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The indications of tautomeric conversion in amorphous bicalutamide drug

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ABSTRACT

The investigation of tautomerization phenomenon in pharmaceutically relevant materials has important implications. The lack of knowledge about tautomeric preferences may negatively impact the formulation and manufacturing process as well as performance of drug product. In this paper we performed theoretical calculations to verify the occurrence of proton transfer in popular anti-androgen drug bicalutamide (BIC). Density functional theory (DFT) calculations determine the activation energy values for possible tautomeric paths providing a basis for comparison with experimental data. To find the indication of the presence of imidic acid and amide tautomers in amorphous BIC we applied infrared spectroscopy (IR). Finally, we performed isothermal broadband dielectric spectroscopy studies (BDS) to select the most likely mechanism of proton transfer in supercooled BIC. We found out that thermal processing applied during glass preparation *via* vitrification method results in the presence of amide and imidic acid forms in glassy BIC. Further heating leads to re-equilibration of supercooled BIC manifested by the growth of viscosity and effective dipole moment. Based on the value of activation energy determined in theoretical and experimental BDS studies we recognized that the observed time evolution of dielectric parameters likely reflects the increasing amount of more stable amide tautomer. The results presented herein indicate that in amorphous BIC in particular conditions the temperature-dependent changes in tautomeric composition due to intramolecular proton transfer are possible.

1. Introduction

The occurrence of tautomerization in drugs, i.e. proton transfer from one place to another, becomes one of the major challenges facing pharmaceutical community today. From practical perspective the occurrence of impurities in drug product, such as distinct tautomeric forms, must be precisely predicted during drug development and strictly controlled at all stages of drug manufacturing and administration. It becomes a necessity since different tautomeric forms of active pharmaceutical ingredients (APIs) may possess completely distinct physicochemical features, diverse biological reactivates and consequently their efficacy can vary distinctly (Katritzky et al., 2010; Martin, 2009). A huge difficulty in exploring APIs tautomerism originates from substantial environmental sensitivity of tautomeric equilibrium which may change unexpectedly in response to mutable external conditions, such as temperature, pH, solvent or mechanical stress. Despite these experimental impediments, and because of great practical importance, the attempts to identify and characterize tautomers in various APIs have been continued throughout the recent years (Skotnicki et al., 2016; Porter, 2010; Malińska et al., 2014; Kasetti and Bharatam, 2012; Bhatt and Desiraju, 2007; Bag et al., 2014; Calvo et al., 2015).

In this paper we focused on amorphous bicalutamide drug (BIC). BIC is an anticancer agent widely used in prostate cancer treatment, which inhibits the production of androgens by competitive binding to the androgen receptor (Tan et al., 2014). According to biopharmaceutical classification system (BCS) the drug belongs to class II containing permeable but insufficient soluble materials. Thus, different strategies have been proposed to enhance its oral bioavailability and in vivo efficiency (Ren et al., 2006; Meer et al., 2013; Surov et al., 2016; Tres et al., 2015; Le et al., 2009). Conversion of crystalline solid into

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amorphous form is considered as auspicious way to overcome the problems arising from APIs poor solubility (Descamps, 2016). Then, if the molten drug is cooled rapidly a glassy state can be attained. The latter due to unique properties such as higher enthalpy, entropy, free energy, and volume offers vital medical benefits, i.e. higher apparent solubility, dissolution rate, and oral absorption (Baghel et al., 2016). In order to benefit these advantages the drug composition must not change with time. Thus, the eventual partial recrystallization of thermodynamically unstable amorphous content must be monitored. Furthermore, in the case of amorphous APIs with tautomerization ability an appropriate control of isomeric composition should be implemented. Very slow or extremely fast conversion rates may not have an adverse impact on the drug properties otherwise an appropriate guideline for proper drug handling during its formulation and storage is required.

Over the last few years the molecular properties of BIC have been investigated several times (Vega et al., 2007; Dhaked et al., 2012; Li et al., 2013; Nemet et al., 2008; Perlovich et al., 2013). Such great research interest originates from BIC molecular architecture and large molecular flexibility giving rise to so-called conformational polymorphism. Insightful studies performed by Vega et al. provide detailed description of two distinct polymorphic forms of BIC (I and II) (Vega et al., 2007). According to DFT calculations the energy difference between both forms is quite small, however, the form II in a gas phase was found to be a higher energy state (Le et al., 2009; Dhaked et al., 2012). Crystalline BIC in form I is actually available on pharmaceutical market while form II can be obtained through recrystallization from amorphous phase. As reported by Német et al. polymorphic conversion between both forms can also occur when sample is subjected to mechanical stress, e.g. during sample milling, grinding or compression (Nemet et al., 2008). The different value of torsion angle in form I and form II, responsible for variations in mutual orientations of phenyl rings, leads to conformationally distinct crystalline structures being described as “open” and “folded”, with various pattern of molecular attractions including hydrogen bonds and π···π stacking interactions (Dhaked et al., 2012; Surov et al., 2016). What is interesting the conformation found for BIC localized inside the binding pocket of androgen receptor is completely different from those reported previously for drug in the solid state (Dhaked et al., 2012; Bohl et al., 2005).

Despite attentive investigation of BIC its tautomerization ability has been never tested before. The chemical structure of BIC drug is presented in Fig. 1. Due to the presence of amide group in BIC structure, the amide–imidic acid conversion being a consequence of intra- or intermolecular proton transfer within BIC molecule cannot be excluded. Previously, proton transfer phenomenon has been reported for other APIs with amide bond, such as glibenclamide or indapamide (Wojnarowska et al., 2010b; Wojnarowska et al., 2013). The relevance of issue raised in our paper is evidenced by relatively new reports identifying tautomers for several well-known APIs, such as warfarin (Guasch et al., 2015), cimetidine (Calvo et al., 2015), valsartan (Skotnicki et al., 2016), omeprazole (Bhatt and Desiraju, 2007). The revival is also observed in the field of computational method (Sitzmann et al., 2010; Cruz-Cabeza and Groom, 2011).

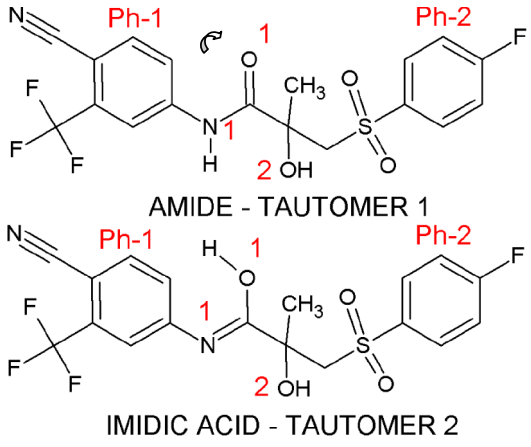

Fig. 1. Tautomeric forms of BIC.

The possibility of tautomeric conversion in amorphous BIC prepared *via* vitrification method was verified by performing density functional theory calculations (DFT) utilizing transition state theory (TST). Then, infrared spectroscopy (IR) was applied to find experimental indication of the presence of distinct tautomeric forms in the amorphous sample. With the hope to get a deeper insight into the tautomerization effect ^{1}H NMR experiments have been performed as well. On the basis of these theoretical and experimental results we propose the possible mechanisms of proton transfer in BIC drug which were further verified during isothermal broadband dielectric spectroscopy (BDS) measurements of supercooled sample. Results presented herein provide a fresh insight into the molecular-level properties of BIC. Our findings, identifying some previously unknown facts, can robustly contribute to better understating of BIC behavior in the solid state.

2. Materials and method

2.1. Investigated material

Bicalutamide (BIC), N-[4-cyano-3-(trifluoromethyl)phenyl]-3-[(4-fluorophenyl)sulfonyl]-2-hydroxy-2-methylpropanamide, was purchased from Hangzhou Hyper Chemicals Limited as a crystalline white powder with 99.8% purity. The amorphous sample was prepared by vitrification method relying on rapid cooling (quenching) of the melt. The crystalline powder (form I) was molten on a heating block prewarmed to a melting temperature of BIC drug which is T = 467 K and quickly transferred to a metal plate pre-cooled to temperature lower than glass transition temperature of investigated material. The glass transition temperature of BIC assessed from differential scanning calorimetry was T_g = 328 K (heating rate = 10 K/min).

2.2. Computational

All the calculations were carried out in Orca program (Neese, 2012) using density functional theory (DFT) on the B3LYP/6-311G** level of theory with the D3 dispersion corrections incorporated (Grimme et al., 2010; Grimme et al., 2011). Transition states were estimated with the Eigenvector following method and were verified by frequency analysis (every transition state has one imaginary frequency). Activation energies for studied reaction have been corrected by the zero point vibrational energy (ZPE). Simulated infrared spectra were rescaled in order to fit experimental peak positions. Frequency scaling factor c = 0.969 satisfactory reproduced measured spectra, which stays in agreement with literature (Merrick et al., 2007).

2.3. Infrared measurements

Infrared measurements were performed using an Agilent Cary 640 FTIR spectrometer equipped with a standard source and a DTGS Peltier-cooled detector. The spectra have been collected using GladiATR diamond accessory (Pike Technologies) in the 4000–400 cm^{-1} range. All spectra were recorded by accumulating of 16 scans with spectral resolution of 2 cm^{-1}. The assignment of vibrational bands was performed by comparison with theoretical spectra obtained using mentioned B3LYP/6-311G** method.

2.4. Broadband dielectric spectroscopy

Isothermal dielectric measurements at ambient pressure were performed using a Novocontrol GMBH Alfa analyzer with frequency values ranging from 10^{-1} Hz to 10^{6} Hz. The dielectric spectra were measured