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Solid State of Tulobuterol : Characterization, Dissolution, Transformation

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ABSTRACT − The objective of this work was to investigate the existence of new crystal forms of tulobuterol which is used to prevent morning asthma attacks by β_2 agonist and the transformation of crystal forms. Three crystal forms of tulobuterol have been isolated by recrystallization and Form 2 was transformed to Form 4 at 52% RH and 95% RH and these four crystal forms are characterized by differential scanning calorimetry (DSC), powder X-ray diffractometry (PXRD) and thermogravimetric analysis (TG). The DSC and PXRD patterns of four crystal forms of tulobuterol were different respectively. The dissolution patterns of these three crystal forms of tulobuterol were studied and they showed significant differences in the dissolution rate. After storage of 2 months at 0% RH (silica gel, 20°C), 52% RH (saturated solution of $Na_2Cr_2O_7 \cdot 2H_2O$ / 20°C) and 95% RH (saturated solution of Na_2HPO_4 / 20°C), Form 1 and Form 3 were not transformed. But Form 2 was transformed to Form 4 at 52% RH and 95% RH.

Key words − Tulobuterol, Polymorphism, Crystal form, DSC, PXRD, Dissolution

Polymorphism defines as the ability of a substance to exist as two or more crystalline phases that have different arrangements and/or conformations of the molecules in the crystal lattice. Polymorphs share the same chemical composition but have different crystal structures. Because of their structural differences, polymorphs may have different physicochemical properties. For example, polymorphs can have different density, habit, melting properties, vapor pressure, solubility, dissolution rate, tableting and mechanical properties (Haleblian et al., 1969; Haleblian, 1975; Hüttenrauch, 1988; Song et al., 2010).

Crystal form includes polymorphs, solvates, and amorphous forms as defined in the International Conference on Harmonization (ICH) Guideline Q6A : Test Procedures and Acceptance Criteria for New Drug Substances and New Drug Products: Chemical Substances. Federal Register, 2000.

Crystal form affects properties such as drug absorption, rate of dissolution, elimination rate and stability in galenic preparations (Opalchenova et al., 1997; Sohn, 2007; Lee et al., 2008).

Because different polymorphs and pseudopolymorphs exhibit significantly different pharmaceutically relevant properties, discovery, preparation, and characterization of polymorphs and pseudopolymorphs are essential preformulation steps in pharmaceutical research and development (Haleblian et al., 1969).

The compound tulobuterol (Figure 1), (RS)-2-tert-Butylamino-1-(2-chlorophenyl)ethanol, is used to prevent morning asthma attacks by β_2 agonist. Caira et al. reported two polymorphs of tulobuterol (Caira et al., 2004). The aim of this study was to investigate the existence of new crystal forms of tulobuterol, the influence of crystal form on dissolution, and the transformation of these crystal forms. In the present study, the identification, solid state stability, and dissolution behavior of tulobuterol crystalline forms were examined. Powder X-ray diffractometry (Bachet, 1997; Yamamura et al., 2001) and thermal analysis (Giron, 1995; Campeta et al., 2010) are clearly useful for the study of polymorphism and pseudopolymorphism. A stability test at various relative humidities was also performed to provide an early and rapid method for predicting stability. In the present study, dissolution patterns of crystal forms of tulobuterol were studied.

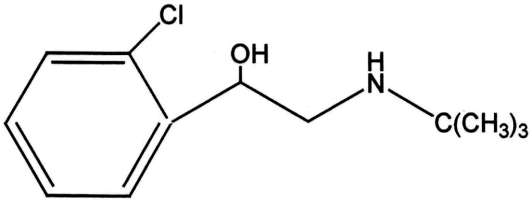

Figure 1. Chemical structure of tulobuterol.

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Materials and Methods

Materials

Tulobuterol was provided from Pacific Pharmaceutical Co. Ltd., Korea. Other extra pure chemicals were purchased from a reagent commercial company.

Preparation of crystal forms

Form 1

Form 1 was the donated one. Form 1 was always stored at 0 - 2°C condition.

Form 2

Form 1 (1 g) was dissolved in acetic acid (1.5 mL) and the suspension was heated to 40°C for 30 minutes. The solution was filtered to remove most nuclei and then left undisturbed for 5 days at room temperature. The resulting solid was filtered and dried for 3 days in the desiccators to give Form 2.

Form 3

Form 1 (1g) was dissolved in formic acid (1 mL) and the suspension was heated to 40°C for 30 minutes. The solution was filtered to remove most nuclei and then left undisturbed for 5 days at room temperature. The resulting solid was filtered and dried for 3 days in the desiccators to give Form 3.

Form 4

Form 2 was transformed to Form 4 at 52% RH and 95% RH.

Powder X-ray diffraction (PXRD)

Powder X-ray diffraction patterns under ambient conditions were collected on Rigaku DMAX-IIIA (Japan) diffractometer using graphite monochromatized CuKα radiation (λ=1.54178 Å). The isothermal measurement conditions were; target, Cu; voltage, 30 kV, current, 20 mA. The PXRD patterns of the samples were compared with regard to peak position and relative intensity, peak shifting, and the presence of lack of peaks in certain angular regions.

Thermal analysis

Thermal analysis methods used in this study included differential scanning calorimetry (DSC), thermogravimetric analysis (TG). DSC patterns were recorded with a Mettler DSC 30 (Mettler, Switzerland). The temperature was usually scanned from 35 to 300°C at 10°C/min. 5 mg of sample was used for each study. TG analysis was performed on all samples indicated by DSC as being possible solvates or hydrates. TG patterns were recorded with a Mettler TG 50 (Mettler, Switzerland). The temperature was usually scanned from 35 to 300°C at 10°C/min. 5 mg of sample was used for each study.

Dissolution

The dissolution rate of tulobuterol was measured by paddle method of USP XXIV using dissolution tester (Duksan pure chemical Co., Korea). A fixed amount (300 mg, 250-600 μm) of tulobuterol crystal forms was put into 900 mL of distilled water and stirred at 100 rpm at 37±0.5°C At an appropriate intervals, an aliquot (1 mL) was withdrawn with a syringe and filtered with 0.45 μm syringe filter. And then it was analyzed spectrophotometrically at 212 nm.

Transformation

A certain amount (20 mg) of crystal forms was taken and placed in weighing dish. They were stored in desiccator of 0% RH (relative humidity) (silica gel, 20°C), 52% RH (saturated solution of $Na_2Cr_2O_7 \cdot 2H_2O$/20°C) and 95% RH (saturated solution of Na_2HPO_4/20°C). The transformation behavior of crystal forms was monitored by PXRD analysis, DSC and TG.

Results and Discussion

UV/Vis spectrophotometer was used for crystal form's chemical identity. The UV/Vis spectrum shows same absorption peak of four crystal forms in wavelength 212 nm.

DSC curves of three crystal forms of tulobuterol are illustrated in Figures 2-4. The DSC curve of Form 1 shows a single melting endothermic peak at 91-92°C The DSC curve of Form 2 shows two endothermic peaks, one endothermic melting peak at 150-151°C and the second broad endothermic peak at 190-210°C. The DSC curve of Form 3 shows three endothermic peaks, one melting endothermic peak at 116-117°C and two endothermic peaks at 175-210°C and 210-255°C. TG anal-

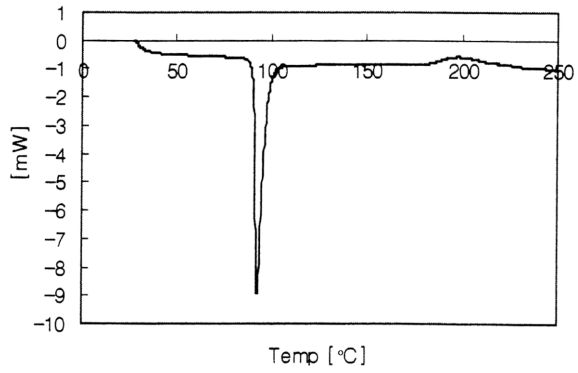

Figure 2. DSC curve of Form 1.

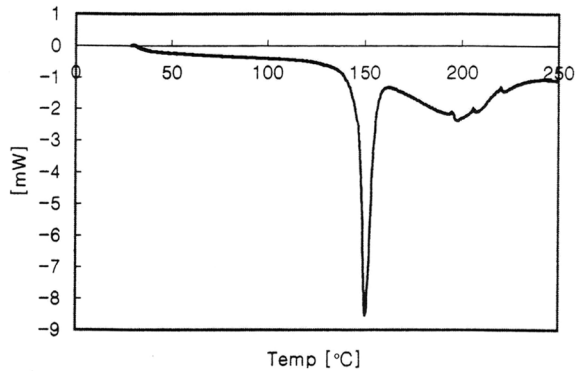

Figure 3. DSC curve of Form 2.

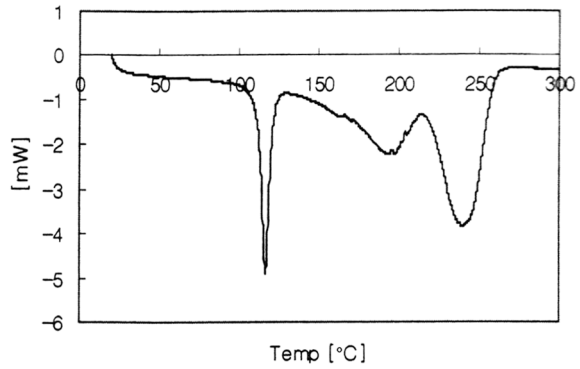

Figure 4. DSC curve of Form 3.

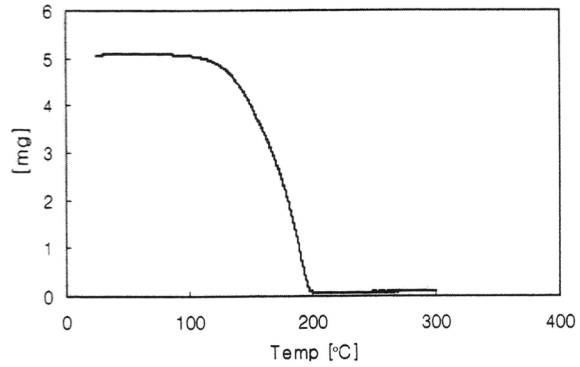

Figure 5. TG curve of Form 2.

ysis represents a powerful adjunct to DSC, since a combination of a DSC study with a TG determination can be used in the assignment of observed thermal events. TG curves of Form 2 - 3 of tulobuterol are illustrated in Figures 5-6 and Form 2 and Form 3 were found to be nonsolvates.

The Powder X-ray diffraction patterns of three crystal forms of tulobuterol are illustrated in Figures 7-9 and they showed distinct differences. Table I - III list 2θ angles, d-spaces and relative intensities of characteristic diffraction peaks up to 30° of three crystal forms.

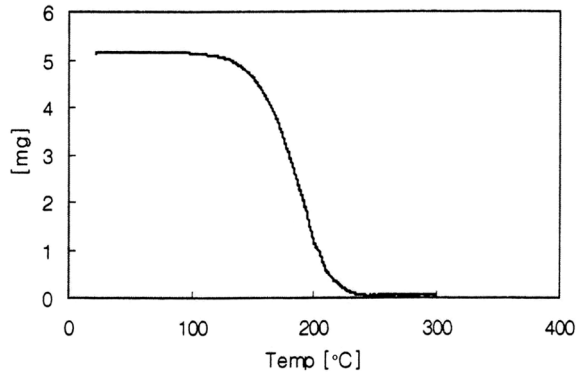

Figure 6. TG curve of Form 3.

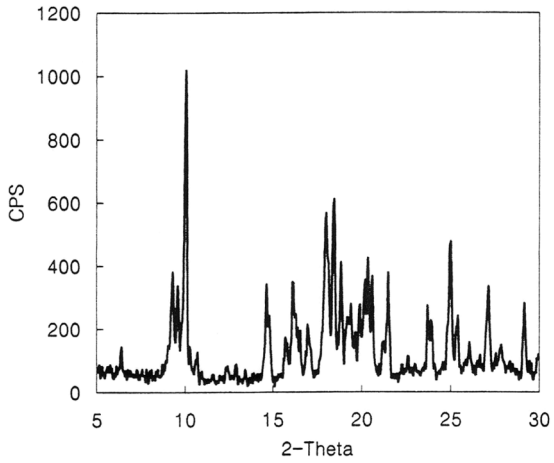

Figure 7. Powder X-ray diffraction pattern of Form 1.

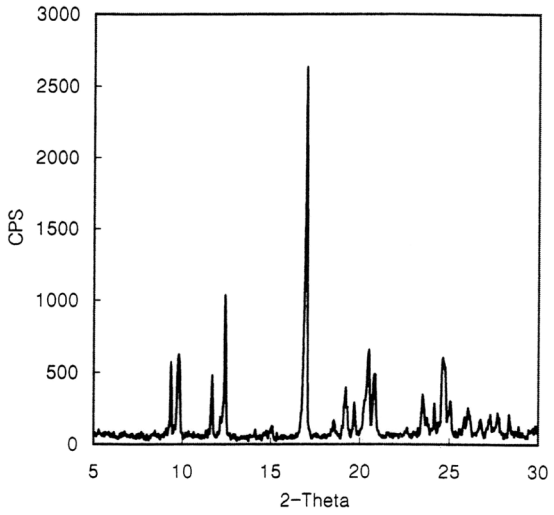

Figure 8. Powder X-ray diffraction pattern of Form 2.

Caira et al. reported the existence of two polymorphs of tulobuterol (Caira et al., 2004). The DSC patterns and PXRD patterns of Form 2, Form 3 and Form 4 are different from those of Form 2 of Caira et al.

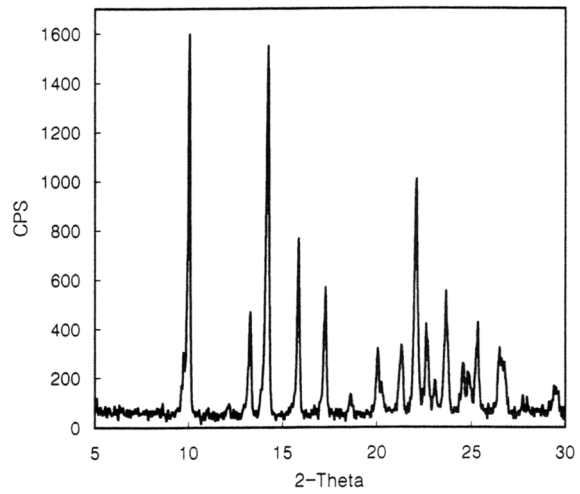

Figure 9. Powder X-ray diffraction pattern of Form 3.

Table I. *Characteristic diffraction peaks of Form 1 up to 30°C*

Angle (2θ)	d-space (Å)	Intensity Ratio
9.3	9.5091	37
9.6	9.2126	33
10.05	8.8011	100
14.65	6.0463	33
16.1	5.5049	34
16.3	5.4378	24
18.0	4.9279	56
18.45	4.8087	60
19.4	4.5753	27
19.9	4.4615	27
20.2	4.3959	35
20.35	4.3638	41
20.6	4.3114	36
21.5	4.1329	37
23.7	3.754	37
25.0	3.5617	47
27.15	3.2843	32
29.15	3.0634	28

After storage of 2 months at 0% RH (silica gel, 20°C), 52% RH (saturated solution of $Na_2Cr_2O_7 \cdot 2H_2O$ / 20°C) and 95% RH (saturated solution of Na_2HPO_4 / 20°C), Form 1 and Form 3 showed no change in DSC, TG and PXRD patterns. But Form 2 was transformed to Form 4 at 52% RH and 95% RH. The DSC, TG and PXRD patterns of Form 4 are illustrated in Figures 10-12 and 2θ angles, d-spaces and relative intensities of characteristic diffraction peaks up to 30° of Form 4 are given in Table IV. Form 4 was found to be nonsolvate.

Table II. *Characteristic diffraction peaks of Form 2 up to 30°C*

Angle (2θ)	d-space (Å)	Intensity Ratio
9.35	9.4584	22
9.8	9.025	24
11.7	7.5633	18
12.4	7.1379	39
17	5.2154	100
19.2	4.6225	15
19.7	4.5063	11
20.5	4.3322	25
20.8	4.2704	19
23.5	3.7855	13
24.15	3.6851	11
24.65	3.6115	23
25.05	3.5547	11
26.05	3.4205	10
27.3	3.2666	8
27.7	3.2203	8
28.35	3.148	8

Table III. *Characteristic diffraction peaks of Form 3 up to 30°C*

Angle (2θ)	d-space (Å)	Intensity Ratio
9.7	9.1178	19
10.05	8.8011	100
13.3	6.6569	29
14.25	6.2151	97
15.85	5.5912	49
17.3	5.1257	36
18.6	4.7702	8
20.05	4.4284	20
21.3	4.1713	21
22.1	4.0221	64
22.6	3.9342	26
23.65	3.7618	35
24.55	3.6259	16
24.8	3.59	14
25.35	3.5133	27
26.5	3.3634	20
26.6	3.351	17
26.75	3.3325	17

The dissolution patterns of three crystal forms of tulobuterol are illustrated in Figure 13. In the dissolution studies in distilled water at 37±0.5°C, the solubility of Form 1 was the high-

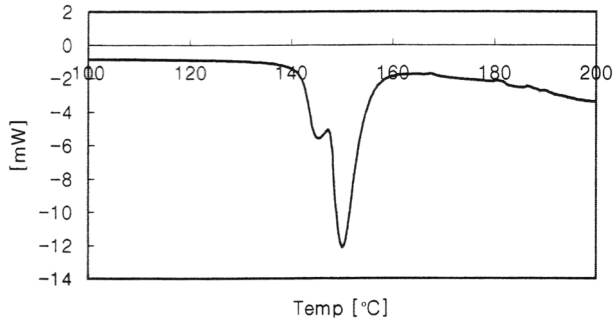

Figure 10. DSC curve of Form 4.

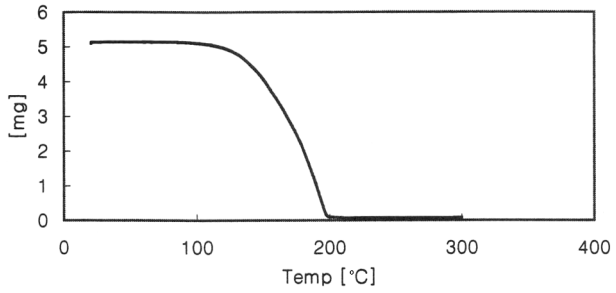

Figure 11. TG curve of Form 4.

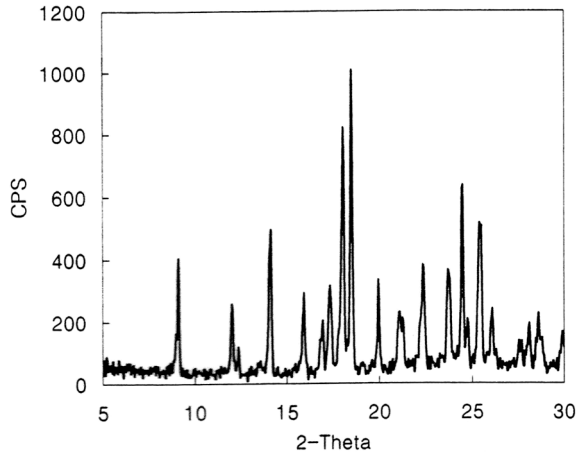

Figure 12. Powder X-ray diffraction pattern of Form 4.

est. And the solubility in water decreased in rank order: Form 1>Form 3>Form 2.

Conclusion

Three crystal forms of tulobuterol were prepared by recrystallization from different solvents. Form 2 was transformed to Form 4 at 52% RH and 95% RH. These four crystal forms were characterized by DSC, TG and PXRD. After storage of 2 months at 0% RH, 52% RH and 95% RH, Form 1 and Form 3 showed no transformation. But Form 2 was transformed to Form 4 at 52% RH and 95% RH. In the dissolution studies in distilled water at 37±0.5°C, three crystal forms showed difference. These four crystal forms were found to be nonsolvates.

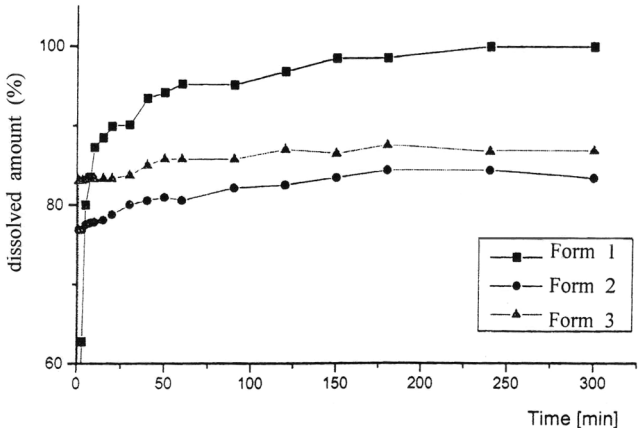

Figure 13. Dissolution patterns of three crystal forms of tulobuterol.

Table IV. *Characteristic diffraction peaks of Form 4 up to 30°C*

Angle (2θ)	d-space (Å)	Intensity Ratio
9.1	9.7176	40
12.0	7.3749	26
14.15	6.2588	49
15.95	5.5563	29
16.95	5.2307	20
17.35	5.111	31
18.05	4.9143	82
18.5	4.7958	100
19.95	4.4504	33
21.1	4.2104	23
21.25	4.181	21
22.35	3.9776	38
23.7	3.754	36
24.5	3.6332	63
24.75	3.5971	21
25.4	3.5065	51
26.1	3.414	24
28.6	3.121	22

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