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Polymorphic forms of bisoprolol fumarate

Preparation and characterization

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Abstract
Bisoprolol fumarate is a beta blocker-type drug substance which has been well known for several decades. However, no relevant data can be found in the literature about its crystal polymorphism. The purpose of this paper was to present two anhydrous forms (Form I and Form II) and a hydrate of bisoprolol fumarate substance. Crystalline forms were studied by various solid-state analytical methods: Fourier transform infrared (FT-IR) spectroscopy, X-ray powder diffraction (XRPD), dynamic vapor sorption (DVS) and thermoanalytical methods (thermogravimetry and differential scanning calorimetry). Thermodynamic stability and solubility of the presented polymorphs were also investigated. Both FT-IR and XRPD methods were found to be suitable for the characterization of the different crystal structures. Thermoanalytical measurements showed that (1) Form I and Form II own clearly different melting points and (2) both Form II and hydrate forms can transform into Form I at higher temperature values. Results of the DVS measurements prove that both Form I and Form II became metastable under extremely humid conditions (> 80% RH) and converted into the hydrate. Thermodynamic stability studies showed that Form I and Form II polymorphs are in enantiotropic relationship with an enantiotropic point at about 40–45 °C. Solubility studies indicated that all of the prepared forms are highly soluble, and no difference was found between them. Considering the recommendations of the corresponding International Conference of Harmonization guideline, it can be stated that no specification is required for crystal polymorphism in case of this substance.

Keywords Bisoprolol fumarate · Crystal polymorphism · Thermodynamic stability · Solubility · Solid-state analysis

Introduction

Crystal polymorphism has become an important issue for the last several decades in the pharmaceutical industry [1–11]. Though different polymorphic forms of a drug substance own exactly the same chemical structure, their physical and physicochemical properties (e.g., melting point, solubility, hygroscopic property, thermodynamic stability, crystallinity) can be significantly different due to their various crystal structures.

One of the most relevant matters is the solubility difference between polymorphs of active pharmaceutical ingredients (API) [12]. The solubility of the API directly influences the bioavailability of the final product; therefore,

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this parameter has a strong effect on its performance. In case of substances with low solubility, this factor is more emphasized. In the biopharmaceutics classification system (BCS), drug substances are categorized based on their aqueous solubility and permeability (Class I: high solubility, high permeability; Class II: low solubility, high permeability; Class III: high solubility, low permeability; Class IV: low solubility, low permeability) [13–15].

The thermodynamic stability is also a very important aspect of the investigation of different polymorphic forms [16–18]. In case of drug substances, the risk of polymorphic transformation during the formulation process or storing is increased by choosing a metastable form for drug product development. Such transformations can threaten the uniform quality and bioavailability of the final product. However, a metastable polymorph can be also suitable for further development if its potential polymorphic transformations are hindered by choosing the appropriate manufacturing and formulation processes and package systems for the storing of the final product. From these reasons, it is very important to analyze the thermodynamic stability of the different polymorphs and the potential transformations between them [19, 20].

Crystalline polymorphic forms of a given substance can show significantly different behavior under humid conditions [21]. Hygroscopic property can result in undesired and uncontrolled water uptake which may cause problems in stability, water content and content uniformity of the drug product. Polymorphic transformations can also occur due to the water uptake (e.g., formation of a hydrate structure from an anhydrous form) [22]. Therefore, this property has to be carefully studied and considered before choosing the appropriate polymorphic form for further development and determining the suitable storing conditions and package systems.

Among many others, these reasons indicate that properties of different polymorphic and pseudopolymorphic (e.g., hydrates, solvates) forms of the actual substance have to be studied before drug product development [23–25]. In accordance with this statement, the guideline prepared by the International Conference of Harmonization (ICH) recommends a polymorphism screen on the drug substance and detailed characterization of the found polymorphs before use [26, 27]. If only one crystal structure of the substance is known or no significant differences were found between the properties of the discovered polymorphs or the detected differences do not affect the safety, performance or efficacy of drug product, no acceptance criterion is needed for crystal polymorphism according to the decision tree #4 presented in the ICH guideline [26].

Bisoprolol (1-(propan-2-ylamino)-3-[4-(2-propan-2-yloxyethoxymethyl)phenoxy]propan-2-ol) is a beta blocker-type drug substance most commonly used for heart diseases (e.g., high blood pressure, cardiac ischemia, heart failure). It was patented in 1976 [28], and its fumarate salt (see Fig. 1) has been used in many various drug products [e.g., Concor (Merck); Zebeta (Teva); Bisoprolol-ratiopharm (Teva); Bisoblock (Actavis); Bisoprolol Sandoz (Sandoz)]. Although the substance has been known for such a long time, no comprehensive report can be found in the literature about its crystal polymorphism.

A monograph about the bisoprolol fumarate substance is present in both the European (Ph. Eur.) and the American (USP) Pharmacopoeia [29, 30]. It is mentioned in the Ph. Eur. monograph that the API shows polymorphism and is very soluble in water.

Solubility of the substance was investigated in some studies, and it was classified as a BCS Class I material [31–33]. Solubility of different polymorphic forms was not mentioned in these articles. Moreover, it was stated in one of these papers published in 2014 that "references to polymorphic forms were not found in the literature" [33].

Bisoprolol fumarate has been used for drug product development purposes at Egis Pharmaceuticals PLC for many years. During the study of the API, it was found out that two different polymorphic forms and a hydrate of the substance exist which have not been published in the literature yet. The purpose of this paper was to present the detailed solid-state analytical investigation of these crystalline forms. The thermodynamic stability relationship between them and their solubility were also studied. Based on the gathered information, it can be decided whether it is necessary to specify the crystal polymorphism of bisoprolol fumarate substance to provide a drug product with acceptable properties and consistent bioavailability.

Experimental

Preparation of the samples

Two different polymorphic forms (Form I and Form II) and a pseudopolymorphic (hydrate) form of bisoprolol fumarate were prepared. Active substance (bisoprolol or its fumarate salt) used for the experiments was produced at Egis Pharmaceuticals PLC. Details of the synthesis and supplier of the starting materials are confidential information. The original form of the substance was Form I, and in our experience it is valid for most of the commercially available products. Preparation processes were carried out as follows.

Form I

Two different processes were applied for the preparation of the Form I polymorphic form.

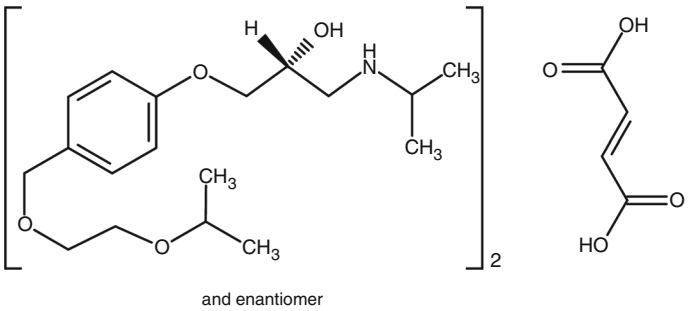

Fig. 1 Chemical structure of bisoprolol hemifumarate drug substance

At the first, one bisoprolol base was used as starting material. In this case, 25.0 g (77 mmol) of bisoprolol base was dissolved in 70 mL of acetone (for synthesis) in an EasyMax^{TM} 102-type reactor system (Mettler Toledo AG) equipped with 100 mL glass reactors, overhead driven anchor stirrers, turbidity probes and temperature sensors. The solution was heated to 40 °C, and 4.45 g (38 mmol) of fumaric acid (for synthesis) was added. While the suspension was heated to reflux, it transformed into a clear solution. After 15 min of reflux, the hot solution was cooled down to 0–5 °C in 2 h: the crystallization of Form I started during the cooling process. After 1 h of crystallization at 2 °C, the suspension was filtered, and the crystalline product was washed with acetone and dried at 50 °C. 25.2 g (85%) of Form I-type bisoprolol fumarate was produced.

The other preparation process started directly from bisoprolol fumarate. 30.0 g anhydrous form of the substance was dissolved in 71 mL of acetone at reflux in the same type reactor mentioned before. The following steps were exactly the same as it was presented at the previous process: after 15 min of reflux the hot solution was cooled down to 0–5 °C in 2 h, the crystallization started, and after 1 h of crystallization at 2 °C the suspension was filtered. The crystalline product was washed with acetone and dried at 50 °C. 25.0 g (83%) of Form I-type bisoprolol fumarate was produced.

Purity of the prepared active pharmaceutical ingredient was found to be 99.88% based on high-performance liquid chromatography (HPLC) results.

Form II

Form II crystalline form of bisoprolol fumarate can be produced via slurry stirring or a seeding process.

In the first case, 30.0 g of bisoprolol fumarate (Form I) was suspended in 70 mL of acetone and stirred for a longer period at ambient temperature in a round bottom flask equipped with a magnetic stirrer. After at least 96 h of stirring, Form I form transformed into Form II polymorph. Afterwards the suspension was filtered and the yield was found to be 29.5 g (98%).

For the seeding process, 30.0 g of anhydrous form of bisoprolol fumarate was dissolved in 71 mL of acetone at reflux in an EasyMax^{TM} 102-type reactor system (Mettler Toledo AG) equipped with 100 mL glass reactors, overhead driven anchor stirrers, turbidity probes and temperature sensors. After 15 min of reflux, the hot solution was cooled down to 0–5 °C in 2 h. At 50 °C, the solution was seeded with 0.35 g of bisoprolol fumarate (Form II or the mixture of Form I and Form II forms). After 1 h of crystallization at 2 °C, the suspension was filtered, and the crystalline product was washed with acetone and dried at 50 °C. 28.55 g (95%) of Form II-type bisoprolol fumarate was produced.

It should be noted that for the second process previous preparation of seeding crystals is necessary (via slurry stirring).

Hydrate

Hydrate form of bisoprolol fumarate substance was produced by storing either of the anhydrous forms (Form I or Form II) under humid conditions. For this purpose, 3.3 g of potassium nitrate (KNO_3, for analysis, $\geq$ 99.0%, Merck) was dissolved in 10 mL of water in order to get a saturated solution which was put into a desiccator. A small additional amount of KNO_3 was given to the system (so the solution contained solid excess of it) to guarantee its saturated state. The equilibrium relative humidity over this solution is about 93–94% at room temperature [28]. A small amount of anhydrous bisoprolol fumarate was put into this desiccator and after at least 24 h of storing the substance transformed into the hydrate form. After removing it from the desiccator, it remained stable under normal conditions (room temperature, $\sim$ 30–60% RH).

Characterization of the samples

Bisoprolol fumarate API samples were characterized by various solid-state analytical methods.

X-ray powder diffraction (XRPD)

XRPD patterns were obtained using a PANalytical Empyrean diffractometer. X-ray radiation was produced by a copper X-ray tube with a wavelength of 1.541874 Å (Cu

Kα) and was focused by a focusing elliptical mirror. Accelerating voltage and anode heating current values were set to 45 kV and 40 mA, respectively. Silicon powder was used as line position and line shape standard, and alumina plate was applied as relative intensity standard (both standards are certified, originated from National Institute of Standards and Technology, NIST). The instrument was used in transmission mode, and the powder samples were placed into the sample holder between two Mylar foils without grinding. Samples were rotated (1 rps) during the measurement. A PIXcel 3D 1 × 1 area detector in scanning line detector (1D) mode was used at the diffracted side. Measurements were taken with a step size of 0.01 °2θ, a measurement range of 2.00–35.00 °2θ and a time per step value of about 110 s. The whole process was carried out at room temperature.

Fourier transform infrared (FT-IR) spectroscopy

FT-IR spectra were taken using a Bruker Alpha FT-IR spectrophotometer in attenuated total reflection (ATR) measuring mode. Instrument was calibrated using a polystyrene film (certified Mid Infrared Transmittance Wavelength Standard, Bruker Optik GmbH). Measurements were taken in the range of 4000–525 cm^{-1} with a resolution of 2 cm^{-1}. The number of scans was 16 for each sample.

Thermogravimetric analysis (TG)

TG measurements were taken using a TA Instruments Discovery Thermogravimetric Analyzer under nitrogen gas flow (25 mL min^{-1}). Measured mass and temperature were calibrated using TGA calibration weights and certified alumel and nickel Curie standards, respectively (both from TA Instruments). About 12–15 mg of powder samples was put into 100 µl platinum pans and was heated up to 120 °C with a heating rate of 10 °C min^{-1}. Additionally two different sorts of isothermal heat treatment were carried out for hydrate samples: in the first process sample was heated up to 80 °C (heating rate: 10 °C min^{-1}) and held at this temperature for 15 min. In the second one, it was heated up to 50 °C (heating rate: 10 °C min^{-1}) and held for 60 min at this temperature value (see "Thermogravimetric analysis (TG)" section for details).

Differential scanning calorimetry (DSC)

DSC curves were obtained using a TA Instruments Discovery Differential Scanning Calorimeter under nitrogen gas flow (50 mL min^{-1}). The instrument was equipped with an RCS90 Refrigerated Cooling System. Measured temperature and enthalpy were calibrated using certified indium standard (NIST). 2–3 mg of powder samples was used for each measurement. Measurements were taken using TA Instruments standard aluminum pans (open pans for hydrate samples and sealed ones for the others, respectively). Samples were heated up to 120 °C with a heating rate of 10 °C min^{-1}. Form II samples were analyzed with moderate heating rate values, too (2; 1 and 0.5 °C min^{-1}, respectively; see "Thermodynamic stability of bisoprolol fumarate polymorphs" section for details). For the determination of melting points, three parallel measurements were performed.

Dynamic vapor sorption (DVS)

DVS measurements were taken using a TA Instruments Q5000 SA sorption analyzer. Mass was calibrated using a certified calibration weight (Mettler Toledo AG). Relative humidity (RH) was calibrated applying salts with different relative humidity values over their saturated aqueous solutions. The used salts (and the corresponding RH values at room temperature based on literature data [34]) were the following: lithium chloride (LiCl, for analysis, Merck, RH: 11.3%), magnesium chloride hexahydrate ($MgCl_2$*$6H_2O$, for analysis, Merck, RH: 32.8%), sodium bromide (NaBr, TA Instruments, RH: 57.6%), sodium chloride (NaCl, for analysis, Merck, RH: 75.3%), potassium chloride (KCl, puriss, Sigma-Aldrich, RH: 84.2%) and potassium nitrate (KNO_3, for analysis, Merck, RH: 93.6%). Measurements were started at the actual relative humidity (40%), and relative humidity was changed within a cycle as follows: 40%→0%→95%→40%, i.e., a cycle contained a desorption (40%→0% RH), a sorption (0%→95% RH) and a second desorption (95%→40% RH) stage. Two cycles were taken for all studied samples. Step size was 5%, and the instrument stepped to the next stage if equilibrium state occurs (or the maximum stage time, set to 360 min expires). Minimum stage time was set to 5 min, and the state was considered as equilibrium if the rate of change in mass per time unit (dm/dt) was below 0.01%. Measurements were taken at 25 °C under nitrogen gas flow (200 mL min^{-1}).

Investigation of the thermodynamic stability

Thermodynamic stability of bisoprolol fumarate polymorphs was investigated via the competitive slurry experiment [35]. In this process, the powder mixture of the studied polymorphs is suspended in different solvents and stirred for a longer period. Dynamic dissolution–recrystallization processes take place during the stirring procedure. Since polymorphs have different solubility in general, the composition of the mixture will change during the process via solvent mediated polymorphic transformations

and the mixture will be enriched with the polymorph with lower solubility (in the actual solvent). If we keep on stirring the suspension for a sufficient time, only one polymorph will remain in the sample (which has the worst solubility in the actually used solvent). Since the thermodynamically stable form owns worse solubility compared to the metastable ones, the stable polymorph can be identified at the end of this experiment by filtering the substance from the suspension and taking its X-ray powder diffractogram. It is important to mention that this method does not work if a hydrate or a solvate forms during the stirring process. In this case, we are not able to provide any information about the thermodynamic stability of the starting polymorphs. From this reason, experiment should be repeated using different solvents with various characteristics. In this case, the 1:1 mixture of Form I and Form II forms of the substance was suspended in six different solvents (ethyl-acetate, isopropanol, tetrahydrofuran, ethanol, diethyl-ketone and acetone) and stirred for 1 day at room temperature (25 °C). All of the used solvents were synthesis grade and manufactured by pharmaceutical raw material producers. Filtered samples were studied by XRPD.

Solubility studies

Solubility of three different bisoprolol fumarate polymorphs or pseudopolymorphs (Form I, Form II and hydrate) was tested in three different dissolution media such as 0.1 M HCl solution, pH = 4.5 phosphate buffer and pH = 6.8 phosphate buffer. Dissolution media were prepared as follows:

1. *0.1 M hydrochloric acid solution* About 750 mL of water was added into a 1000-mL volumetric flask, and then 8.4 mL of 37% hydrochloric acid (excipient grade, 37%, Merck) was weighed accurately into the flask. Finally, it was filled up to volume with purified water and mixed.
2. *pH = 4.5 phosphate buffer solution* 13.61 g of potassium dihydrogen phosphate (KH_2PO_4, excipient grade, Thomasker) was accurately weighed into a 1000 mL volumetric flask and dissolved in 750 mL of purified water. The pH of the solution was adjusted to 4.50 ± 0.05 using 0.2 N phosphoric acid (excipient grade, 85%, Merck) if it was necessary. Then, the solution was filled up to volume with water and mixed.
3. *pH = 6.8 phosphate buffer solution* 6.81 g of KH_2PO_4 and 0.9 g of sodium hydroxide (NaOH, excipient grade, Molar) were accurately weighed into a 1000-mL volumetric flask and dissolved in 750 mL of purified water. The pH of the solution was adjusted to 6.80 ± 0.05 using 0.1 M NaOH solution. Finally, it was filled up to volume with water and mixed.

The equilibrium solubility values for bisoprolol fumarate substance were measured by the saturation shake-flask method [36] using temperature-controlled orbital agitation platform (GFL 1029 Shaking Water Bath).

For carrying out a measurement, 10 mg of the actually studied bisoprolol fumarate polymorph was weighed into a 25-mL Erlenmeyer flask and then 10.0 mL of the appropriate medium was added. The solution was stirred for a period of 6 h (saturation time) at controlled temperature (37.0 ± 0.1 °C) for achieving the thermodynamic equilibrium. After a further 18 h of sedimentation at the same temperature value (to achieve the separation of the excess solid from the solution), samples were filtered and ten times diluted solutions were studied applying a suitable HPLC method described in "High-performance liquid chromatography (HPLC)" section. Three parallel solubility experiments were performed in each medium.

High-performance liquid chromatography (HPLC)

Amount of dissolved bisoprolol fumarate substance in the solutions prepared for solubility studies (see "Solubility studies" section) was measured by HPLC. For the measurements, an Agilent 1200 Series instrument was used equipped with a UV detector (operating at a wavelength value of 226 nm). The used column was Nucleosil C8, 5 μm, CC 250 mm × 4 mm (Macherey–Nagel). Measurements were taken at 60 °C and 85 bar, and the flow rate was set to 1.0 mL min^{-1}. 250:750 (V/V) mixture of acetonitrile and triethylamine (TEA) buffer (pH = 3.0) was used as eluent applying isocratic elution. TEA buffer was prepared by adding 7.0 mL of TEA (for synthesis) to about 980 mL of water (HPLC grade). pH of the buffer was adjusted to 3.0 using concentrated phosphoric acid (H_3PO_4, excipient grade), and finally, it was filled up to 1000 mL with water. The injected sample volume was 20 μL, and samples were stored at 25 ± 5 °C before the measurement. Time of the measurement was 16 min, as the retention time for the bisoprolol substance was found to be about 5.4 min. Dissolved amount of bisoprolol was determined based on the area under the appropriate peak and using external standard calibration.

Results and discussion

Characterization of crystal structure

Polymorphic forms of bisoprolol fumarate substance were investigated by XRPD and FT-IR spectroscopy.

XRPD results showed that all of the studied forms are crystalline, diffractograms contained sharp and well-defined peaks (see Fig. 2). Diffractograms of the three presented forms are clearly different, and they can be unambiguously distinguished.

FT-IR spectra taken from the three forms of bisoprolol fumarate are plotted in Fig. 3. FT-IR is a suitable method for the investigation of the chemical structure of the molecule, since characteristic bands and the corresponding wavenumber values are typical for the appropriate functional groups. Carrying out the measurements in solid-state FT-IR can be also suitable for distinguishing different polymorphic forms of substances with the same chemical structure.

FT-IR spectra were evaluated as characteristic vibrations, and functional groups are given in Table 1 together with the corresponding wavenumber values [37]. There are slight differences between the corresponding wavenumber values for each polymorphic form depending on the actual crystal structure. The vibration belonging to the crystalline water obviously appears only in the spectrum of the hydrate form (first line in Table 1).

Both XRPD and FT-IR methods are suitable for the characterization of the crystal structure of the substance in solid state.

Thermoanalytical investigations

Thermogravimetric analysis (TG)

Bisoprolol fumarate samples of Form I, Form II and hydrate were analyzed applying the TG method (see Fig. 4). Samples were heated up to 120 °C with a heating rate of 10 °C min^{-1}.

No significant decrease was observed in the sample mass for Form I and Form II forms in the whole studied range. It shows that these modifications are hydrate and

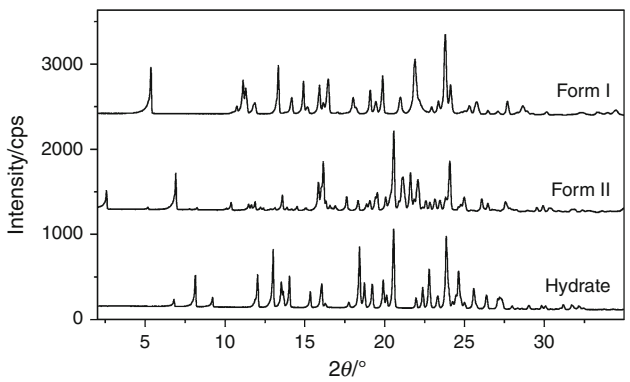

Fig. 2 X-ray powder diffraction patterns for polymorphic (Form I and Form II) and pseudopolymorphic (hydrate) forms of bisoprolol fumarate substance

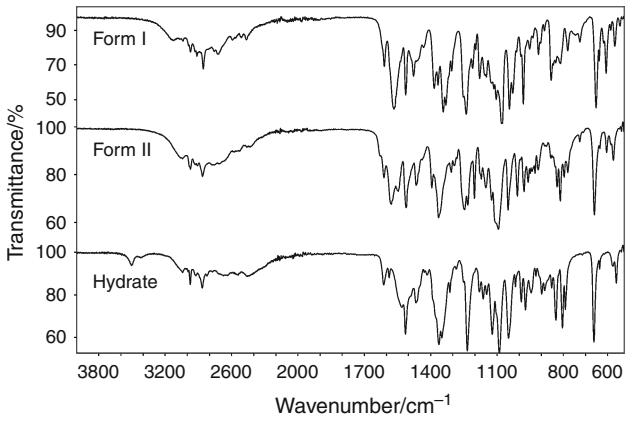

Fig. 3 Fourier transform infrared spectra for polymorphic (Form I and Form II) and pseudopolymorphic (hydrate) forms of bisoprolol fumarate substance

solvate free (anhydrous); they do not contain any volatile components.

For the hydrate form, a mass loss of 2.0% was detected between 30 and 120 °C which is probably related to the evaporation of its water content. The measured value is in reasonable agreement to the water content of a monohydrate (molar ratios: 2:1:1 for bisoprolol base, fumaric acid and water, respectively, calculated water content: 2.3%). Derivative TG (DTG) curve for hydrate was also plotted. It clearly showed that dehydration underwent via more than one steps. The process can be divided into three different stages with the corresponding temperature ranges of 25–75, 75–90 and 90–120 °C, respectively.

Water was evaporated from the studied sample by isothermal heat treatment, too, carried out in the TG instrument. Two different processes were applied:

1. In the first case, sample was heated up to 80 °C and held at this temperature for 15 min. After all the volatile compounds were removed from the sample (the mass change was found to be 1.9%), it was studied by XRPD and TG again. Its diffractogram was considered to be identical to that of Form I form, and no significant decrease was detected in the sample mass up to 120 °C during the TG measurement (see Figs. S1–S2 in the Supplementary Material). It means that the drying of the bisoprolol fumarate hydrate form results in a solid-state transformation into the anhydrous Form I polymorph.
2. In the second process, heat treatment was carried out under milder conditions. Sample was heated up to 50 °C and held for 60 min at this temperature value. The detected mass decrease was 1.9% again. The X-ray powder diffractogram taken after the heating process was considered to be identical to that for the starting hydrate form of bisoprolol fumarate. TG

Table 1 Assignment of the characteristic absorption peaks in Fourier transform infrared spectra taken from polymorphic (Form I and Form II) and pseudopolymorphic (hydrate) forms of bisoprolol fumarate substance

Functional group/assignment	Group frequency/cm^{-1}		
	Form I	Form II	Hydrate
ν (O–H) stretching vibration (free)	–	–	3505
ν (O–H) stretching vibration (H-bonded)	3300–2200	3300–2200	3300–2200
ν (NH_2^+) and ν (NH) stretching vibrations of associated amine			
ν (= CH), ν_{as} (CH_3), ν_{as} (CH_2), ν_s (CH_3),	3037, 2971, 2913, 2856	3042, 2974, 2908, 2861	2973, 2866
ν_s (CH_2) stretching vibrations in chains and ring			
ν (C=C) aromatic skeletal vibration	1610, 1512	1611, 1509	1611, 1587
ν_{as} (COO^-) stretching vibration	1566	1577, 1548	1530, 1513
β_s (CH_2) scissoring vibration	1477	1462	1466
δ_{as} (CH_3) bending vibration			
β (OH) deformation vibration,	1383, 1366, 1343, 1331	1394, 1360	1363, 1349
ν_s (COO^-) stretching vibration			
ν (C–O(H)) stretching vibration	1239, 1210	1248, 1232, 1202	1232
ν_{as} (COC) stretching vibration (aliphatic–aromatic ether)			
ν_{as} (COC) stretching vibration (aliphatic ether)	~ 1070	1099	1121, 1090
ν_s (COC) stretching vibration (aliphatic–aromatic ether)	1043, 1029	1047	1046
γ_{as} (=CH) bending vibration of "E" isomer	981	978	971
γ (=CH) out of-plain CH bending vibration of 1,4-disubstituted aromatic ring	855	828, 814	834, 804

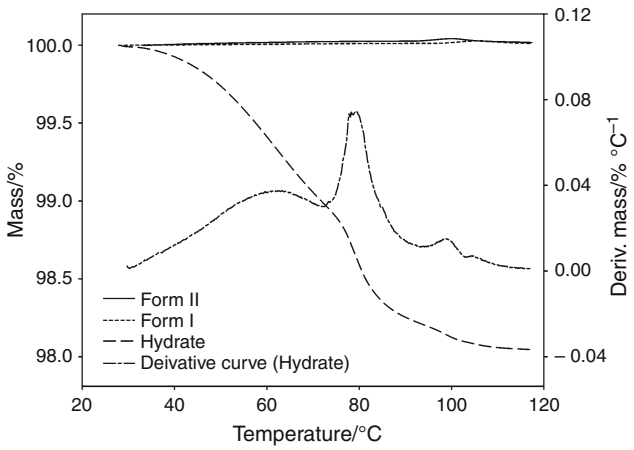

Fig. 4 TG curves for polymorphic (Form I and Form II) and pseudopolymorphic (hydrate) forms of bisoprolol fumarate substance

measurement showed 1.5% of mass loss between 30 and 120 °C (see Figs. S1-S2 in the Supplementary Material). These results indicate that the crystal structure of the hydrate did not collapse during the heat treatment due to the leaving of water molecules. Probably a metastable dehydrated form was evolved, which picked back water spontaneously from the moisture content of the environment. So the evaporation of water was found to be a reversible process under these conditions.

Differential scanning calorimetry (DSC)

DSC studies were also carried out for each crystalline form (see Fig. 5). Samples were heated up to 120 °C with a heating rate of 10 °C min^{-1}. Three parallel measurements were taken.

In case of Form I and Form II samples, standard sealed pans were used and no relevant signals were detected in the DSC curves before the melting. Onset values of melting points and enthalpy of fusion values are displayed in Fig. 5.

Because of its water content, hydrate form was studied in standard open pans. At the beginning stage of the DSC curve, a broad endothermic peak could be observed with a low intensity (between about 30 and 75 °C, see its enlarged view in the inset of Fig. 5) which was followed by another, much sharper and more intensive endothermic peak with an onset temperature value of 76.3 (± 1.0) °C. Considering the results of TG analysis, these two peaks probably represented the reversible and irreversible evaporation of water, respectively: only the second process resulted in significant change in the crystal structure (see "Thermogravimetric analysis (TG)" section). DTG curve also showed that the dehydration process consists of more steps, which is in agreement to DSC results. The next endothermic peak represented the melting of the sample, and its onset temperature was 101.3 (± 0.1) °C. This value shows

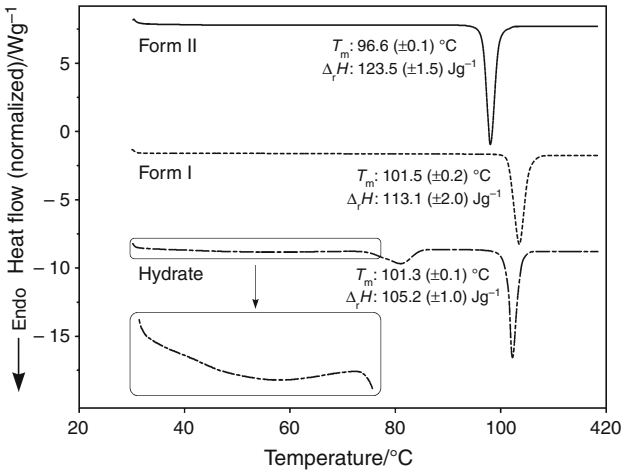

Fig. 5 DSC curves for polymorphic (Form I and Form II) and pseudopolymorphic (hydrate) forms of bisoprolol fumarate substance. (Curves were shifted in order to separate them visually.) Melting point (T_m) and enthalpy of fusion ($\Delta_r H$) data are displayed for each form. (Standard deviation values are in brackets, $n = 3$.) Inset shows an enlarged view from the marked part of the curve for the hydrate form

very good agreement to the melting point of the Form I form, which confirms our previous assumption that the hydrate form transforms into this crystalline modification by its water loss at higher temperature.

Dynamic vapor sorption (DVS) measurements

Form I, Form II and hydrate forms of bisoprolol fumarate were studied by the DVS method. The anhydrous forms of the substance (Form I and Form II) showed very similar behavior (see Fig. 6 and Fig. S3 in the Supplementary Material): in the first desorption stage (40%→0% RH), no significant mass change was observed and neither was in the sorption stage until reaching the relative humidity value of 80%. In this range (0–80% RH), water uptake stayed below 0.2% for both forms, i.e., they did not show hygroscopic property. Above 80% of relative humidity, a significant mass gain was detected (Form I: 2.9% and Form II: 2.4–2.6%, respectively), probably due to the formation of a hydrate structure. Further increase in the relative humidity to 95% results in a huge mass gain (Form I: 17.7% and Form II: 18.0%, respectively). In this stage presumably, the dissolution of the sample occurs, followed by a recrystallization during the desorption process. In this stage (95%→40% RH), the mass change value stabilized at 2.7–2.9% for both crystalline forms, which indicates that a hydrate was formed again in the course of the recrystallization process and it remained stable until the end of the first cycle (i.e., it did not transform back to the starting or any other anhydrous form of the substance). The second measurement cycle clearly differed from the first one;

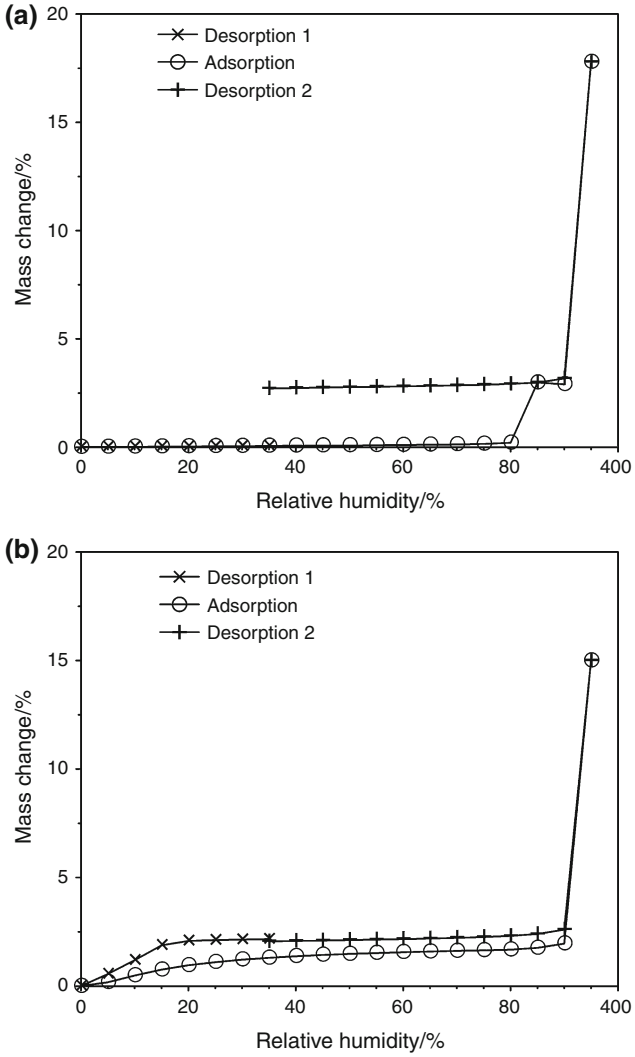

Fig. 6 DVS sorption isotherms for bisoprolol fumarate Form I sample (**a** first and **b** second measurement cycles)

however, it was found to be practically the same for the two anhydrous polymorphs of the substance. In the first desorption stage (below 20% of relative humidity), the mass started to decrease continuously (2.0–2.1%→0.0%). The evolved dehydrated state was not found to be stable since the sample mass started to increase again immediately in the sorption stage and reached the value of 2.0–2.1% at 90% RH (20% RH: 1.0%; 50% RH: 1.5% and 80% RH: 1.7–1.8%, respectively). Under extremely humid conditions (95% RH), the dissolution of the sample happened again, attended by an extraordinary mass gain (Form I: 15.0% and Form II: 17.1%, respectively). In the desorption stage, the mass change value resumed promptly below 3.0% and stabilized at 2.1–2.2%.

In case of the hydrate form, there was no significant difference between the two measurement cycles and they were found to be very similar to the second cycle of the

anhydrous forms (see Fig. S4 in the Supplementary Material). It confirms our previous supposition that a hydrate was formed both from Form I and Form II during the first measurement cycle.

All of the samples were studied by XRPD after finishing the DVS measurements (see Fig. S5 in the Supplementary Material). Diffractograms were found to be the same for all three samples (starting materials: Form I, Form II and hydrate forms of bisoprolol fumarate, respectively), and they were identical to that of the hydrate form, reinforcing the supposed polymorphic transformations based on the sorption isotherms.

It can be stated that both anhydrous forms of bisoprolol fumarate substance (Form I and Form II) are stable in a broad humidity range (0–80% RH). Under extremely humid conditions ($> \approx$ 80% RH), they transform into the known hydrate form and at 95% RH dissolve and recrystallize as the same hydrate. Hydrate was found to be stable, too; however, it loses its water content under extremely dry conditions ($< \approx$ 20% RH), probably forming a metastable dehydrate. It transformed back to the starting hydrate form spontaneously as the relative humidity started to increase. In this case, probably the same solid-state transformation processes take place as it was observed by the isothermal drying of the hydrate carried out under mild conditions (50 °C) using the TG instrument (see "Thermogravimetric analysis (TG)" section). During the DVS studies only one hydrate form of the substance was identified, which is probably a monohydrate (molar ratios: 2:1:1 for bisoprolol base, fumaric acid and water, respectively).

Thermodynamic stability of bisoprolol fumarate polymorphs

Thermodynamic stability of bisoprolol fumarate polymorphs was investigated via the competitive slurry experiment [35]. The 1:1 mixture of Form I and Form II polymorphs was suspended in six different solvents (ethyl-acetate, isopropanol, tetrahydrofuran, ethanol, diethyl-ketone and acetone) and stirred for 1 day at room temperature (25 °C). Filtered samples were studied by XRPD. At the end of the experiment, only the Form II modification was detectable in the samples, and Form I was disappeared in all cases, indicating a complete Form I→Form II transition (see Fig. S6 in the Supplementary Material). It clearly shows that Form II is the thermodynamically stable (less soluble) form of the substance at room temperature.

The temperature dependence of stability relations was also studied. Previously performed DSC measurements showed that the polymorph with a lower melting point (Form II) has a higher enthalpy of fusion (see Fig. 5), which indicates an enantiotropic relationship between Form I and Form II forms of bisoprolol fumarate according to the heat-of-fusion rule by Burger and Ramberger [16]. It means that the thermodynamical stability relations reverse at a defined temperature value.

1:1 mixture of Form I and Form II bisoprolol fumarate polymorphs was suspended in acetone at different temperature values (0, 25 and 50 °C) and stirred for 1 day. At the end of the process, samples were filtered and studied by XRPD. Results showed that diffractograms of the samples stirred at 0 and 25 °C were found to be identical to that of Form II as the sample stirred at 50 °C contained only the Form I polymorph of the substance. This observation indicates that the transition temperature lies between 25 and 50 °C; therefore, experiments were repeated at 35, 40 and 45 °C. After one day of stirring in acetone, samples were filtered and studied by XRPD. Samples stirred at 35 and 40 °C were found to be identical to the Form II polymorph as the sample stirred at 45 °C contained mainly the Form I form with a very small amount of Form II polymorphic contamination. It shows that at 45 °C Form II→Form I is the direction of the transformation; presumably, the stirring time was not enough to the complete conversion. The difference between the stabilities (solubilities) of the two polymorphic forms is probably smaller at this temperature value, which resulted in a slower transformation process (see Fig. S7 in the Supplementary Material for the diffractograms).

In summary, it can be stated that the enantiotropic point is between 40 and 45 °C: below this temperature Form II, above this value Form I is the stable polymorph of bisoprolol fumarate substance from the thermodynamic point of view.

Since it was proven that Form I is the stable polymorph of the substance above 40–45 °C, DSC measurements were taken for studying the potential Form II→Form I transformation at higher temperature values. For this purpose, Form II samples were analyzed with moderate heating rate values (2, 1 and 0.5 °C min^{-1}, respectively). Aside the endothermic peak related to the melting of Form II (onset temperature: 96.4–96.5 °C), another endothermic peak appears in all of the DSC curves with an onset temperature of 101.0–101.1 °C (see Fig. 7). Between these peaks, an exothermic peak was also observed (especially in the DSC curves recorded with lower heating rates). This endothermic–exothermic–endothermic peak triplet probably represents the melting of Form II form, followed by the crystallization of Form I form from the melt and finally the melting of the crystallized Form I form. Measured melting points also confirmed this hypothesis (see Fig. 5). The intensity of the exothermic and the second endothermic peak as well increased by reducing the heating rate. The results show that Form I polymorph of bisoprolol fumarate can crystallize from the melt of Form II form, and the

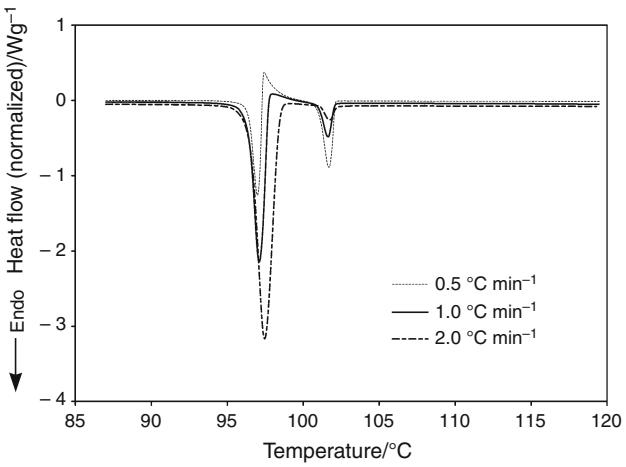

Fig. 7 DSC curves for bisoprolol fumarate Form II samples recorded with different heating rate values

effectiveness of the process can be improved by slowing down the heating procedure.

The potential polymorphic transformations between the presented forms are collected in Fig. 8.

Solubility of bisoprolol fumarate polymorphs

The equilibrium solubility values for all the three known crystalline forms of bisoprolol fumarate substance (Form I, Form II and hydrate) were measured at 37 °C in three different dissolution media with the pH values of 1.0, 4.5 and 6.8, respectively. Results are collected in Tables 2–4.

It has to be mentioned that according to our experience under lower than neutral pH conditions, bisoprolol substance can be transformed to Bisoprolol Impurity A ((2RS)-1-(4-hydroxymethyl-phenoxy)-3-isopropylaminopropan-2-ol; relative retention time ≈ 0.42). The degree of transformation is increased by the decrease in pH. This component can be separated by the used HPLC method.

The high recovery percentages show that the measured solubility values do not represent the real thermodynamic solubility: the latter value is definitely higher. The about 10% loss in recovery detected at the pH value of 1.0 (see Table 2) can be explained by the degradation of bisoprolol in acidic environment. pH value was checked at the end of each measurement, and it did not change significantly during the solubility tests.

For the classification of the solubility of each polymorph dose/solubility ratio (q) and V_{max} (volume in which the highest dose strength is dissolved), values were calculated using Eqs. 1, 2 (M: maximum dose; S_{min}: minimum solubility over the physiological pH range; V: volume of gastric fluid):

$$q = \frac{M}{S_{min} * V}; \tag{1}$$

$$V_{max} = \frac{M}{S_{min}}. \tag{2}$$

The maximum dose (M) for the bisoprolol fumarate substance is 10 mg [38]. According to the BCS, a drug substance is considered highly soluble when the highest dose strength is soluble in 250 mL (volume of gastric fluid) or less of aqueous media over the pH range of 1.0–6.8 [15]. Based on these data and the measured solubility values, the following results were calculated (see Table 5).

It can be seen that the solubility properties of the three studied bisoprolol fumarate forms do not differ significantly in the physiological pH range, and all of them are unambiguously highly soluble (BCS Class I or III).

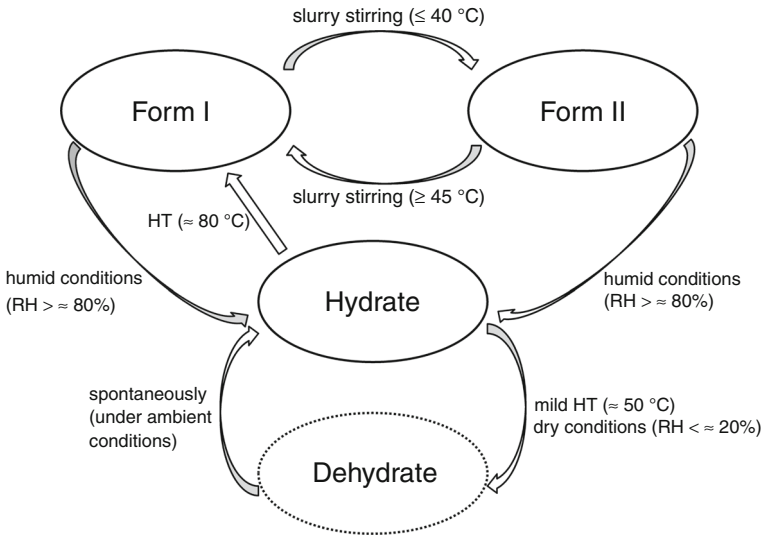

Fig. 8 Potential polymorphic transformations between the crystalline forms of bisoprolol fumarate and their circumstances (dehydrate form was not isolated: its existence is not proven; HT: heat treatment, RH: relative humidity)

Table 2 Solubility of bisoprolol fumarate polymorphs in 0.1 M HCl solution (Mean represents the average values for the three parallel measurements; standard deviation values are in brackets; recovery means the percentage of the dissolved amount compared to the weighed amount; V_{max} is the volume in which the highest dose strength (10 mg for bisoprolol fumarate) is dissolved.)

Polymorphic form	Results						V_{max} mL
	Amount/mg			Recovery/%	Solubility/mg mL^{-1}	pH (end)	
	Weighed	Dissolved	Dissolved (mean)	Mean			
Form I	10.167	9.24	9.45 (± 0.27)	90.4 (± 0.48)	**> 0.945**	1.08	< 11
	10.840	9.75					
	10.341	9.35					
Form II	10.857	9.92	9.78 (± 0.21)	91.4 (± 0.31)	**> 0.978**	1.08	< 11
	10.779	9.88					
	10.474	9.54					
Hydrate	10.487	9.39	9.39 (± 0.13)	89.6 (± 0.32)	**> 0.939**	1.09	< 11
	10.360	9.25					
	10.587	9.52					

Table 3 Solubility of bisoprolol fumarate polymorphs in pH = 4.50 ± 0.05 phosphate buffer solution (Mean represents the average values for the three parallel measurements; standard deviation values are in brackets; recovery means the percentage of the dissolved amount compared to the weighed amount; V_{max} is the volume in which the highest dose strength (10 mg for bisoprolol fumarate) is dissolved.)

Polymorphic form	Results						V_{max} mL
	Amount/mg			Recovery/%	Solubility/mg mL^{-1}	pH (end)	
	Weighed	Dissolved	Dissolved (mean)	Mean			
Form I	10.220	10.06	10.27 (± 0.22)	98.9 (± 0.46)	**> 1.027**	4.71	< 10
	10.604	10.49					
	10.339	10.27					
Form II	10.385	10.28	10.31 (± 0.02)	99.7 (± 0.57)	**> 1.031**	4.70	< 10
	10.336	10.32					
	10.314	10.33					
Hydrate	10.836	10.62	10.74 (± 0.56)	98.4 (± 0.32)	**> 1.074**	4.70	< 10
	10.411	10.25					
	11.499	11.35					

Table 4 Solubility of bisoprolol fumarate polymorphs in pH = 6.80 ± 0.05 phosphate buffer solution (Mean represents the average values for the three parallel measurements; standard deviation values are in brackets; recovery means the percentage of the dissolved amount compared to the weighed amount; V_{max} is the volume in which the highest dose strength (10 mg for bisoprolol fumarate) is dissolved.)

Polymorphic form	Results						V_{max} mL
	Amount/mg			Recovery/%	Solubility/mg mL^{-1}	pH (end)	
	Weighed	Dissolved	Dissolved (mean)	Mean			
Form I	9.904	9.88	10.17 (± 0.25)	99.4 (± 0.37)	**> 1.017**	6.81	< 10
	10.397	10.33					
	10.399	10.30					
Form II	10.433	10.25	10.53 (± 0.28)	98.8 (± 0.41)	**> 1.053**	6.81	< 10
	10.936	10.82					
	10.614	10.51					
Hydrate	10.371	10.14	10.30 (± 0.17)	97.7 (± 0.17)	**> 1.030**	6.81	< 10
	10.558	10.29					
	10.725	10.48					

Table 5 Dose/solubility classification of bisoprolol fumarate polymorphs (q: dose/solubility ratio; V_{max}: volume in which the highest dose strength is dissolved)

Polymorphic form	Maximum dose/mg	q	V_{max}/mL	BCS solubility class	Dose/solubility volume
Form I	10	0.042	10.58	Highly soluble ($V_{max} < 250$ mL)	High solubility ($q < 1$)
Form II		0.041	10.22		
Hydrate		0.043	10.65		

Conclusions

The aim of this work was to study the polymorphic and pseudopolymorphic forms of bisoprolol fumarate drug substance. Three different crystalline forms (two polymorphs called Form I and Form II and a hydrate) of the API were prepared and analyzed by various solid-state analytical methods (XRPD, FT-IR, TG, DSC and DVS). Thermodynamic stability and solubility properties of the prepared forms were also investigated.

XRPD and FT-IR measurements showed that both powder diffractograms and IR spectra of the three different forms are clearly distinguishable from each other, i.e., both techniques are suitable for the characterization of these crystal structures.

TG and DSC analyses prove that Form I and Form II are water and solvent free forms of bisoprolol fumarate with melting points of 101.5 °C for Form I and 96.6 °C for Form II, respectively. Water content of the hydrate could be removed via isothermal heat treatment at 80 °C resulting in a solid-state transformation into the anhydrous Form I form. Carrying out the heat treatment under milder conditions (at 50 °C), water evaporation was found to be reversible as the dried sample (possibly a metastable dehydrate form) took back the water from air moisture forming a hydrate again (identical to the starting structure). Mass loss data detected during TG measurements indicated that the prepared pseudopolymorphic form of the API is a monohydrate (molar ratios: 2:1:1 for bisoprolol base, fumaric acid and water, respectively).

According to DVS data, Form I and Form II forms were stable and non-hygroscopic between 0 and 80% of relative humidity at room temperature (25 °C). Under extremely humid conditions (> 80% RH), both forms turned into the known monohydrate structure. The hydrate remained stable and lost its water content only under dry conditions (< 20% RH). Possibly a metastable dehydrate formed at this point which transformed back to the hydrate immediately as relative humidity increased over 0%. At 95% of relative humidity, all three forms dissolved and as relative humidity decreased again below 95% always the presented hydrate recrystallized (irrespectively of the starting crystal structure).

Competitive slurry experiments demonstrated that Form I and Form II are in enantiotropic relationship: below the temperature value of ca. 40–45 °C Form II, above that Form I is the thermodynamically stable polymorph of bisoprolol fumarate substance. DSC studies additionally confirmed that Form I can crystallize from the melt of Form II at high temperature.

Solubility studies were also carried out investigating all three forms at 37 °C and at three different pH values (1.0; 4.5 and 6.8, respectively). Results showed that all of the presented polymorphs are highly soluble under all of the studied conditions: no significant deviation was found between the behavior of the different crystalline forms. Experienced high solubility of the API is in agreement to literature data; however, solubility of different polymorphs of this substance has never been studied so far.

On the whole, two anhydrous forms and a hydrate of bisoprolol fumarate were studied in this work. Though significant differences were found between their physical or physicochemical properties (melting point, thermodynamic stability, behavior under humid conditions), due to their uniformly high solubility it is not necessary to specify the crystal polymorphism of the substance. Considering the recommendations of the ICH guideline, all of the three presented crystalline forms can be suitable for the development of a drug product with predictable and well-defined bioavailability and characteristics since the detected differences do not affect the safety, performance or efficacy of drug product.

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