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Investigation of intrinsic dissolution behavior of different carbamazepine samples

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ABSTRACT

The aim of the present study was to investigate the effect of the variability of commercially available carbamazepine (CBZ) samples on the intrinsic dissolution behavior in order to recommend a strategy to maintain product quality by monitoring the variability of critical parameters of the bulk drug.

Extensive physical characterization of nine anhydrous CBZ samples from three different sources and their respective dihydrates showed that the commercial anhydrous CBZ samples exhibited the same polymorphic form, but different morphology and particle size distribution which led to a variation in the kinetics of conversion from anhydrous to the dihydrate CBZ and therefore to variation in the kinetics of solubility.

Disc intrinsic dissolution rate (DIDR) tests showed different intrinsic dissolution behavior of the samples, whereby the transition points of anhydrous to dihydrate conversion varied between 15 and 25 min. On the other hand, converting the anhydrous CBZ's to dihydrate eliminated the variation in intrinsic dissolution behavior.

Tablets of the different CBZs and Ludipress® were prepared by direct compression. The amount of CBZ dissolved after 15 min showed the same rank order as the rank order of the transition points determined by intrinsic dissolution test. Therefore, the intrinsic dissolution test with specific acceptance criteria can be a valuable and simple tool for monitoring, respectively reducing the variability of the CBZ bulk material.

1. Introduction

Carbamazepine (CBZ) is a well established drug used in the treatment of epilepsy. It is a poorly water soluble substance, classified as class II according to the Biopharmaceutics Classification System. There are at least four anhydrous polymorphs of this molecule and dihydrate (structure) as well as other solvates (i.e. monoacetonate) reported in the literature (Krahn and Mielck, 1987, 1989; Kobayashi et al., 2000; Rustichelli et al., 2000). CBZ polymorphs have different crystal structures and exhibit different melting points, chemical reactivity, solubility and compactibility, all of which can contribute to the differences in their bioavailability (Murphy et al., 2002; Ono et al., 2002). Among them, the anhydrous form III (CBZ A), the thermodynamically stable form at room temperature and the dihydrate form (CBZ D) are the most commonly encountered forms. CBZ A form III (P-monoclinic form) is thermodynamically the most stable form at room temperature and it is the only form used in formulation of marketed products. On the other hand, CBZ D is the most stable form in aqueous solution and this is why all other polymorphs convert (although with different kinetics) to the dihydrate form in aqueous solution via solution-mediated mechanism (Rodriguez-Hornedo and Murphy, 2004). The two molecules of water bonded at amino and carboxyl group of CBZ, correspond theoretically to 13.2% of water. Crystals of dihydrate are stable at room temperature and relative humidity above 52% (McMahon et al., 1996).

Commercially available generic raw material can contain a mixture of CBZ polymorphs and crystal modifications as well as amorphous parts or can exhibit variations in the crystal habit depending on the process used for its manufacturing.

CBZ is one of the most extensively researched drug because of its polymorphism and because it undergoes solution-mediated transformation in aqueous media. In spite of this, generic products of CBZ have suffered failure, instability and bioinequivalence (Meyer et al., 1992; Meyer et al., 1998).

During the dissolution in aqueous medium, anhydrous CBZ forms undergo a transformation to dihydrate CBZ. The kinetics of this transformation is a critical point for the bioavailability

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because it results in a reduction of dissolution and bioavailability and explains partly the erratic dissolution of CBZ tablets stored at different temperatures and relative humidites (Wang et al., 1993). Transformation from anhydrous to dihydrate form may be the reason why some CBZ generic tablets have shown bioinequivalence and clinical failures.

The development of a drug formulation for the commercial market requires a tremendous effort in order to assure constant quality of the product, i.e. its ability to deliver the therapeutic effect for which it was intended. This lack of robustness may be attributed to the variability of the primary materials both active and excipients which can "spoil" the enormous efforts spent on development when the effects of such variability are not well understood.

Yet no comprehensive study has been made on the variability of the commercially available bulk drug. Understanding the cause of the problem, i.e. the mechanisms which are responsible for the variability of the primary materials can help to find a solution to the current problems of CBZ generic formulations. This is one example which illustrates the necessity to identify and characterize polymorphic modifications as precisely as possible. In this respect, measurement of a critical attribute of the raw materials is essential in insuring final product quality.

We hypothesize that the variation in dissolution may be avoided by establishing an adequate strategy to maintain product quality by defining and monitoring the variability of critical parameters of the bulk drug and by using specific excipients in the formulation which will control the CBZ anhydrate to dihydrate conversion and thereby stabilize the variation in dissolution behavior of the final CBZ product.

The aim of the study was to investigate the variability of commercially available CBZ from different manufacturers of the drug by fully characterizing the physical and chemical characteristics in order to recommend a strategy to maintain product quality by monitoring the variability in the active ingredient and/or by selecting a well controlled pre-treatment method to obtain a "primary" material independent of the supplier of CBZ.

2. Materials and methods

2.1. Materials

The following samples of carbamazepine were used for the investigation:

- CBZ meets USP specifications purchased from Sigma–Aldrich, Lot 093K1544, named USP standard in the manuscript.
- Carbamazepine anhydrous: the samples were provided from three different manufacturers and for the reasons of confidentiality will be designated as CBZ A, CBZ B and CBZ P. Each of the producers donated three batches of carbamazepine (A1, A2, A3, B1, B2, B3, P1, P2 and P3) and thus batch to batch variability was investigated as well. There was no special specification for the drug substance from our side, e.g. for particle size distribution, therefore all delivered samples were of commercial grade.
- CBZ dihydrates (CBZ D) were prepared by crystallization of all 10 available CBZ anhydrous samples by suspending CBZ in distilled water and stirring with a magnetic stirrer for 24 h at room temperature. The precipitated crystals were filtrated on a filter paper and dried at room temperature. Dihydrates were stored at room temperature and a relative humidity of 55–60% to maintain hydrate conditions (McMahon et al., 1996).
- For the second part of the study co-processed excipient Ludipress® purchased from BASF, was used.
- All other chemicals and reagents purchased from commercial sources were of analytical grade.

2.2. Methods

2.2.1. Fourier transform infrared spectroscopy (FTIR)

FTIR spectroscopy was accomplished with a microscope Olympus BX/51-52 (Olympus, Japan) modified with an infrared microspectrometer IlluminatIR® (Smith Detection, USA), and equipped with an ATR objective (attenuated total reflectance objective). Setting parameters were: resolution 4 cm^{-1}, data region 4000–700 cm^{-1}, aperture 100 μm, energy 27,000, detector type MCT (mercury–cadmium–telluride) and number of runs per spectrum 32.

2.2.2. Determination of residual moisture content

Loss on drying measurements were carried out according to European Pharmacopoeia Ph.Eur.5, using an infrared balance Type LP 16M (Mettler Toledo, Switzerland). Samples of approximately 1 g were heated up for 2 h at temperature of 105 °C.

2.2.3. Scanning electron microscopy (SEM)

The morphology of the bulk materials and the lower surface of CBZ compacts before and after intrinsic dissolution were determined by scanning electron microscopy. SEM images were taken using an ESEM XL 30 FEG (Philips, The Netherland). The samples were mounted with carbon adhesive on aluminum holder, sputtered with gold and photographed at a voltage of 10 kV. In the case of compacts, Ag was used as additional conductor.

2.2.4. Particle size analysis (PSA)

The average particle size and particle size distribution were determined by laser diffraction technique with a Malvern Mastersizer 2000 (Malvern Instruments, United Kingdom) using dry unit Scirocco 2000 (Malvern Instruments, United Kingdom). The measurement was carried out six times and five consecutive values with the smallest variation were taken into account.

2.2.5. True density

The true density was measured with a helium pycnometer AccuPyc 1330 (Micrometrics, USA) with nominal cell volume of 10.0 ml. As the true density is expressed as a quotient of mass and volume, the samples were weighed on balance AX204 (Mettler Toledo, Switzerland) and placed in the cell. The volume was determined by purging each sample 10 times with helium.

2.2.6. X-ray powder diffraction (XRPD)

X-ray powder diffraction profiles of powders were taken at room temperature with a Diffractometer D5000 (Siemens, Germany). The operating conditions were as following: Ni filtered Cu-Kα radiation (λ = 1.5406 Å), voltage 40 kV, current 30 mA, step 0.02°, step time 1.2 s, angular scanning speed 1° 2θ/min and angular range between 2° and 40° 2θ scale.

2.2.7. Thermal analysis

2.2.7.1. Differential scanning calorimetry (DSC). DSC measurements were performed using a Differential Scanning Calorimeter, Pyris 1, (PerkinElmer, USA). CBZ samples of 4–6 mg were weighed into 30 μl perforated aluminum pans and scanned from 40 to 220 °C at the heating rate of 10 and 40 °C/min and at the flow rate of nitrogen gas of 20.0 ml/min. Baseline runs were performed by scanning empty perforated Al pans in the temperature range between 40 and 250 °C in order to test the thermal behavior of the measuring system itself and the degree and influence of unavoidable asymmetries. DSC measurements were performed in triplicate.

2.2.7.2. Thermogravimetric analysis (TGA). Thermogravimetric analyses were conducted on the Pyris TGA 6 (PerkinElmer, USA). Samples of 8–10 mg were weighed into sample pans and scanned

from 40 to 220 °C at the heating rate of 10 °C/min with nitrogen gas flow rate of 100.0 ml/min. Loss of mass was registered on instruments microbalance. TGA measurements were conducted in triplicate.

2.2.7.3. Hot stage microscopy (HSM). Physical changes in the samples during heating were monitored by hot stage microscopy. Lynkam THMS 600 (Lynkam, United Kingdom) was used as a hostage unit. A small amount of each sample was placed on a glass slide and scanned at the same heating rate and the same temperature range as used for DSC measurements, (see Section 2.2.7.1). The changes in the samples were observed via an optical microscope Olympus BX51 (Olympus, Japan) with 10× magnification.

2.2.8. Solubility

Equilibrium solubility was determined by adding an excess amount of sample into 100.0 ml of distilled water. The samples were placed in a shaker, set at 100 rpm, and tempered by water bath (Heto, Denmark) at 37 ± 1 °C for 72 h (Murphy et al., 2002). Samples of 2.0 ml were withdrawn at the following time intervals: 5, 10, 15, 20, 25, 30, 45, 60, 90 min, 2, 4, 8, 12, 24, 36, 48 and 72 h. Subsequently they were filtered and analyzed by UV–vis spectrophotometer (Beckman, USA) at λ = 285 nm. Solubility measurements were performed in triplicate.

2.2.9. Intrinsic dissolution rate

The intrinsic dissolution rate was measured by the rotating disk method. A compact of 11 mm diameter was prepared by compacting 400.0 mg of sample using Zwick 1478 material tester (Zwick, Germany) with flat faced round punches. CBZ samples were compacted to a constant porosity of 12 ± 0.3%. Compaction, decompression and ejection speed were kept constant, 10, 25, and 10 mm/min, respectively. The punch and die were constantly lubricated before each compaction process with magnesium stearate powder. Five compacts were prepared for each examined sample and they were stored at constant conditions at room temperature and a relative humidity of 42% (using the saturated solution of K_2CO_3).

Prior to dissolution test, compacts were embedded in paraffin wax which was melted at the temperature of 70–80 °C. Only the lower punch side with a surface of 0.95 cm^2 was left to the outside to be in direct contact with dissolution medium.

Disc intrinsic dissolution was performed at 100 rpm in 400.0 ml of distilled water as dissolution medium at 37 ± 1 °C for 120 min. Additionally, in order to get a homogeneous dissolution medium, a magnetic stirrer was placed at the bottom of each vessel. Aliquots of 5.0 ml were withdrawn every minute during the first 20 min, then every 5 min during the first hour, and afterwards every 10 min during the second hour. Dissolution medium was replaced after every sampling. Concentration of CBZ in solution was measured by UV spectrophotometer DU 530 (Beckman, USA) at wavelength of 285 nm. The sink conditions were maintained during the entire dissolution experiment.

2.2.10. Preparation of CBZ formulations

Five different formulations were prepared consisting of CBZ (USP standard, CBZ A, CBZ B, CBZ P and CBZ D), Ludipress® and magnesium stearate. The formulations were mixed in blender Turbula T2C (W. Bachofen, Switzerland) for 5 min. Tablets of 350.0 mg for each formulation were produced using Presster™ compaction simulator (MCC, USA) with flat faced round punches of 10 mm diameter. The tablet press Korsch PH336 was simulated, with desired press speed of 10800 TPH (tablet per hour) and resulting dwell time of 32.0 ± 1.9 ms. The porosity of the tablet was kept constant (12.7 ± 0.2%), simulating the porosity of marketed CBZ tablets, and in respect to that the gap value was adapted in the range from 2.75 to 2.90 mm.

2.2.11. Dissolution

The dissolution test for the five CBZ formulations (CBZ, Ludipress® and magnesium stearate) was performed according to USP 31 for dissolution of carbamazepine immediate release tablets, dissolution apparatus II (USP 31, 2008). The dissolution apparatus used was VK7025 (Varian, USA). Dissolution medium was 900.0 ml of distilled water containing 1% of sodium lauryl sulfate at 37 ± 1 °C, with a rotation speed of 75 rpm during 60 min. 5.0 ml samples were withdrawn every 5 min during the dissolution, and replaced by the same amount of dissolution medium. Concentration of CBZ in solution was measured by UV spectrophotometer (PerkinElmer, USA) at wavelength of 288 nm. The weight of each tablet was used in the calculation of dissolved percentage of the drug.

3. Results and discussion

3.1. Carbamazepine anhydrous characterization

3.1.1. FTIR microspectroscopy

The band characteristics for CBZ are in the range of 3490–3460 cm^{-1} where NH valence vibration occurs, then in the range of 1700–1680 cm^{-1} where –CO–R vibration is pronounced, and in the range around 1600 cm^{-1} absorption bands are happening due to –C=C– and –C=O vibration, and –N–H deformation. These are also the main regions for identifying and distinguishing among different polymorphs of CBZ by IR spectrum. Generally, there is a shift of band position to higher wave numbers starting from polymorphic form III to form I, respectively. In particular the bands at 3464, 1676 and 1383 cm^{-1} of form III, are shifted to the bands 3473, 1673 and 1393 cm^{-1} in the case of form II and to 3484,1684 and 1397 cm^{-1} for form I (Rustichelli et al., 2000). Intensity of the peaks and peaks at any additional band are valuable information for the polymorphic characterization. In the case of polymorphic form III the peak at 1471 is less intense than the peak at 1245, while for polymorphic form I these two peaks have the same intensity.

The FTIR spectra of anhydrous CBZ A, CBZ B, CBZ P corresponded to the spectra of polymorphic form III showing the characteristic absorptions at 3463–3462, 1673–1672, 1604, 1593–1592, 1379–1378, 1269, 1244 and 851 cm^{-1} (Grzesiak et al., 2003; Krahn and Mielck, 1987; Rustichelli et al., 2000). CBZ USP standard was also analyzed by FTIR microspectroscopy, and found to be polymorphic form III. There was no variation in the FTIR results of the commercial samples in comparison to the FTIR spectrum of CBZ USP standard.

3.1.2. Synthetic pathways

Crystallization is widely used in pharmaceutical and chemical industries for purification of the final crystal form or for particle size control. In the crystallization process it is sometimes necessary to use mixture of solvents in order to reduce the solubility of the solute or to change the dependence of the solubility on temperature, to increase the yield of cooling crystallization. However, the concentration of the second solvent used in that process can affect polymorphism, pseudopolymorphism and morphology of the crystalline product. Consequently, production of the pharmaceutical solid products has to be optimized in terms of final solid form, crystal habit and size distribution, final yield and batch to batch reproducibility (Qu et al., 2006). Crystal properties such as polymorphism, habit, and particle size also can be changed by using different crystallization techniques. The nature and the extent of these changes depend on the crystallization conditions such as presence of impurities, and cooling rate (Nokhodochi et al., 2004). Crystal shape and crystal disruption formed during or after the

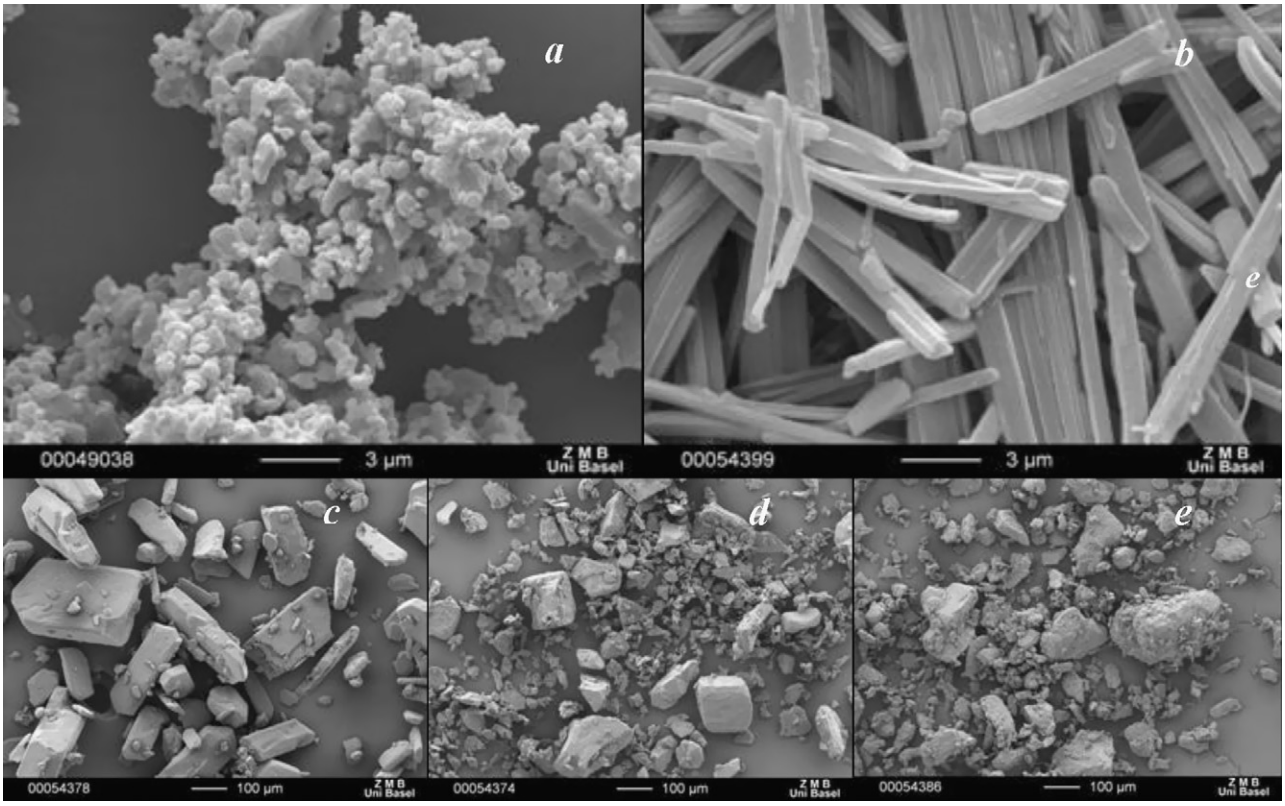

Fig. 1. Scanning electron micrographs of CBZ USP-a, USP dihydrate-b, CBZ A-c, CBZ B-d and CBZ P-e (100×).

crystallization process are important for the processability of raw materials and therefore, for the efficacy and performance of the final solid dosage form.

As declared in the open part of the carbamazepine's Drug Master Files (DMF), different processes were employed for the manufacturing of CBZ A, CBZ B and CBZ P. The crystallization process took different time periods and in some cases grinding was used as final step, for which is known that it can produce some amorphous parts of CBZ (Murphy et al., 2002), while in the recrystallization process different solvents were used, such as methanol/acetone in case of CBZ A, toluene/ethanol in case of CBZ B and toluene/methanol in case of CBZ P. All of these factors could cause variations in the examined samples.

3.1.3. Morphology and particle size distribution (SEM and PSA)

CBZ USP is a chemically pure standard exhibiting very small particles of prismatic shape, while the dihydrate made from the USP standard, USP Dihydrate exhibited the needle-like formation typical for CBZ dihydrate. The scanning electron micrograph pictures of USP standard and dihydrate are presented in Fig. 1(a and b).

Although all commercial anhydrous CBZ samples appeared to be prismatic (corresponding to the polymorphic form III), particle shape varied among them. CBZ A crystals were more elongated and of tabular shape, whereas CBZ B and CBZ P crystals appeared as polyhedrons or prisms with different sizes of particles (Fig. 1c–e). Since solvent type and crystallization conditions have a significant effect on CBZ crystal modification (Bolourtchian et al., 2001), the variations in the morphology of the commercial samples were probably caused by the type of solvent used in the second stage of CBZ manufacturing, i.e. acetone for CBZ A, and alcohols for CBZ B and CBZ P. In the case of CBZ P where the last step in the production of raw material involved grinding, ruptures on the crystal were noticed. Grinding, in particular prolonged grinding can influence the polymorphism of the primary material, and can promote a formation of amorphous parts of CBZ.

A reproducible PSA technique was developed based on dry method in order to characterize the size distribution. The results are presented in Table 1 from which it can be seen that the commercial samples differed in sizes, the CBZ B samples having the smallest particles and the lowest standard deviation within its batches.

3.1.4. X-ray powder diffraction (XRPD)

As every crystalline solid phase has a unique XRPD pattern, this technique is very powerful for identification of crystalline solid states. It is the most reliable method to distinguish the four polymorphic forms of CBZ and its dihydrate.

The anhydrous CBZ samples showed patterns corresponding to polymorphic form III reported in the literature (Krahn and Mielck, 1987; Grzesiak et al., 2003), see Fig. 2.

The absence of characteristic peaks of form I and II in the range between 5 and 10° of 2θ excluded the possibility of having these two forms present in the commercial samples. Patterns of form

Table 1
Particle size distribution for anhydrous CBZ.

Sample ($n=5$)	d (0.1) µm	d(0.5) µm	d(0.9) µm
CBZ A1	103.67 ± 3.08	307.52 ± 7.93	741.83 ± 17.18
CBZ A2	105.89 ± 2.33	327.86 ± 8.19	821.88 ± 27.23
CBZ A3	93.33 ± 4.06	299.43 ± 11.22	722.13 ± 23.03
CBZ B1	36.29 ± 0.45	174.20 ± 0.46	362.79 ± 2.54
CBZ B2	44.79 ± 0.91	191.37 ± 1.64	384.65 ± 3.65
CBZ B3	43.76 ± 1.16	196.69 ± 2.30	396.22 ± 4.30
CBZ P1	95.93 ± 6.26	377.53 ± 20.44	778.71 ± 29.75
CBZ P2	65.20 ± 2.28	295.83 ± 10.40	668.31 ± 11.51
CBZ P3	79.48 ± 3.18	375.24 ± 10.60	720.19 ± 13.26

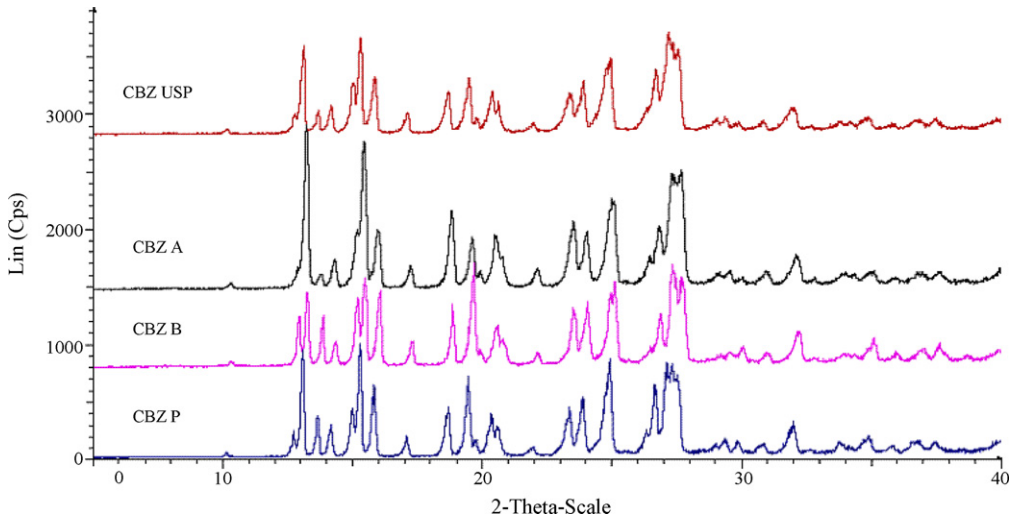

Fig. 2. X-ray diffractograms of CBZ USP, CBZ A, B and P samples.

IV reported by Grzesiak et al. (2003) and Rustichelli et al. (2000)) were not detected, which led to the conclusion that there was no mixture of polymorphs present in the commercial samples or at least not in the detectable quantities. However, the relative height (intensity) of some peaks (e.g. peaks around 13–17° of 2θ) varied between different samples. This discrepancy may be attributed to the differences in crystal sizes and habits. Differences in the peak intensity may also be caused by the crystal lattice disruption formed during the manufacturing of CBZ samples, especially in the case when milling or grinding step was performed (CBZ P).

3.1.5. Thermal behavior (DSC, HSM, TGA)

The DSC thermogram of CBZ USP obtained at 10 °C/min showed characteristic behavior of CBZ form III: melting of form III at 176.6 °C, followed by exothermic crystallization of form I (solid–solid transformation of form III to form I) and then a sharp melting endotherm of form I at 191.9 °C with enthalpy of ΔH = 107.3 J/g. These results agreed with literature data (Krahn and Mielck, 1987; Rustichelli et al., 2000; Grzesiak et al., 2003).

Although all analyzed samples were shown to exhibit the same polymorphic form, differences in the thermal behavior and deviation from the CBZ USP standard were observed in some of them. CBZ A and CBZ B exhibited melting endotherms similar as CBZ USP with transition peaks at 178.8 and 176.3 °C, respectively, the melting of form I at 194.9 and 192.9 °C, respectively and the melting enthalpies ΔH = 106.4 J/g and ΔH = 107.3 J/g, respectively. These results confirmed that samples A and B are polymorphic form III of CBZ. However, the sample CBZ P exhibited thermal characteristic atypical of CBZ USP standard (see Table 2). In the DSC thermograms of all CBZ P samples an additional endothermic peak can be seen at 164.6 °C with enthalpy of ΔH = 11.3 J/g. This was followed by a melting/recrystallization event at 184.6 °C, and subsequently by the melting endotherm of form I at 192.9 °C with an enthalpy of ΔH = 109.0 J/g, see Table 2 and Fig. 3.

To study the effect of heating rate, DSC measurements were conducted additionally at 40 °C/min (Rustichelli et al., 2000) where the thermograms of samples CBZ A, B and P showed some differences as well. The melting of form III and form I were, as expected, shifted to higher temperatures. CBZ A samples exhibited the highest enthalpy for III to I transition and the lowest enthalpy of fusion of form I. The enthalpy of III to I transition for CBZ B increased, but enthalpy of melting of form I was almost the same as with the heating rate of 10 °C/min. Thus, changing of heating rate had no influence on melting of form I in case of CBZ B. The additional peak observed in the thermogram of CBZ P at heating rate of 10 °C/min, was shifted from 164.4 to 177.1 °C and with the higher enthalpy (13.9 J/g). The melting event was shifted to 187.6 °C and had lowest enthalpy of fusion among all CBZ samples. The results of the thermal analysis at 40 °C/min are presented in Table 2.

Comparing the enthalpies obtained at the two applied heating rates, it can be seen that there was an increase of enthalpy of melting of form III with increase in heating rate. Additionally, the variations in obtained values among samples were found to be significant. For example enthalpy of fusion of form III at 40 °C/min for CBZ USP was double than at lower heating rate; for CBZ A was increased almost three times and obtained value corresponded to enthalpy of form I recorded at 10 °C/min; for CBZ B increased (from 5.12 to 47.38 J/g), while in case of CBZ P there was only slight differences between two enthalpies.

At both heating rates used in this study, the samples of CBZ A and to large extent the samples of CBZ B exhibited similar behavior as the CBZ USP standard. On the other hand, the behavior of the samples of CBZ P at both 10 and 40 °C/min differed from that of the USP standard.

To explain the atypical thermal events seen on the DSC thermograms of CBZ P the following mechanisms are considered. One explanation for the additional endothermal peak in case of all CBZ P samples is sublimation of CBZ which is an endothermic reaction

Table 2
Transition temperatures, melting enthalpy of CBZ USP, A, B and P at 10 and 40 °C/min.

Sample (n = 3)	Solid-solid trans formation form III ? Form I ± SD (°C)		Melting point of form I ± SD (°C)		Enthalpy ± SD (J/g)	
	10 °C/min	40 °C/min	10 °C/min	40 °C/min	10 °C/min	40 °C/min
CBZ USP	176.57 ± 0.12	177.75 ± 0.35	191.88 ± 0.23	194.95 ± 0.19	107.32 ± 1.13	91.26 ± 2.35
CBZ A	178.16 ± 1.37	179.62 ± 0.38	194.98 ± 1.46	195.31 ± 0.18	106.47 ± 2.52	68.34 ± 2.94
CBZ B	176.37 ± 0.21	17923 ± 0.14	192.95 ± 0.36	195.59 ± 0.16	107.32 ± 2.13	110.83 ± 2.48
CBZ P	184.63 ± 0.23*	187.60 ± 0.49*	192.98 ± 0.30	195.64 ± 0.37	109.01 ± 2.169	97.59 ± 8.42

* Atypical event.

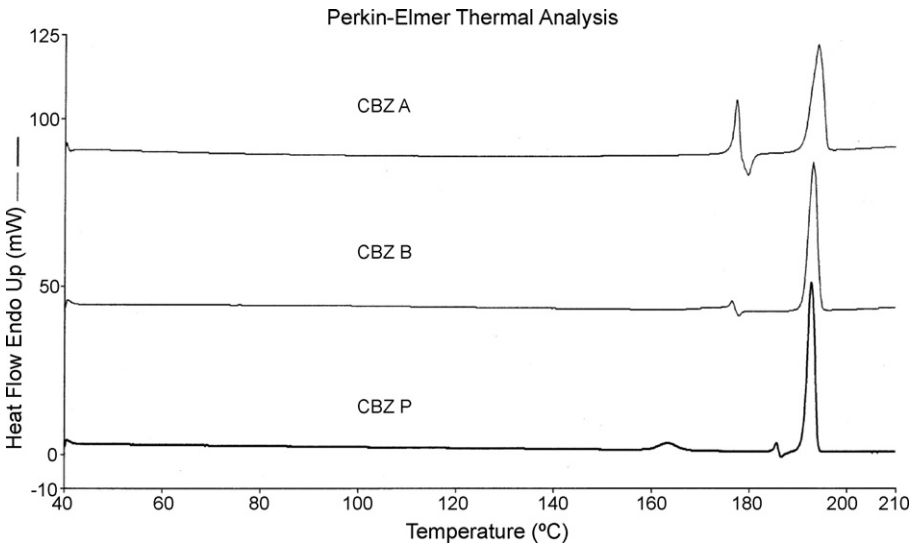

Fig. 3. DSC thermograms of CBZ A, B and P at heating rate of 10 °C/min.

that occurs around 150 °C. But since all measurements where done with the same type of pan (open pan), it is not clear why it was not registered with the other samples. On the other hand, sublimation (which can be observed with the hot stage microscope) results in a loss of compound and it would be possible to record it by TGA.

The possibility of presence of small amount of form II can be considered as well because the form II exhibits the thermal reaction between 140 and 160 °C as reported by Krahn and Mielck (1987). However, according to Grzesiak et al. (2003), Umeda et al. (1984) and Kaneniwa et al. (1984), solid–solid transition in the case of monotropic pair of polymorphs is exothermal. Other possibilities are to have a small solvent inclusion (entrapped solvent) in the crystal pores, which may play a role in the crystal stability or to have presence of amorphous material, created by grinding during the manufacturing of the raw material. Amorphous CBZ would exhibit crystallization around 80 °C (glass transition and then crystallization), and this could be detected by TGA.

The DSC results of anhydrous forms were confirmed by the visual images obtained with HSM operated at the same heating rate as the one used for DSC. The examination by optical microscope revealed, in agreement with previous results from XRPD and PSA tests, the presence of prismatic particles of CBZ in all analyzed samples and the difference in particle size. The prisms appeared brightly colored under polarized light.

During the heating of CBZ USP at 10 °C/min heating rate, the melting of the form III could be observed around 176 °C followed by formation of the needle-like particles of form I which then melted around 192 °C. The findings are in agreement with literature data (Rustichelli et al., 2000). CBZ is known to undergo sublimation in the temperature range of 145–155 °C which was noticed in all samples as formation of small crystals at the upper lid of the THSM unit.

The anhydrous samples CBZ A exhibited analog behavior as CZB USP standard. With increase in the temperature, at 176–178 °C melting of form III particles began, and around 179 °C some needles started to spread out from the edges of the prisms. The transformation into needles was completed at 182 °C. Subsequently, these crystals melted in the 189–192 °C range, according to the endothermic event previously registered by DSC. A very similar situation was observed analyzing the sample CBZ B. During heating, the smallest particles disappeared, turning into needles at temperatures higher than 175 °C. Finally, the fusion of the crystals occurred at 193–195 °C. CBZ P samples on the other hand showed discrepancies from the other samples. At temperature of 149 °C there were slight changes in the intensity of the color of the samples. Starting from 160 °C, the formation of needle-shaped crystals could be observed, followed by intense needle formation at 184–188 °C and their melting between 192 and 194 °C.

TGA in this study was used in combination with DSC measurements for better understanding of the thermal behavior of the carbamazepine samples. No sharp drop of TGA curve, characteristic for desolvation or decomposition, was recorded. In the case of all examined samples ΔY was in the range between 0.169% and 0.214%. In comparison to the TGA findings, the water content in the CBZ samples determined by the loss on drying tests was slightly lower, i.e. between 0.1% and 0.14%. This small variation in the values can be explained as volatile impurities. It can be concluded, that no substantial weight loss or glass transitions were evident from the TGA data (Sehic, 2008). Therefore, the atypical DSC thermograms of CBZ P can be a result of partial melting of the CBZ crystals at lower temperature, i.e. around 160 °C. Stressed and aged CBZ crystals have been reported to exhibit endothermal event at lower temperatures, than the ones characteristic of form III (Krahn and Mielck, 1989). Higher density of crystal defects and mechanical stress caused by grinding could have caused the atypical melting of CBZ P. The formation of needle-like crystals (corresponding to a metastable polymorph form of CBZ) was observed in the melt by HSM but no exothermic effect was seen by DSC (this can be due to the dispersion of the small crystals in the melt and to the high rate of heating). As the temperature rises the newly formed crystals melt together with the remaining form III crystals pushing the melting event to higher temperature than that of the melting of pure form III. The form I then crystallize from the melt and finally melt at the temperature characteristic for form I. And because the observed phenomena took place at elevated temperature, they could not be detected by XRPD which is conducted at room temperature. However, the performance of the commercial product under normal room conditions (i.e. its solubility and dissolution behavior) will be affected by variability in the primary material caused by the variations of the processes used for its manufacturing.

3.1.6. Solubility

The equilibrium solubility test was carried out by shake agitation method until the equilibrium concentration was achieved. Since carbamazepine anhydrous is known to undergo a phase transformation in aqueous solutions, after 72 h, only CBZ dihydrate form was present in solution. The solubility of the metastable form is very

Table 3
Results for solubility and IDR of anhydrous CBZs.

Sample anhydrous ($n = 3$)	Solubility estimated (μg/ml) ± SD	Solubility measured (μg/ml) ± SD	IDR (μg/min/cm^2) ± SD	Sample dihydrate ($n = 3$)	Solubility measured ± SD (μg/ml)	IDR (μg/min/cm^2) ± SD
A	491.1 ± 7.70	277.4 ± 4.10	69.11 ± 1.96	DA	283.3 ± 3.0	23.09 ± 0.40
B	507.8 ± 26.1	284.9 ± 3.90	77.45 ± 2.04	DB	274.2 ± 1.5	22.43 ± 0.63
P	497.4 ± 48.2	283.7 ± 11.2	73.92 ± 2.00	FP	275.7 ± 2.9	22.52 ± 0.20

difficult to determine in anhydrate/hydrate systems, since transformation happens quickly in most of the cases.

During solubility measurement of anhydrous CBZ, samples were withdrawn in predefined time intervals to compare the kinetics of solubility in water of different CBZ samples. The shortest time to reach the maximum amount of dissolved anhydrous carbamazepine was found to be 30 min for CBZ USP standard, due to the very small particle size. In the samples CBZ A and CBZ P, the maximum quantity dissolved was observed after 12 h, while in the CBZ B samples which showed the highest amount of dissolved material, the maximum dissolved was reached only after 36 h.

Calculations of solubility of anhydrous CBZ were made as well using the results obtained by the disc intrinsic dissolution rate (DIDR) tests, whereby only the initial part of the curve was used for the calculations. The estimated solubilities had higher values than the measured ones, because during the equilibrium method after 72 h CBZ was almost completely converted to dihydrate form which has lower solubility (see Table 3).

ANOVA tests were performed to evaluate batch to batch variation (variation within the same sources of CBZ) and variation between the different samples. Results within one group of samples did not show significant batch to batch variation. However, comparing the solubility results between CBZ A, B, P and USP, differences were found. CBZ P samples exhibited the highest variation from CBZ USP, but that discrepancy was statistically significant (confidence interval $\alpha = 0.05$, $p < 0.1$).

3.1.7. Disc intrinsic dissolution behavior

During dissolution in aqueous medium, anhydrous CBZ undergoes transformation to CBZ dihydrate. The kinetics of anhydrous CBZ to dihydrate (CBZ D) transformation in water was investigated by Murphy et al. (2002) and it was shown that the transformation process is solution-mediated and the kinetics and the rate-controlling step of this transformation depend on the processing and storage conditions of anhydrous CBZ. A solution-mediated transformation involves three processes: dissolution of metastable form (CBZ A); nucleation of stable form (CBZ D) and crystal growth. Grinding of CBZ A shortens the transformation time and changes the rate-limiting step from crystallization of CBZ D form to dissolution of the CBZ A. The kinetics were shown to depend on the prehistory of the anhydrous form (for example, grinding can shorten the transformation time and change the rate-limiting step as a result of amorphousness, which significantly increases the crystallization rate of CBZ D). Surfactants such as sodium lauryl sulfate (SLS) when added to the dissolution medium can promote the surface-mediated nucleation of CBZ D on CBZ A crystals at the concentration below critical micellar concentration (CMC) and (can promote) the transformation by increasing the bulk nucleation of CBZ D above CMC (Rodriguez-Hornedo and Murphy, 2004).

DIDR measurements are used mainly to determine the intrinsic dissolution rate constant, which is expressed in mg/cm^2/min and values below 1.0 mg/cm^2/min indicate dissolution rate-limiting absorption (Hanson and Gray, 2004). DIDR is a rate phenomenon and as such correlates more closely with in vivo dissolution behavior than the solubility, which is an equilibrium phenomenon. It was even proposed (Yu et al., 2004) that DIDR be used for drug classification instead of solubility (the latter being the basis of the biopharmaceutics classification system or BCS). Moreover, actual solubility may not "capture" changes in polymorphs, etc. during the solubility experiments. DIDR on the other hand can be used to determine drug solubility before the changes actually occur. This is in accordance with the aim of the CBZ study, since CBZ dissolution is a solution-mediated phase transformation from anhydrous form to dihydrate form and the polymorphism-related variations in the primary material can affect the dissolution mechanism. The kinetics of the solution-mediated process of the commercial samples was determined from the dissolution profiles of CBZ obtained from disk intrinsic dissolution rate tests.

The CBZ samples used in this study varied in their particle size, particle shape and bulk density. As it was impossible to obtain compacts with less than 12% porosity, the further investigations were carried out with constant porosity compacts (12 ± 0.3%) by varying only the compaction force in the range between 8 and 12 kN (10 kN for CBZ A and 8 kN for CBZ B and CBZ P samples). By performing disc intrinsic dissolution test, it should be possible to exclude the influence of particle size on the dissolution. However, preliminary tests showed that tablettability of the CBZ samples (and thus appearance of the surface of the compacts) were affected by the difference in particle size and morphology. In order to minimize the problem during compaction, the CBZ A, B and P samples were sieved into fractions with the same particle sizes. The fraction of particle size from 250 to 355 μm was selected for the further experiments, because it was the most representative one of all samples.

The slope of the intrinsic dissolution curve is usually linear, but that was not the case for carbamazepine because anhydrous CBZ was shown to undergo a phase transformation into the dihydrate form during the test (Kobayashi et al., 2000; Murphy et al., 2002; Ono et al., 2002). This phase transformation is indicated by the change of slope in the profile, which means that during the intrinsic dissolution, the crystallization of CBZ dihydrate occurs at the surface of tablets resulting in a decrease of intrinsic dissolution rate of CBZ anhydrous.

The disc intrinsic dissolution profiles of the CBZ anhydrous materials showed the above mentioned typical behavior. Nevertheless some "jumps" on the dissolution curve were observed following the transformation period. That could be explained by the fact that in some regions of the compacts the transformation was not completed, and the "jumps" were probably created when some newly exposed regions of anhydrous CBZ began to dissolve.

The surfaces of CBZ compacts were analyzed before and after intrinsic dissolution testing by SEM and the pictures are presented in Fig. 4. It can be observed that the compacts prepared from CBZ A had flatter surfaces, than those of samples B and P, the latter being rougher, with gaps which could eventually increase the starting area of compacts. Some discrepancies were found also by analyzing the pictures of the compacts after the intrinsic dissolution. These variations resulted from the conversion to dihydrate form which varied among samples, i.e. the change in the dissolution slope occurred at different time periods. For some samples (i.e. CBZ P) the transition was not fully completed even after 120 min. Therefore, based on the observation of the SEM pictures, the conversion of anhydrous to dihydrate form of CBZ A was completed after 120 min

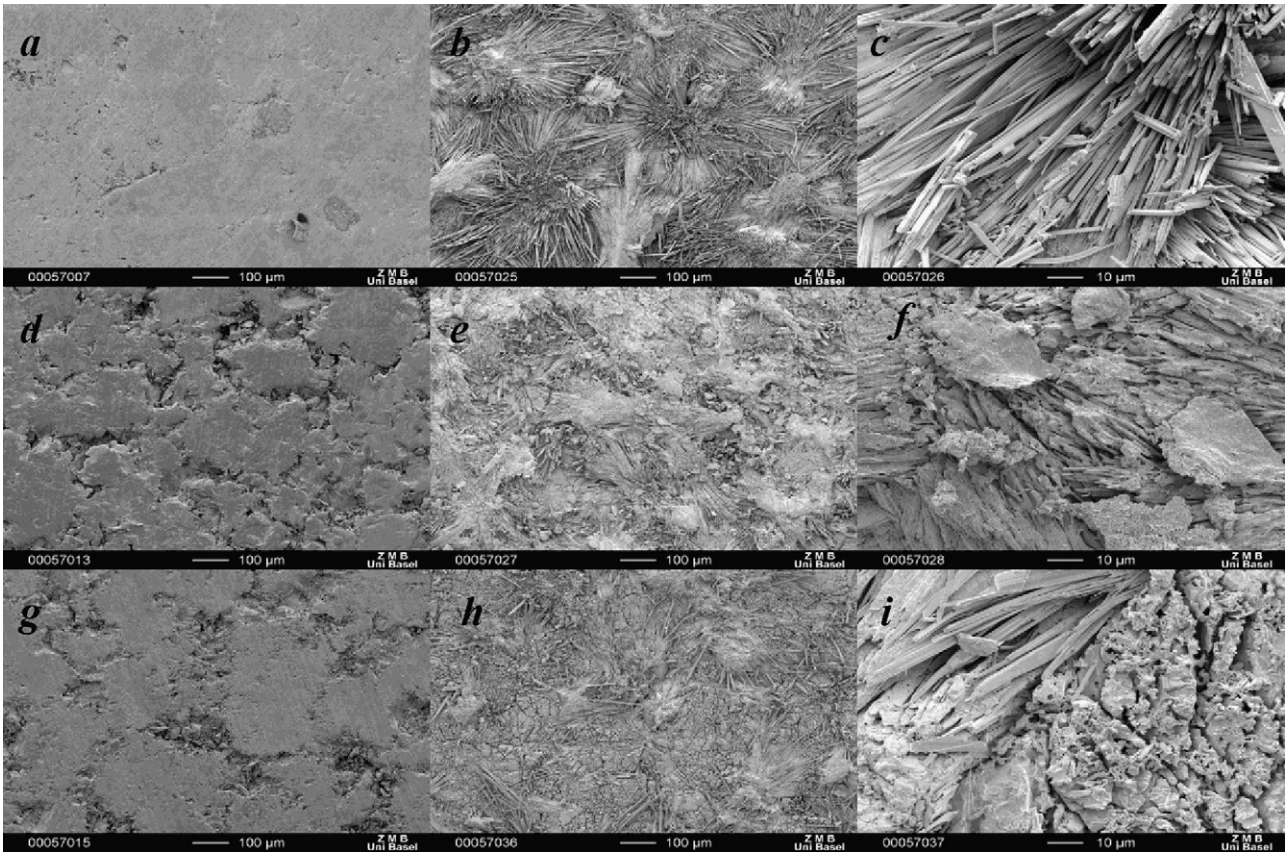

Fig. 4. Surface of compacts before (CBZ A-a,b, CBZ B-d,e, CBZ P-g,h) and after disc intrinsic dissolution (CBZ A-c, CBZ B-f, CBZ P-i).

of dissolution (as confirmed by needle-like formations which were covering the surface of the compacts). In the case of CBZ P, after 120 min of dissolution, besides the needle-like formations, some prismatic particles were still present.

One-way ANOVA was used to test if the differences in the intrinsic dissolution profiles and/or in the conversion kinetics within each CBZ of same producers were statistically significant. According to the results obtained, no significant difference was found in the intrinsic dissolution behavior among the batches of samples A and batches of samples B, while in the CBZ P batches, the difference was statistically significant (for confidence interval $\alpha = 0.05$, $p < 0.1$).

One-way ANOVA test was also used for the comparison between the CBZ A, CBZ B and CBZ P samples for intrinsic dissolution after 20 and 120 min. The time interval of 20 min was selected because the transition from anhydrous to dihydrate CBZ normally would take place within the first 20 min of intrinsic dissolution. Therefore, it is important to determine the variations in the intrinsic dissolution behavior of the commercial CBZ samples during that particular time interval. It was shown that the intrinsic dissolution behavior for the initial 20 min was different, but the differences were not statistically significant when the intrinsic dissolution profiles of CBZ A, B and P were compared over the period of 120 min. This leads to the conclusion that most probably the main differences between CBZ A, B and P are expressed in the initial part of dissolution, i.e. where anhydrous–dihydrate conversion occurs.

The variability can be attributed to variations in the manufacturing process most likely resulting from different solvent systems used and different drying and/or grinding regimes applied in the final stage of the manufacturing all of which could influence the final shape and size of the CBZ crystals. Moreover, crystal defects and ruptures could also lead to differences in the conversion kinetics of CBZ anhydrous to CBZ dihydrate.

As mentioned previously the intrinsic dissolution rate can become complicated when one or more of the studied polymorphs interconvert to another polymorph during the time of the measurement. If one of the polymorphic forms is hydrate, the dissolution rate of the anhydrous phase normally exceeds that of any corresponding hydrate phase.

This phenomenon is observed for carbamazepine which undergoes phase transformation during dissolution and intrinsic dissolution. Unlike the linear intrinsic dissolution profiles of many substances, CBZ exhibited inflection in the curve, resulting in two distinct slopes: the initial slope, which describes dissolution of anhydrous phase and the final slope, which represents the dissolution of dihydrate phase. The inflection point between these two segments of the intrinsic dissolution curve is defined as the transition point of the two phases.

Segmentation of the DIDR curves of the different samples into initial and final segments was obtained by splitting the data in such a way that the linear regression lines for each segment resulted in the best correlation coefficient. The slopes of each segment and the intercept of the final segment for each sample were obtained from statistical analysis using nonlinear model with Systat software (Systat Software Inc., USA). The segmented graphs and the average curves of each sample are presented in Fig. 5a from which the transition points and the difference in the final slope are observed.

It can be seen that the times required for conversion of anhydrous CBZ to dihydrate differed in each group of carbamazepines (around 20 min, around 15 min and between 20 and 25 min for CBZ A, CBZ B and CBZ P respectively). According to literature data, the transformation occurs after 5–10 min of intrinsic dissolution test

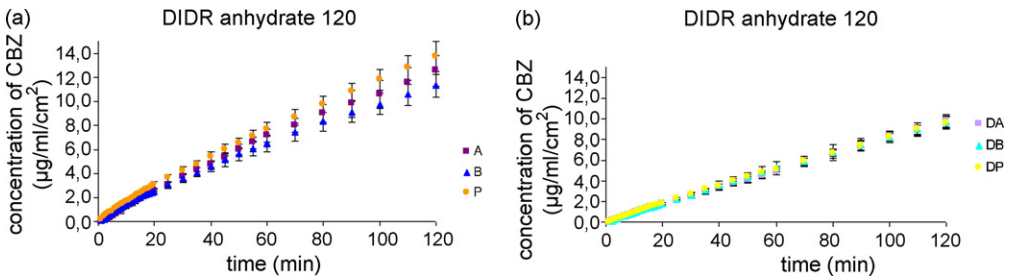

Fig. 5. Intrinsic dissolution profiles of CBZ A, B and P –*a* and dihydrates CBZ DA, DB and DP–*b* with standard deviations.

(Kobayashi et al., 2000; Ono et al., 2002). The discrepancies found in the present work can be explained by the different raw material used.

The segmented plots were analyzed employing the method of Nogami et al. (1969) and Ono et al. (2002) to calculate the dissolution parameters using the following equations:

$$\frac{dC}{dt} = k_t C_{SH} \quad (1)$$

$$\left(\frac{dC}{dt}\right)_{t=0} = k_t C_{SA} \quad (2)$$

$$b = \frac{k_t(C_{SA} - C_{SH})}{k_r} \quad (3)$$

where, C represents the concentration of carbamazepine in bulk solution (μg/ml), t is the time (min), k_t is the rate constant of the transport process (min), k_r is the rate constant of the phase transformation process (min), C_{SH} is the saturated concentration of dihydrate (μg/ml), C_{SA} is the saturated concentration of anhydrous form (μg/ml), b represents the intercept obtained by the extrapolation of the linear portion of the dissolution curve of the anhydrous form.

The parameters k_t, k_r and C_{SA} were calculated from the above equations. k_t or the rate constant of the transport process was calculated from the slope of the dihydrate curve (Eq. (2).) using the solubility value for the respective dihydrate C_{SH} obtained from equilibrium solubility testing. C_{SA} was calculated from Eq. (1). using the estimate of the slope of the initial segment and the k_t value was calculated from Eq. (2). The intercept of the final segment was calculated using Eq. (3). The results are presented in Table 4. Values for the final slope in the anhydrous part were lower than the initial slope (because of phase transformation). However, these values are markedly higher than those of pure dihydrates. The reason may be that even after 2 h of disc intrinsic dissolution test, there were some parts of the compact remaining in anhydrous form which increased the intrinsic dissolution rates. It is interesting to note that CBZ B, which gave the highest amount of drug released in 20 min, had a final slope relatively close to the slope of dihydrate, suggesting the most complete transformation from anhydrous to dihydrate.

The turning point on the DIDR curves (transition point), where the slope changes from dissolution of anhydrous CBZ to dissolution of dihydrate, varied from sample to sample: CBZ A ~20 min; CBZ B ~15 min; CBZ P 20 ~ 25 min. In addition to that, variations were found in the value for the constant of phase transformation process (k_r) (see Table 4). The highest k_r value means that the sample has ability to undergo the fastest transformation from anhydrous to dihydrate form, which consequently shows the highest IDR. In the study of Ono et al. (2002), the k_r value was higher than the values obtained in this study, and the reason is probably because the conversion to dihydrate happened in the first 5 min. In all examined samples, the highest k_r value was observed for CBZ B and the lowest one was observed for CBZ P samples. Considering these statements, the correlation between the intrinsic dissolution parameters and the rate of transformation is promising and may help to determine the kinetic of transformation for each examined CBZ which eventually can help to predict its behavior in the final formulation by verifying these parameters in the preformulation stage.

The intrinsic dissolution parameters were used for the calculation of estimated solubility of anhydrous CBZ (Eq. (1).). The calculated solubilities were higher than the experimentally obtained equilibrium solubilities (measured after 72 h), because during the solubility measurements transformation of CBZ anhydrous to CBZ dihydrate took place. The estimated solubilities (C_A) were ranked C_ACBZ B > C_ACBZ P > C_ACBZ A and variations within each manufacturer were maximum 10%, i.e. for CBZ B 507.8 ± 26.1 (CV = 5.1%), for CBZ P 497.4 ± 48.2 (CV = 9.6%) and for CBZ A 491.1 ± 7.7 (CV = 1.4%). The estimated solubility data were in agreement with literature data of form III (Kobayashi et al., 2000).

Intrinsic dissolution rates were calculated from the initial part of each profile using Eq. (3) (Kobayashi et al., 2000; Sethia and Squillante, 2004)

$$\text{IDR} = \frac{C}{t} \times \frac{V}{S} = kC_s \quad (4)$$

where, S is the surface area of the tablet (cm²), V is volume of test solution (ml), k is intrinsic dissolution rate constant, C_s represents solubility (mg/ml).

The obtained results have the same ranking as reported in the estimated solubility, i.e. IDR CBZ B > IDR CBZ P > IDR CBZ A, see Table 3. The values of IDR in this study were slightly higher than data for IDR of polymorphic form III found by Kobayashi et al. (2000). The differences in the intrinsic dissolution rates described by a single value, for different samples were not relatively high. However, the differences in the intrinsic dissolution behavior described by a set of parameters were found to be of great importance. Considering that carbamazepine has narrow therapeutic

Table 4
Results for dissolution parameters of anhydrous and dihydrate groups of CBZs.

Sample n = (3)	Initial slope (μg/ml min)	Final slope (μg/ml min)	Intercept (μg/ml)	k_t (per min)	k_r (per min)
A	0.141 ± 0.002	0.098 ± 0.002	0.925 ± 0.130		0.0649 ± 0.016
B	0.152 ± 0.002	0.090 ± 0.001	0.870 ± 0.084		0.0811 ± 0.008
P	0.148 ± 0.002	0.107 ± 0.001	1.137 ± 0.068		0.0583 ± 0.002
DA		0.082 ± 0.000	0.084 ± 0.014	0.00029	
DB		0.082 ± 0.001	0.126 ± 0.029	0.00030	
DP		0.082 ± 0.001	0.129 ± 0.032	0.00030	

index and very narrow dissolution range of acceptance criteria in USP monographs, the variation in the kinetics of conversion from anhydrate to dihydrate form among different raw material of CBZ represent useful information. Therefore the point of transformation as well as the kinetics of the phase transformation may be considered as critical parameters which should be investigated during the characterization of carbamazepine.

3.2. Carbamazepine dihydrate characterization

It was already seen that samples of the commercial anhydrous CBZ, although being form III, showed variations in DSC behavior, intensity of XRPD peaks, solubility (estimated) and intrinsic dissolution behavior. In particular, the variations in intrinsic dissolution behavior were similar to those found in the dissolution of some CBZ immediate tablet formulations previously investigated. Therefore it was proposed to convert the anhydrous CBZ into their respective dihydrate and to evaluate the impact of this strategy on the variability seen in commercial CBZ products. As discussed earlier variability due to previous history of the samples (e.g. manufacturing process, grinding, etc.) influenced their intrinsic dissolution behavior. Thus the question: Can the observed variability be eliminated or indeed "erased" from the previous history of anhydrous commercial products by converting them to their dihydrate form? For this purpose, dihydrates of all commercial anhydrous samples were prepared and were fully characterized prior to subjecting them to intrinsic dissolution testing.

3.2.1. X-ray powder diffraction of CBZ dihydrates

CBZ dihydrate has its own unique XRPD pattern. CBZ dihydrates prepared from the nine available samples (CBZ DA, DB, DP) had characteristic reflections at $2\theta = 8.9^\circ$, 18.9° and 19.4° which correspond to literature data for CBZ D (Carino et al., 2006; Kobayashi et al., 2000). It was therefore confirmed that CBZ dihydrate was properly formed. In addition it was also observed that the differences in the intensity of the peaks found on the X-ray diffractograms of CBZ A, B, and P disappeared after their conversion to dihydrate, see Fig. 6.

3.2.2. Loss on drying of CBZ dihydrates

Complete hydration of the anhydrous samples was confirmed by the loss on drying method. The water content of the anhydrous CBZ was $0.1 \pm 0.05\%$, whereas the dihydrate samples had a loss on drying of 13.2% for CBZ DA, 13.3% for CBZ DB and 13.1% for CBZ DP. These values are nearly equal to the stoichiometric value calculated for the water content of CBZ dihydrate (13.2%, w/w) reported in literature (McMahon et al., 1996). The weight loss is attributed to the dehydration of CBZ D, i.e. removal of lattice water (Surana et al., 2003).

3.2.3. Differential scanning calorimetry (DSC) of CBZ dihydrates

The DSC thermograms of dihydrate prepared from the USP standard (CBZ USP D) obtained at heating rate of 10 °C/min showed two endotherm peaks: a broad peak in the range of 75–87 °C representing dehydration/vaporization of water and a sharp peak at 192 °C representing melting process of CBZ with enthalpy $\Delta H = 95.8$ J/g. These findings were in good agreement with the results reported for CBZ dihydrate (Li et al., 2000; Surana et al., 2003). DSC results of the dihydrate samples prepared from all anhydrous CBZ confirmed that dihydrates were formed successfully. All of them exhibited the same behavior as CBZ USP D. Contrary to DSC results of CBZ anhydrous forms which exhibited the differences in enthalpies of melting of form I (among CBZ A, B and P) and shift of the peak for CBZ P, the DSC results of all dihydrate samples prepared from CBZ A, B and P showed no variation among each other, see Fig. 7.

In other words, differences in the thermal behavior which were detected in same polymorphic forms of CBZ samples obtained from different sources were practically deleted by converting commercial anhydrous form to dihydrates.

DSC measurements on dihydrate samples were conducted also at the heating rate of 40 °C/min as in the case of the anhydrous samples. As the heating rate was increased the dehydration process was shifted to higher temperature range. These profiles revealed that the faster the heating rate was chosen, the wider was the temperature range over which dehydration of water occurred. No significant differences between USP dihydrate and CBZ DA, DB and DP behavior were found (Sehic, 2008).

3.2.4. Solubility of CBZ dihydrates

Dihydrate of CBZ is the stable form in aqueous conditions; therefore no phase transformation during this test was expected. The solubility of dihydrates was determined by supersaturation, where an excess amount of CBZ dihydrate solid phase was added to distilled water at 37 ± 1 °C and was shaken for 72 h to equilibrium.

The obtained results are presented in Table 3 for comparison with the solubility data of anhydrous CBZ obtained previously with the same method.

One-way ANOVA analysis showed that there were no significant differences in solubility between dihydrates prepared from CBZ A, CBZ B and CBZ P. Furthermore, no differences were found within dihydrates prepared from different batches of the same producers.

It can be noted that the solubility values of dihydrates measured after 72 h were approximately equal to the values of anhydrous CBZ measured in the same time interval, except for CBZ B. The reason

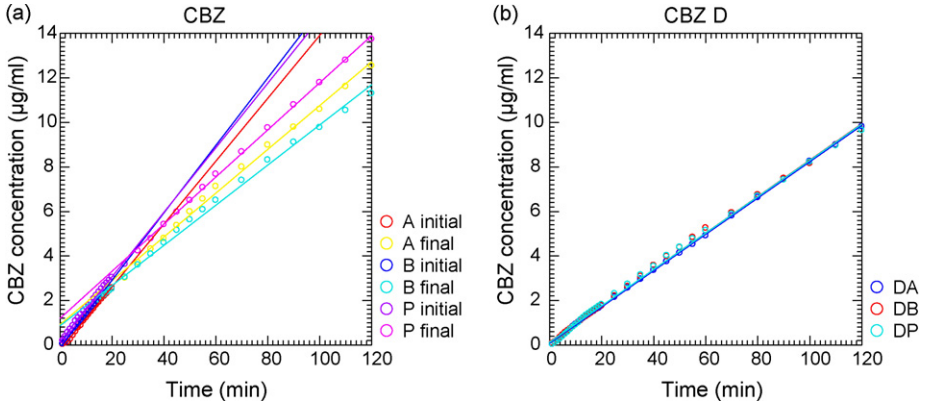

Fig. 6. Transition points of anhydrates CBZ A, B and P – *a* and dihydrates CBZ DA, DB and DP–*b* analyzed by Systat software.

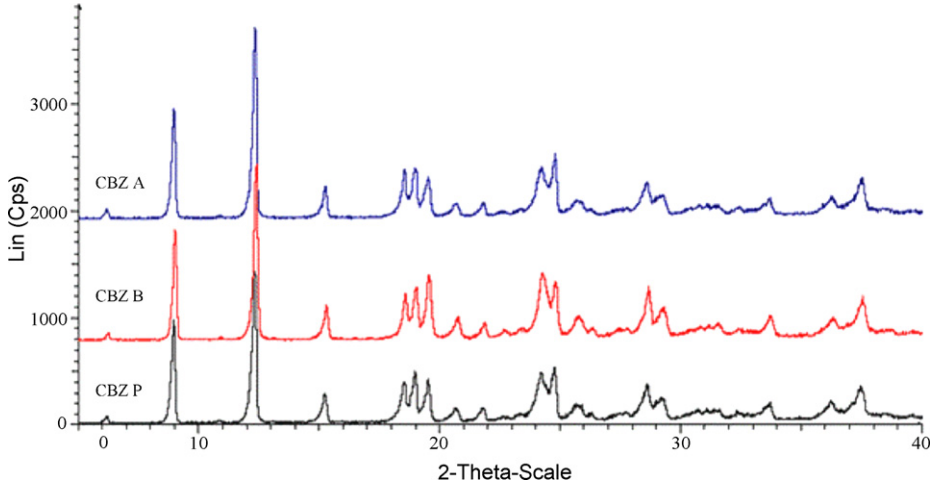

Fig. 7. X-ray diffractograms of CBZ DA, CBZ DB, CBZ DP.

for this is that during the measurement time, the anhydrous CBZ was converted completely or almost completely to dihydrate. In the case of CBZ B, the measured solubility of the anhydrate was slightly higher than in the other samples, most likely because the transformation was not yet fully completed. Therefore, for correct determination of DIDR of the anhydrous forms, the estimated solubilities of the anhydrous forms should be taken into account, not the measured ones.

Comparing the solubilities of the dihydrates with the results of the respective anhydrous forms obtained by the estimation from IDR results, those of the dihydrate are 1.7–1.8 times lower, which is in agreement with literature data (Kaneniwa et al., 1987; Kobayashi et al., 2000).

3.2.5. Disc intrinsic dissolution of CBZ dihydrates

During the preparation of compacts for the intrinsic dissolution tests of anhydrous forms, carbamazepine showed poor compactibility properties, such as capping and lamination (lamination being major problem in the case of CBZ USP samples), but after converting commercial CBZ samples to the respective dihydrate forms, their compactibility was improved. There were no problems with capping or fragmentation of the compact's edges. In order to obtain compacts with the same porosity of 12% as for the anhydrous CBZ, lower compaction force was applied (between 6 and 8 kN). Dihydrate compacts were robust, easy to handle and had much higher crushing strength than the anhydrous compacts (ranging between 120 and 135 N). The surfaces of the dihydrate compacts were analyzed by SEM before and after dissolution and their pictures are presented in Fig. 8. As a result of the improved mechanical property, the surfaces of dihydrate compacts prior to dissolution appeared smoother and more homogeneous without the gaps observed in the anhydrous compacts. The SEM analysis confirmed absence of prismatic particles (of form III) which indicated that the dihydrate was prepared properly. Regarding the surface of the compacts after dissolution, only needle-like forms (of dihydrate) were found at the end of dissolution tests (after 120 min).

Under constant hydrodynamic conditions, the intrinsic dissolution rate is usually proportional to the solubility of the dissolving solid. Consequently, in a polymorphic system, the most stable form will ordinarily exhibit the slowest intrinsic dissolution rate. It also has been noted in the earliest dissolution work (Shefter and Higuchi, 1963) that, for many substances, the dissolution rate of an anhydrous phase usually exceeds that of any corresponding

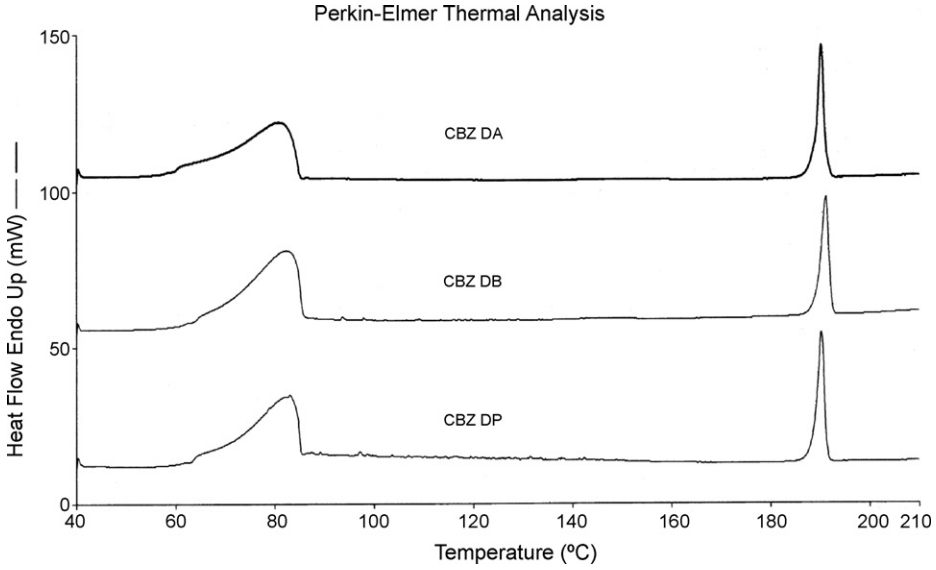

Fig. 8. DSC thermograms of CBZ DA, DB and DP at heating rate of 10 °C/min.

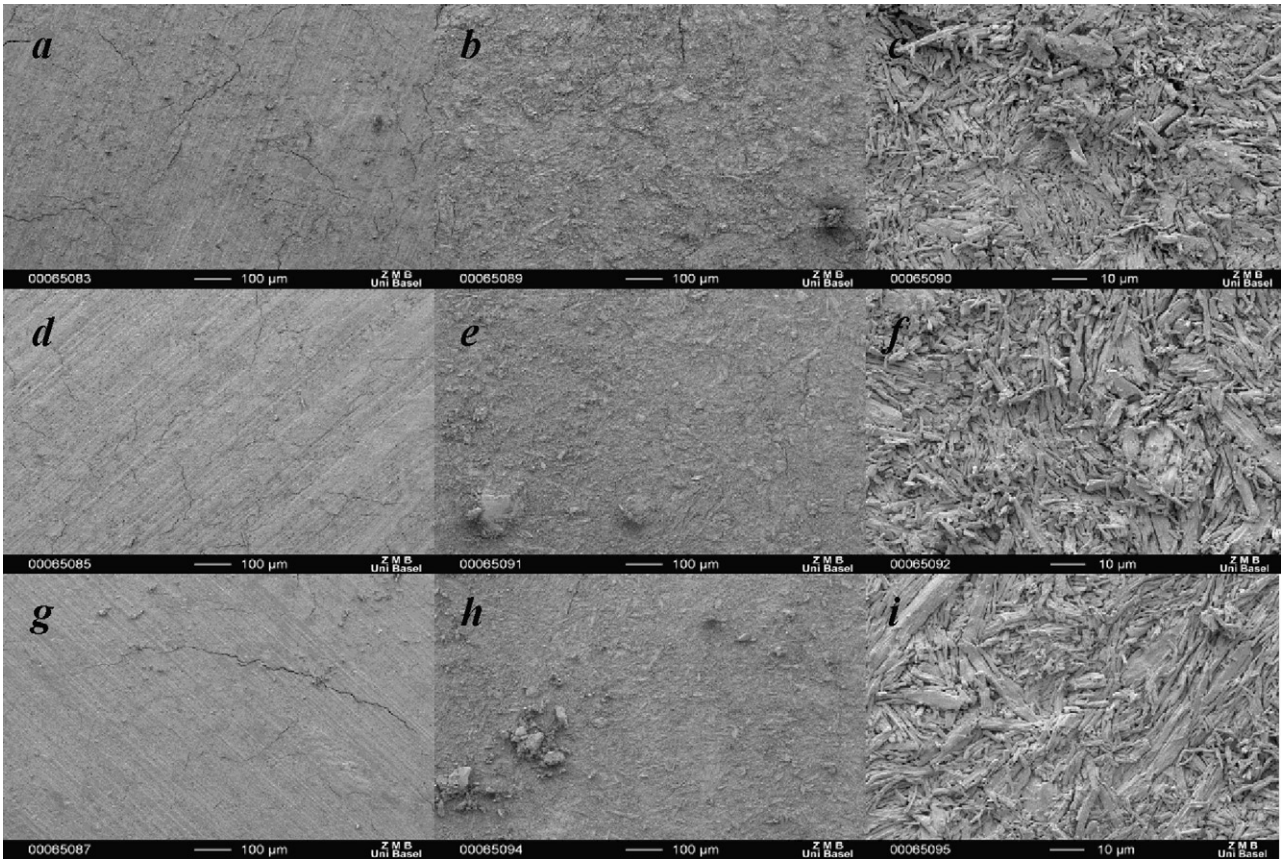

Fig. 9. Surface of compacts before (CBZ A-a,b, CBZ B-d,e, CBZ P-g,h)and after disc intrinsic dissolution (CBZ A-c, CBZ B-f, CBZ P-i).

hydrate phase. These observations were explained by thermodynamics, where it was reasoned that the drug in the hydrates possessed a lower activity and would be in a more stable state relative to its anhydrous forms. This general rule was found to hold for anhydrous/hydrate phases of carbamazepine (Brittain, 1999).

The conditions for intrinsic dissolution of dihydrates were kept the same as for the intrinsic dissolution rate measurements of anhydrous forms.

During intrinsic dissolution of dihydrates, no phase transformation is expected and therefore the dissolution profiles exhibited the characteristic linear shape without segmentation, which means that estimated and measured solubility of dihydrate should be practically identical. This is illustrated in Fig. 9b, which represents the intrinsic dissolution profiles of each group of different dihydrates (CBZ DA, DB and DP).

One-way ANOVA was used to evaluate the dissolution profiles. It was confirmed that there was no significant difference within different batches and between dihydrates of CBZ DA, DB and DP. Also the observed faster dissolution of DP2 was not found to be significantly different from other CBZ DP dihydrates.

In the previous section, it was discussed that CBZ dihydrates, as the most stable form in aqueous solution, exhibited lower solubility than the respective CBZ anhydrates.

Consequently, because of lower solubility, the amount of CBZ dissolved from the surface of the compact decreased in comparison with the amount dissolved form anhydrate CBZ surfaces, and in addition the intrinsic dissolution rate was slower.

It is well known that different polymorphic forms may show different dissolution behavior. In the case of the examined CBZ samples, differences in dissolution behavior were observed even if the samples exhibited the same polymorphic form. Moreover, high standard deviations were found within each anhydrous CBZ. By converting the commercial CBZ samples to dihydrate form, the step of phase transformation A → D, as the most problematic and critical property of CBZ was skipped. Thus, the dissolution behavior of dihydrates was found to be more uniform. Furthermore, in all examined CBZ D samples standard deviation was found to be much lower than in their respective anhydrates, therefore the dissolution behavior was considerably improved. Systat software was used to calculate intrinsic dissolution parameters, as presented in Table 4. The slopes of CBZ DA, DB and DC were 0.082 ± 0.001 and this value was used in the calculations of IDR (see Table 3 and Fig. 5b).

IDR of CBZ DA, DB and DP showed no variations, but they were slightly higher than those reported by Murphy et al. (2002). However, because of lower solubility of dihydrate form, the values were three times lower than the IDR of the anhydrous forms (Sehic, 2008).

3.3. Carbamazepine formulations

Carbamazepine presents formulation challenges due to its inherent poor compressibility and compactibility, high dose and low water solubility. The wet granulation process may be utilized to improve the flow and compression/compaction properties of the formulation. However, during wet granulation, if combination of ethanol and water is used, solution-mediated transformation of CBZ occurs (Otsuka et al., 1997; Otsuka et al., 1999) and the amount of dihydrate formed during this process may be appreciable. During the drying process, the dihydrate may convert back to initial form III, or eventually may convert to form I, thus a mixture of form I and III is created and that will influence the dissolution process of the final product. If only aqueous solution is used, the process has to be carefully controlled to insure that phase transformation does not occur. Therefore CBZ is a good example to illustrate how a wrong

Table 5
Settled gap in Presster™, obtained compaction force, porosity of tablets, crushing strength and disintegration time.

Sample ($n=6$)	Gap (mm)	Compaction (kN)	Thickness (mm)	Porosity (%)	Crushing strength* (N)	Disintegration time** (s)
Formulation USP	2.85	9.5–10.9	3.62 ± 0.02	12.55 ± 0.62	90 ± 10	77 ± 15
Formulation A	2.75	11.4–12.4	3.57 ± 0.01	12.71 ± 0.47	105 ± 5	14 ± 3
Formulation B	2.85	9.6–9.8	3.59 ± 0.01	12.78 ± 0.29	79 ± 3	24 ± 4
Formulation P	2.85	9.3–9.9	3.59 ± 0.02	12.75 ± 0.41	77 ± 1	24 ± 6
Formulation D	2.90	8.7–10.1	3.64 ± 0.03	12.51 ± 0.75	132 ± 4	110 ± 20

* $n=3$.
** $n=6$.

choice of the manufacturing process may lead to an increased phase transformation, processing difficulties and problems with dissolution.

In order to avoid the possible phase transformation of CBZ, direct compaction was chosen for this study as process for producing CBZ tablets using a co-processed excipient for direct compaction, Ludipress®.

During the preparation of tablets, porosity was kept constant. The only parameter set on Presster™ was the gap, and it is presented for each formulation, together with the measured compaction force, thickness of the tablets and calculated porosity in Table 5.

The formulation D had the highest hardness of the tablets (132 ± 4 N), due to good tabletting properties of CBZ dihydrate. Regarding the commercial samples, the highest crushing strength of 105 ± 5 N was measured in the formulation A while for formulations B and P the crushing strengths were of 79 ± 3 N and 77 ± 1 N, respectively. These differences are most probably caused by the differences in the morphological properties of commercial CBZ samples (see Table 5). On the other hand, disintegration times were longer for formulation B and P (24 ± 4 s and 24 ± 6 s, respectively) than for formulation A (14 ± 3 s), although it was expected that the harder tablet will need more time to disintegrate.

Dissolution of CBZ formulations was conducted according to USP monograph for the CBZ immediate release tablets (USP 31, 2008). Six tablets were examined for all formulations. The requirements for the time and tolerance for CBZ drug release in USP pharmacopoeia are the following:

45–75% of drug has to be dissolved in the first 15 min.
>75% of drug has to be dissolved in 60 min.

The first range is the critical one and a lot of previously investigated CBZ formulations failed to meet this requirement during the dissolution test.

According to USP monograph for CBZ, immediate release tablets will meet requirements if the dissolved amount of drug agrees with the interpretations specified in the Acceptance Table 2 in USP pharmacopoeia.

In the first 15 min all six examined tablets of formulation A met the USP requirements (dissolved amount was in the range of 67.5–74.7%, see requirements above). It may be emphasized that two tablets almost reached the upper limit (by dissolving 73.15% and 74.67% of drug). The variation (variation coefficient) was at maximum 4.58%. After 60 min, the percentage of dissolved CBZ was 94.9–100.0%.

Formulation B did not pass the first criteria of acceptance of dissolution, because more than 75% of drug was released. Actually, in the first 15 min dissolved carbamazepine was in the range between 70.7% and 79.2%, and two of six examined tablets which failed the requirement dissolved between 78.61% and 79.19%. If dissolution was continued in the next step, considering the L2 limit, the results would probably meet the requirement (because the variation was lower than 5% allowed). Variation coefficient during the dissolution was at maximum of 5.61%.

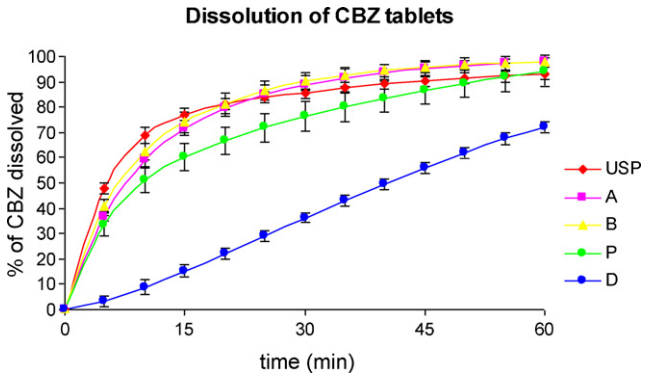

Fig. 10. Dissolution profiles of modell formulations.

After 60 min, the percentage of dissolved CBZ was between 95.1% and 99.6%.

Formulation P showed the highest variation especially in the first 15 min where the variation coefficient was 11.8%. This formulation had the lowest amount dissolved in the first 15 min which was within 53.2% and 66.9%, thus it met the USP requirements. After 60 min, within 86.3% and 100% of carbamazepine was dissolved.

Formulation made with USP CBZ showed the fastest dissolution, caused by micronized particles of USP standard, while the dissolution with CBZ dihydrates was the slowest because of the lower solubility of carbamazepine dihydrate but with the smallest variation within 6 examined tablets. During the first 15 min only 15 ± 2.5% of CBZ from formulation D was dissolved, and in 60 min the amount of measured drug in the dissolution media was 71.2 ± 1.2%. The findings for CBZ dihydrate are in agreement with our previous statement, which means that although compactibility properties of the drug were improved and dissolution behavior was more uniform, the solubility of the drug decreased and this caused decrease in amount of drug dissolved (see Fig. 10).

Considering that in this study the same excipients were used in all formulations, the variation found in the formulation A, B and P are caused only by differences in the CBZ sample which was used.

Intrinsic dissolution behavior of examined carbamazepines showed differences in the phase transformation, and it was found that for CBZ B, the transition point is around 15 min, for CBZ A around 20 min, and for CBZ P between 20 and 25 min. If direct compaction is the process which is used, all possible phase transitions during the manufacturing of CBZ dosage forms are avoided and CBZ should keep its intrinsic properties. In that case, using intrinsic dissolution it is possible to predict the dissolution behavior of CBZ in a formulation. Intrinsic dissolution behavior of the drug may be one of the critical parameters during the preformulation study of CBZ.

4. Conclusion

Understanding variations in the characteristics of raw material during preformulation stage is necessary in order to ensure reproducibility of results, and predict the cause of eventual instability or

poor performance of a dosage form. One of the tasks of preformulation studies is defining the critical parameters which influence the product quality and furthermore to suggest a method to monitor it during development of a product.

In this work a robust formulation for CBZ immediate release tablets was suggested which is within the frame of PAT initiative to build in the quality into the product. Using thermal analysis, X-ray, and FTIR, the nine samples of anhydrous CBZ obtained from different sources, were shown to exhibit the same polymorphic form (form III), but further investigation showed that CBZ anhydrous samples exhibited different morphology, had different particle size and size distribution all of which led to a variation in solubility and in the intrinsic dissolution behavior. These variations were attributed to the different solvents used in the crystallization process and to different drying and/or grinding regimes in the final stage of the manufacturing process. The samples varied as well in particle size distribution. In order to compare their properties, intrinsic dissolution tests were conducted with samples containing the same particle size fraction. CBZ A, B and P showed different intrinsic dissolution behavior and exhibited different transformation points of anhydrous to dihydrate form.

This leads to the conclusion that selecting just a stable and pure polymorphic starting material is not sufficient in assuring the constant quality of a product, because polymorphs can exist in different habits which can influence dissolution or stability of the final dosage form. Therefore, critical parameters for CBZ formulation which may assure the desired quality of the product beside the polymorphic form are particle size, size distribution and morphology.

All of these characteristics had an effect on the intrinsic dissolution behavior of CBZ and thus the intrinsic dissolution test is suggested as a valuable and simple monitoring tool. It also can be used for determination of anhydrous to dihydrate transition point for CBZ and therefore for prediction of CBZ behavior in the final dosage form.

In this study the properties of CBZ dihydrate were also examined. As commercial samples were converted to CBZ dihydrate form, variations in solubility, thermal and intrinsic dissolution behavior were significantly reduced from batch to batch and within one sample. Due to the lower solubility of dihydrate, dissolution rate was decreased in comparison with dissolution of anhydrous form and this factor must be considered for the bioavailability of CBZ.

How to avoid problems with CBZ?

By determining the transformation point of anhydrous to dihydrate CBZ, it became possible to predict the dissolution behavior. There are two possible strategies suggested at the raw material level in order to decrease the variability and improve constant dissolution of CBZ formulation:

- To control synthetic pathway of raw material and to keep it constant with no variation in order to produce the specific particle size (narrow distribution) and desired morphology of crystal which can help to control the dissolution (CBZ A to CBZ D transformation steps).
- To delete the prehistory of the active material by converting it to dihydrate form prior to formulation, in order to have a constant dissolution rate with less variation (this step then requires the improvement of solubility of dihydrate itself by co-crystallization or other methods).

References

Bolourtchian, N., Nokhodochi, A., Dinarvand, R., 2001. The effect of solvent and crystallization conditions on habit modification of carbamazepine. Daru 9, 12–22.

Brittain, H.G., 1999. Methods for characterization of polymorphs and solvates. In: Brittain, H.G. (Ed.), Polymorphism in Pharmaceutical Solids, vol. 95. Marcel Dekker Inc., New York, Basel, pp. 228–280.

Carino, S.R., Sperry, D.C., Hawley, M., 2006. Relative bioavailability estimation of carbamazepine crystal forms using an artificial Stomach–Duodenum model. J. Pharm. Sci. 95, 116–125.

Grzesiak, A.L., Lang, M., Kim, K., Matzger, A.J., 2003. Comparison of the four anhydrous polymorphs of carbamazepine and the crystal structure of form I. J. Pharm. Sci. 92, 2260–2271.

Hanson, R., Gray, V., 2004. Dissolution testing of solid dosage forms. In: Hanson, R., Gray, V. (Eds.), Handbook of Dissolution Testing, 3rd ed. Dissolution Technologies, Inc., Hockessin, Delaware, pp. 33–71.

Kaneniwa, K., Yamagouchi, T., Watari, N., Otsuka, M., 1984. Hygroscopicity of carbamazepine crystalline powder. Yakugaku Zasshi 104, 184–190.

Kaneniwa, N., Ichikawa, J., Yamaguchi, T., Hayashi, K., Watari, N., Sumi, M., 1987. Dissolution behavior of carbamazepine polymorphs. Yakugaku Zasshi 107, 808–813.

Kobayashi, Y., Ito, S., Itai, S., Yamamoto, K., 2000. Physicochemical properties and bioavailability of carbamazepine polymorphs and dihydrate. Int. J. Pharm. 193, 137–146.

Krahn, F.U., Mielck, J.B., 1989. Effect of type and extent of crystalline order on chemical and physical stability of carbamazepine. Int. J. Pharm. 53, 25–34.

Krahn, F.U., Mielck, J.B., 1987. Relation between several polymorphic forms and the dihydrate of carbamazepine. Pharma. Acta Helv. 62, 247–254.

Li, Y., Han, J., Zhang, G.G.Z., Grant, D.J.W., Suryanarayanan, R., 2000. In situ dehydration of carbamazepine dihydrate: a novel technique to prepare amorphous anhydrous carbamazepine. Pharm. Dev. Technol. 5, 257–266.

McMahon, L.E., Timmins, P., Williams, A.C., York, P., 1996. Characterization of dihydrates prepared from carbamazepine polymorphs. J. Pharm. Sci. 85, 1064–1069.

Meyer, M.C., Straughn, A.B., Jarvi, E.J., Wood, G.C., Pelsor, F.R., Shah, V.P., 1992. The bioinequivalence of carbamazepine tablets with a history of clinical failures. Pharm. Res. 9, 1612–1616.

Meyer, M.C., Straughn, A.B., Mhatre, R.M., Shah, V.P., Williams, R.L., Lesko, L.J., 1998. The relative bioavailability and in vivo-in vitro correlations for four marketed carbamazepine tablets. Pharm. Res. 15, 1787–1791.

Murphy, D., Rodriguez-Cintron, F., Langevin, B., Kelly, R.C., Rodrriguez-Hornedo, N., 2002. Solution-mediated phase transformation of anhydrous to dihydrate carbamazepine and the effect of lattice disorder. Int. J. Pharm. 246, 121–134.

Nogami, H., Nagai, T., Yotsuyamnagi, T., 1969. Dissolution phenomena of organic medicinals involving simultaneous phase changes. Chem. Pharm. Bull. 17, 499–509.

Nokhodochi, A., Bolourtchian, N., Dinarvand, R., 2004. Dissolution and mechanical behaviors of recrystallized carbamazepine from alcohol solution in the presence of additives. J. Cryst. Growth 274, 573–584.

Ono, M., Tozuka, Y., Oguchi, T., Yamamura, S., Yamamoto, K., 2002. Effects of dehydration temperature on water vapor adsorption and dissolution behavior of carbamazepine. Int. J. Pharm. 239, 1–12.

Otsuka, M., Hasegawa, H., Matsuda, Y., 1999. Effect of polymorphic forms of bulk powders on pharmaceutical properties of carbamazepine granules. Chem. Pharm. Bull. 47, 852–856.

Otsuka, M., Hasegawa, H., Matsuda, Y., 1997. Effect of polymorphic transformation during the extrusion-granulation process on the pharmaceutical properties of carbamazepine granules. Chem. Pharm. Bull. 45, 894–898.

Qu, H., Louhi-Kultanen, M., Kallas, J., 2006. Solubility and stability of anhydrate/hydrate in solvent mixtures. Int. J. Pharm. 321, 101–107.

Rodriguez-Hornedo, N., Murphy, D., 2004. Surfactant-facilitated crystallization of dihydrate carbamazepine during dissolution of anhydrous polymorph. J. Pharm. Sci. 93, 449–460.

Rustichelli, C., Gamberini, G., Ferioli, V., Gamberini, M.C., Ficarra, R., Tommasini, S., 2000. Solid-state study of polymorphic drugs: carbamazepine. J. Pharm. Biomed. Anal. 23, 41–54.

Sehic, S., 2008. Investigation of variability of primary materials on the intrinsic dissolution behavior of carbamazepine. PhD Thesis, University of Basel, Switzerland.

Sethia, S., Squillante, E., 2004. Solid dispersion of carbamazepine in PVP K30 by conventional solvent evaporation and supercritical methods. Int. J. Pharm. 272, 1–10.

Shefter, E., Higuchi, T., 1963. Dissolution behavior of crystalline solvated and nonsolvated forms of some pharmaceuticals. J. Pharm. Sci. 52, 781–791.

Surana, R., Pyne, A., Suryanarayanan, R., 2003. Solid–vapor interactions: influence of environmental conditions on the dehydration of carbamazepine dihydrate. AAPS Pharm. Sci. Tech. 4, 1–10.

Umeda, T., Ohnishi, N., Yokoyama, T., Kuroda, K., Kuroda, T., Matsuda, Y., 1984. Kinetics of the thermal transition of carbamazepine polymorphic forms in the solid state. Yakugaku Zasshi 104, 786–792.

US Pharmacopoeia 31, 2008. Carbamazepine immediate release tablets. The United States Pharmacopoeial Convention, Rockville, MD, pp. 1631–1632.

Wang, J.T., Shiu, G.K., Ong-Chen, T., Vitswanathan, C.T., Skelly, J.P., 1993. Effects of humidity and temperature on in vitro dissolution of carbamazepine tablets. J. Pharm. Sci. 83, 1002–1005.

Yu, L.X., Carlin, A.S., Amidon, G.L., Hussain, A.S., 2004. Feasibility studies of utilizing disk intrinsic dissolution rate to classify drugs. International Journal of Pharmaceutics 270 (1–2), 221–227.