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Multivariate analysis of quaternary carbamazepine–saccharin mixtures by X-ray diffraction and infrared spectroscopy

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ABSTRACT

Co-crystallization brings new opportunities for improving the solubility and dissolution rate of drugs with the chance of finely tuning some relevant chemical–physical properties of mixtures containing bioactive compounds. As co-crystallization process involves several molecular species, which are generally solid at room conditions, its control requires accurate knowledge and monitoring of the different phase that might appear during the formulation stage. In the present study the suitability of X-ray powder diffraction (XRPD) and Fourier-transformed infrared (FTIR) spectroscopy in quantifying mixtures of carbamazepine polymorphs (forms I and III), saccharin, and carbamazepine–saccharin cocrystals (form I) is assessed. Quaternary crystalline mixtures typically produced in the process of co-crystal production were analyzed by multivariate methods. Principal component analysis (PCA) was used for the identification of the crystal phases, while unsupervised simultaneous fitting of the spectra from pure phases, or supervised partial least squares (PLS) methods were used for their quantitative determination. The performance of data analysis was enhanced by applying peculiar pre-processing methods, such as SNIP filtering in case of FTIR and PCA filtering in case of XRPD. It was found that, for XRPD data, the automatic multi-fitting procedures and PLS models developed in this study are able to quantify single phases in mixtures to an accuracy level comparable to that obtained by the widely used Rietveld method, which, however, requires knowledge of the crystal structures. For FTIR data the results here obtained prove that this technique can be used as a fast method for polymorph characterization.

1. Introduction

An extremely hard and ambitious goal for pharmaceutical companies is the optimization of the physicochemical properties of Active Pharmaceutical Ingredients (APIs) and their market formulation. It is well known that the solubility, dissolution rate, melting point, moisture sorption tendency, and mechanical properties of APIs can dramatically affect the bioavailability, manufacturing, and stability of the resultant dosage form [1]. As a result, the control of these properties represents a valuable option to modulate both the therapeutic effect and processability of a given drug. The vast majority of API's are sold as solids for their relative ease of isolation, for the rejection of impurities inherent to the crystallization process, and for the higher physicochemical stability of crystalline solid state. Nevertheless, a great challenge for pharmaceutical companies is still that of finding new routes for enhancing solubility and dissolution of solid formulations [2]. According to the Biopharmaceutics Classification System (BCS) [3], drugs are categorized into four groups depending on their solubility and permeability properties. For instance, poor bioavailability is expected for weakly soluble APIs, despite they can easily permeate through mucous membranes (BCS class II); in such cases, severe difficulties can occur for the dosage form design and manufacturing. In this respect, it is worth reminding that about 40% of marketed drugs have low solubility [4–6]. Besides low solubility, the existence of more crystalline forms has to be taken properly into account when considering crystalline APIs [7]. It is in fact well documented that API's can exist in several polymorphic, solvated and/or hydrated forms [7,8]. In this respect, it should be said that the multifaceted aspects of polymorphism would represent both a drawback and an opportunity. Different physicochemical properties associated to different polymorphs of the same materials can provide lack of reliability of manufacturing and physical and chemical instability of a given polymorph, while a discovered novel polymorph can provide options for generic pharmaceutical competition.

In such scenarios, in order to improve the solubility and thereby the dissolution rate, research strategies often turned to various

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basic approaches such as salt formation, changes in the solid-state structure, complexation, and encapsulation [9–11]. Though salt formation is a widely implemented and convenient method of improving solubility, it suffers from some disadvantages given that its applicability is limited to those APIs containing and however to nontoxic salts [12]. Additionally, solid-state manipulation approaches of APIs may increase the risk of phase conversion under normal storage conditions [13]. Unlike the traditional solid forms, the pharmaceutical cocrystallization represents a promising alternative, based on crystal engineering approaches, for improving the solubility and dissolution rate and even controlling other pivotal physical properties of APIs [14,15]. Pharmaceutical cocrystals are composed by two or more organic compounds, which consist of at least one active pharmaceutical ingredient and complementary molecules called cocrystal formers, that is solid under ambient conditions [16,17]. The interest in cocrystallization is triggered by the fact that cocrystals have distinct physical properties with respect to the solid forms of the pure API. Thus, they may be optimized to meet the requirements for drug preparation, as shelf life, dissolution rate, bioavailability and stability [18–20]. Therefore, cocrystals clearly open up a vast space of possibilities for exploring a wide range of dissolution characteristics, and facilitate co-optimization with other parameters, such as stability and processability. Cocrystallization has the potential not only for enhancing the dissolution rate, but also for reducing the practical extent of polymorphic diversity of solid dosage forms, although there may be exceptions [17]. Fascinating examples of pharmaceutical cocrystals, including cocrystal polymorphs, were presented in recent publications and reviews describing the characterization methods used to better elucidate their physical properties [21].

In the route to discover new cocrystal forms and/or control cocrystallization processes, outmost importance resides in the methods for the characterization of the crystallization products, which can be also applied online during continuous manufacturing processes. Solid-state forms of pharmaceutical compounds can be analyzed by using several techniques, such as X-ray powder diffraction (XRPD), infrared (IR) and Raman spectroscopy, differential scanning calorimetry and solid-state nuclear magnetic resonance. It is generally advisable to use them in combination to obtain a reliable characterization of the solid forms [22]. In fact, they all have their merits and faults. For example, robust and informative techniques, such as XRPD, may suffer of problems like preferred orientation and sample transparency effects, affecting the accuracy of the analysis, while vibrational spectroscopic methods are more simple and fast, albeit less informative. Worth of note, in many industrial and academic environments, XRPD equipment is not commonly available, while FTIR spectrometers are widespread, and much cheaper, instruments. FTIR also provide a robust technique for on line product control directly on production site. Moreover, all the analytical techniques rely on the computational methods used in the analysis of experimental data, aiming at extracting qualitative and quantitative information.

Among the several pharmaceutically active molecules, carbamazepine (CBZ) [5H-dibenz(b,f) azepine-5-carboxamide] is an important drug for the treatment of epilepsy and trigeminal neuralgia. CBZ represents an excellent test case because of its limited bioavailability [23] and the fact that it exists in multiple crystalline forms; at present, five polymorphs and two solvates have been reported in the literature [24–26]. This makes CBZ an ideal candidate for a crystal engineering case study [27]. Pharmacokinetic (PK) studies have revealed the influence of physical form and formulation on oral bioavailability of carbamazepine. Polymorphs and hydrates differ in their PK characteristics in a manner that is correlated with dissolution profiles [23]. Control over form and formulation is therefore critical to achieve the desired biopharmaceutical performance of carbamazepine oral products. While the polymorph transiently supersaturates in the aqueous medium and subsequently precipitates to eventually form the known dihydrate, the cocrystal supersaturates to a sustained two-fold equilibrium solubility of the dihydrate. Generally, such supersaturation behavior has been found to influence the bioavailability of carbamazepine [23]. In this respect, the dissolution profile of carbamazepine–saccharin cocrystal [28] shows a higher dissolution of the bioactive molecule in that context [29] in comparison with one of the pure anhydrous polymorphs, in terms of relative lack of polymorphism and equivalent chemical stability to the anhydrous polymorph, of favorable dissolution properties and suspension stability. In the same manner, a similar oral absorption profile is observed when compared with the commercial product.

On these bases, in the present work we studied and developed statistical methods to extract quantitative information from XRPD and IR data, by considering carbamazepine–saccharin powder mixtures as case study for a general cocrystallization process. This kind of mixtures have been used in membrane-based crystallization technology [30] from water/ethanol solvent mixtures, a process that offers the chance to directly formulate SAC crystals, CBZ crystals in one of its polymorphic or hydrates forms [31], or CBZ–SAC cocrystals by choosing the appropriate initial solution composition [32]. The occurrence of diverse polymorphic forms of CBZ and co-crystals gave us the chance of quantifying the diverse phases and thus represented an unprecedented opportunity for studying the control of the entire process. Until now, the quantification of CBZ polymorphs has been addressed only for binary [33–35] and ternary [36] mixtures. Thus, quaternary mixtures formed by CBZ polymorphs I and III, SAC and CBZ–SAC cocrystals form I are a relevant test case for assessing the performances of the techniques and the data analysis methods used for classification and quantification purposes.

2. Materials and methods

2.1. Materials

Carbamazepine (polymorphic form III, CBZ III) and Saccharin (SAC) were provided by Sigma–Aldrich. CBZ polymorphic form I (CBZ I) was prepared by heating CBZ III at 170 °C for 4 h according to established procedures [25]. CBZ–SAC cocrystal in its polymorphic form I (CBZ–SAC) was produced by membrane crystallization [37]. Synthetic mixtures of crystalline samples of CBZ I, CBZ III, SAC, and CBZ–SAC were prepared. An analytical scale accurate to 0.01 mg was used to determine the mass ratios of pure phases. They were chosen to scan as uniformly as possible the quaternary diagram, while keeping a reasonable number of samples. Therefore we decided to use only the fractions ¼,⅓ and ½, forming 1 sample ¼, ¼, ¼, ¼ (n. 0), 6 samples 1/2, 1/2, 0,0 (n. 1–6), 4 samples 1/3, 1/3, 1/3, 0 (n. 7–10), 11 samples 1/2, 1/4, 1/4, 0 (n.11-21), and 4 samples 1,0,0,0 (n. 22–25). The actual weight fractions are listed in Table 1.

2.2. Experimental methods

2.2.1. X-ray powder diffraction

X-ray powder diffraction data were collected on a D8 ADVANCE BrukerAXS diffractometer in Bragg-Brentano geometry, using Ni-filtered Cu-K_a radiation ($\lambda = 1.5418$ Å) and a PSD LYNXEYE^{TM} detector simultaneously using about 70 separate channels and measuring the scattering intensity in continuous scanning mode. The samples were placed in the hollow of an aluminium plate equipped with a zero background quartz monocrystal; diffraction profiles were collected in reflection mode by using a sampling step of 0.02° and a scan rate of 2.5 s step^{-1}.