al., 2002).

Solid dispersions that have different dissolution properties and apparent crystallinity were prepared with different water contents in physical mixture as

Table 1
Physicochemical properties of solid dispersions

Sample	1	2
Water content in physical mixture (g/g solid)	0.065	0.182
Dissolution percentage of troglitazone at 60 min	65.5	100.0
Apparent crystallinity (%)	36	0

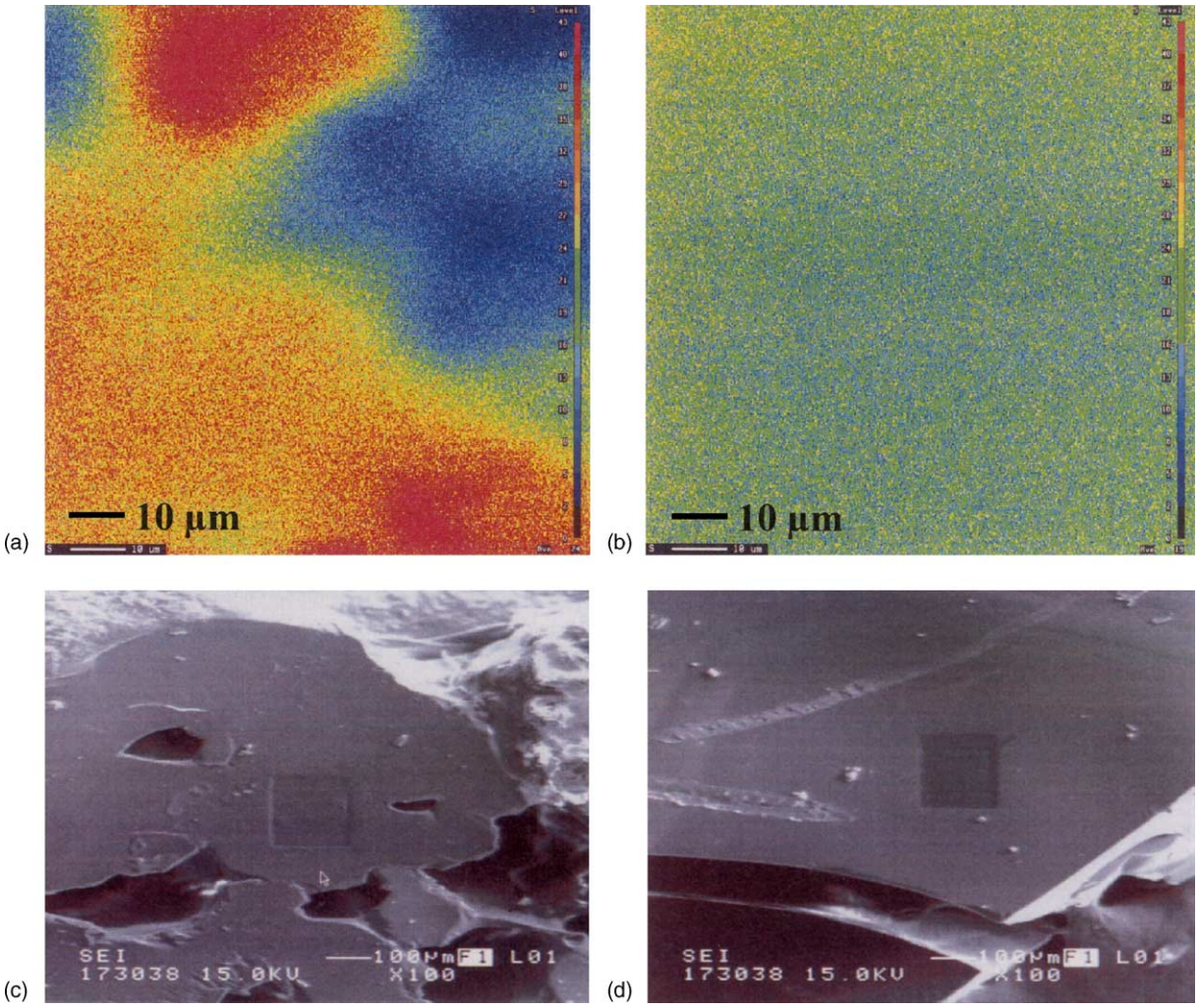

Fig. 4. Electron probe microanalysis images of (a) sample 1 (apparent crystallinity: 36%) (b) sample 2 (apparent crystallinity: 0%), SEM of (c) sample 1 (apparent crystallinity: 36%) and (d) sample 2 (apparent crystallinity: 0%).

shown in Table 1 . As the DSC thermogram of troglitazone in the presence of water under closed conditions did not change (data not shown), it was considered that there was little interaction between troglitazone and water. Therefore, it was thought that water mainly interacted with PVP and acted as a plasticizer for PVP.

EPMA was performed on solid dispersions that had different crystallinities in order to visualize the homogeneity of troglitazone. As shown in Fig. 1, troglitazone contains a sulfur element. On the other hand, there is no sulfur element in PVP. Therefore, the intensity of the characteristic X-ray of sulfur element can indicate the amount of troglitazone molecules present. Fig. 4a and b are typical EPMA images of solid dispersions that had different apparent crystallinities. Similar results were observed with good reproducibility (data not shown). As shown in Fig. 4a, distinct red and blue parts were clearly observed and this demonstrated the heterogeneous localization of troglitazone molecules in sample 1 that had a high apparent crystallinity (36%). In contrast, there was no color difference in sample 2 whose apparent crystallinity was 0%, as shown in Fig. 4b. These results indicated that troglitazone molecules were homogeneously dispersed in sample 2.

SEM images after the EPMA scanning are shown in Fig. 4c and d. A dark square of 100 μm in length was observed on both sample surfaces, after the scanning in EPMA. When conducting EPMA for the organic compounds, it is necessary to take into account their degradation such as due to melting and rotting, because the EPMA exposes the compounds to high-energy primary electrons on their sample surface. In this study, to avoid sample degradation, the irradiation time was reduced as much as possible. In addition, although EPMA could be used to visualize the homogeneity of specific components in multicomponent systems such as in solid dispersions as described above, it cannot determine the physical states of the sample.

A μTA 2990 micro thermal analyzer was used to take topographic and thermal conductivity images. Square images of 100 μm in length were taken. Fig. 5a and b show the topographic and thermal conductivity images, respectively, of the solid dispersion surface having an apparent crystallinity of 36%. The topographic image indicated that the surface of this sample was very smooth and uniform. This result corresponds to the SEM image shown in Fig. 4c. In contrast, non-uniformity in thermal conductivity on the surface of the solid dispersion was observed as shown in Fig. 5b. Local thermal analysis (μTMA and μMDTA) was, therefore, performed at point 1 and point 2, which were observed as having a difference in thermal conductivity. The derivative of the power signal was used for data analysis to identify the exothermic and endothermic events. μMDTA data at point 1 showed two endothermic peaks at ca. 120 and ca. 175 °C, as shown in Fig. 6. It was very similar to the DSC thermogram of troglitazone drug substance shown in Fig. 2. However, the peak at ca. 120 °C observed by μMDTA was smaller than that by DSC, as the sample was prepared by heating at 130 °C. On the other hand, in the DSC thermogram of the sample, the peak at ca. 120 °C was not observed although the peak at ca. 175 °C was observed (data not shown). In addition, μTMA data showed that the

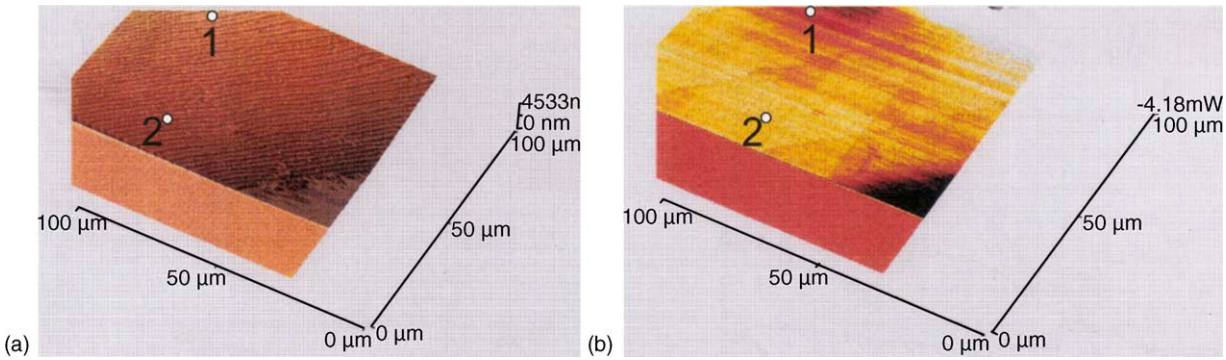

Fig. 5. Topographic and thermal conductivity images of solid dispersion (apparent crystallinity: 36%).

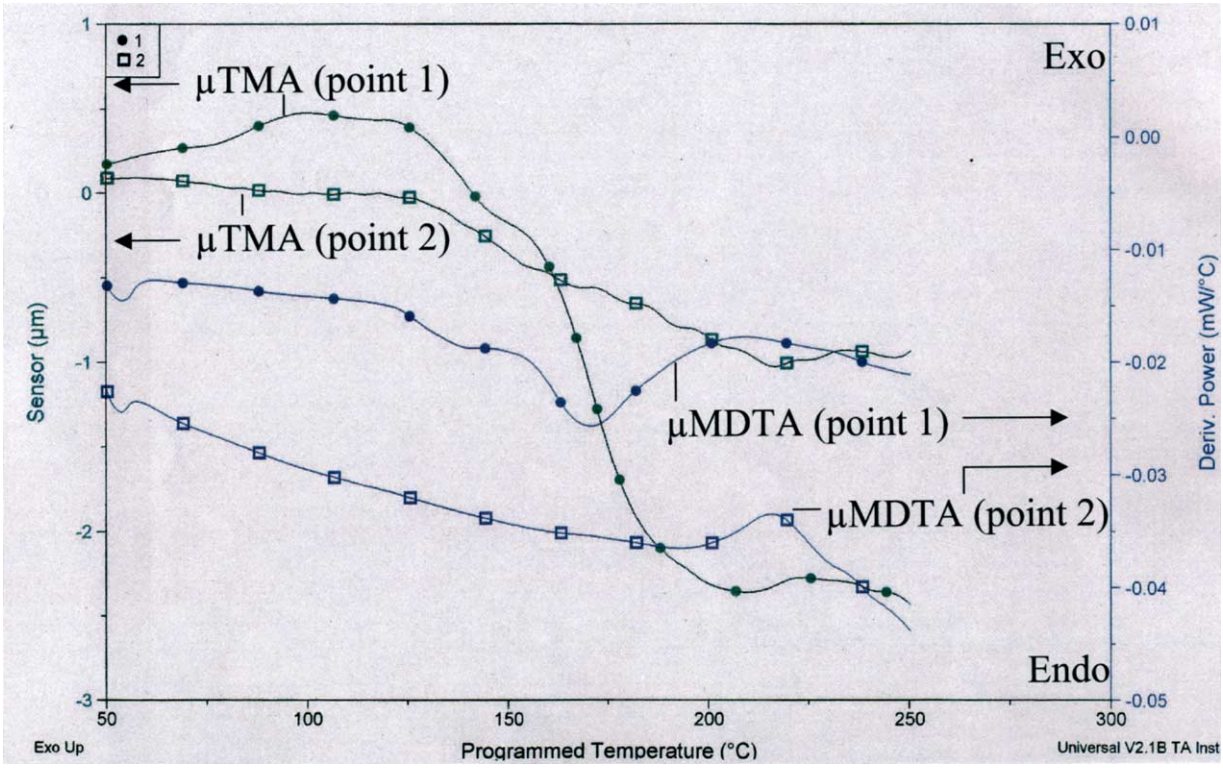

Fig. 6. Local thermal analysis of solid dispersion (apparent crystallinity: 36%). μTMA data (left vertical axis) and μMDTA data (right vertical axis) are shown as sensor position and as derivative of power, respectively.

sensor reading decreased at ca. 120 and ca. 175 °C in accordance with the melting of troglitazone crystal. The sensor reading increased in the range from 50 to 100 °C because of the thermal expansion of the sample. Consequently, it was suggested that troglitazone crystals were presented at point 1. On the other hand, there was no remarkable change in the μMDTA at point 2 although the sensor reading decreased at ca. 130 °C. It was considered that the change in sensor reading was caused by a change in the physical state, from the glassy to a rubbery state, of either PVP K30 or the solid dispersion. A specific heat change could not be detected by μMDTA in this study. The glass transition temperature of the amorphous salbutamol prepared using a spray dryer also could not be observed by μMDTA (Murphy et al., 2003). As the glass transition temperature of PVP K30 and the amorphous solid dispersion containing 0% of water was observed at ca. 160 and ca. 126 °C, respectively, by DSC (data not shown), the amorphous component at point 2 showing a sensor reading change at ca. 130 °C was speculated to indicate a solid dispersion. However, even though the sample was dried after preparation and stored with desiccant, it was measured by μTA under ambient conditions and thus, water could have been absorbed or adsorbed. As the glass transition temperature of PVP is lowered by water, the amorphous component at point 2 might be PVP. According to the literature (Six et al., 2003), the glass transition temperature of Eudragit® E100 determined by μTMA was 65 °C higher than that determined by the DSC because of the low fragility of Eudragit® E100 although glassy itraconazole and an amorphous solid dispersion containing itraconazole (10%) and Eudragit® E100 (90%) did not show such a discrepancy. In this study, no such difference between the μTMA and DSC results was observed. The fragility (m) of PVP K30 was calculated to be about 50 based on the literature (Hancock et al., 1998). This fragility (m) was almost the same as that of Eudragit® E100.

Thus, if the amorphous component at point 2 was PVP K30, the sensor reading would have decreased at a much higher temperature than 130 °C in the μTMA study. However, here, the presence and/or effect of water was not taken into account.

As described above, the μTA is thought to be a useful method to evaluate the physical states and homogeneity (heterogeneity) of multicomponent systems such as solid dispersions.

4. Conclusion

The homogeneity of troglitazone in solid dispersions that have different apparent crystallinities was confirmed to be clearly different by EPMA. The physical states of troglitazone in solid dispersions having an apparent crystallinity of 36% could be evaluated by μTA. It was, therefore, concluded that EPMA and μTA were useful tools to evaluate the uniformity and physical state of troglitazone in solid dispersions, respectively.

Acknowledgements

Our μTA investigations were supported by TA Instruments.

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