Short-term stability studies of amorphous cyclodextrin complexes prepared by co-grinding

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Abstract: Cyclodextrins (CD) are used for various purposes in the pharmaceutical industry. Conventionally complexes are prepared via solvent methods to encapsulate active pharmaceutical ingredients (API). Co-grinding is an effective alternative way of preparing CD complexes. However, stability of these products is not yet fully studied. Our aim was to execute short-term stability studies of complexes with 2 APIs (Terbinafine HCl and Fenofibrate) and a CD derivative. The results of these tests showed that different products had stable amorphous properties during real-time stability studies and different stability of the amorphous form under accelerated stability tests.

Keywords: milling, XRPD, DSC, recrystallization, Terbinafine, Fenofibrate

1. Introduction

One of the tools to increase the dissolution rate of water-insoluble active substances is reducing their particle size [1]. Grinding devices for this purpose are widely known and can be used in laboratory and industrial settings. However, scientific publications increasingly focus on grinding as a process to induce chemical transformations [2–4]. The use of mechanochemical activation-based reactions allows both the synthesis of raw materials and the production of intermediates (e.g., cyclodextrin (CD) complexes) [5]. CDs are water-soluble cyclic oligosaccharides that can encapsulate poorly soluble drugs in their lipophilic cavities, thereby increasing their solubility and stability [6–8]. As cyclodextrin complexation by milling does not require solvents, it is both environmentally and economically advantageous [9–11].

In our previous work, we prepared amorphous complexes with Terbinafine hydrochloride (TER) and Fenofibrate (FEN) with enhanced in vitro dissolution properties [12,13]. Among the two APIs TER has only one known polymorphic form, while different polymorphic forms and their different physicochemical properties have been described several times for FEN [14,15]. Amorphous nature of a product can modify solubility properties and consequently bioavailability, but these non-crystalline materials tend to recrystallize during storage, so it is important to study the stability of the physical state [16]. Differential Scanning Calorimetry (DSC) and X-ray Powder Diffractometry (XRPD) are important tools in stability studies because they provide valuable information about the crystalline properties of materials. This information is critical for ensuring product quality, regulatory compliance, and long-term stability [17]. In this work, we aimed to prepare complexes based on the previously developed products, and to study samples stored under different conditions. In our study, we used TER and FEN as APIs and dimethyl-β-cyclodextrin (DIMEB) as a CD derivative. Grinding was carried out in a planetary mill and the process was monitored by crystallographic measurements of batch samples. The prepared samples were stored in sealed vials and subjected to real-time and accelerated stability tests.

2. Materials and Methods

2.1. Materials

TER and FEN were kindly donated by Gedeon Richter Plc. (Budapest, Hungary). Heptakis-(2,6-di-O-methyl)-β-cyclodextrin (degree of substitution: 14.00; molecular weight: 1331.0 g mol^{-1}) was obtained from Merck KGaA (Darmstadt, Germany).

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2.2. Methods

2.2.1. Preparation method

Co-ground mixtures of the APIs and DIMEB were prepared by grinding substances in a 1:1 API:CD molar ratio (1:4.1 and 1:3.76 mass ratio for TER and FEN, respectively). Products were prepared with a Fritsch Planetary Micro Mill Pulverisette 7 premium line (Fritsch GmbH, Idar-Oberstein, Germany) planetary mill with a rotational speed of 1000 rpm.

2.2.2. Differential Scanning Calorimetry (DSC)

DSC analysis of each component and product was performed using a Mettler Toledo DSC 821e (Mettler-Toledo GmbH, Greifensee, Switzerland) using STARe software (version 9.30). During the tests, the heating rate was 5 °C min^{-1}, and 10 l h^{-1} of Argon gas was used as the purge gas. The weight of our test samples ranged from 2-5 mg in each case. Tests were performed using a 40 µl covered aluminum sample holder.

2.2.3. X-ray Powder Diffractometry (XRPD) at varying temperatures (HOT-XRPD)

XRPD measurements were performed with a BRUKER D8 Advance diffractometer (Bruker AXS GmbH, Karlsruhe, Germany) equipped with a Våntec-1 line detector system with Cu KαI radiation (λ = 1.5406 Å) over the interval 3-40°/2θ. The measurement parameters were as follows: target, Cu; filter, Ni; voltage, 40 kV; current, 40 mA; time constant, 0.1 s; angular step 0.010°. A low-background silicon sample holder was used in our studies.

The crystallographic changes caused by the temperature change were monitored with the same diffractometer equipped with an MRI Basic hot-humidity chamber (MRI Physikalische Geräte GmbH, Karlsruhe, Germany) controlled by an Ansyco Sycos H-Hot (Analytische Systeme und Componenten GmbH, Karlsruhe, Germany). HOT-XRPD measurements were carried out in the temperature range of 30-240 °C, in 5 °C increments over the interval 3-30°/2θ.

2.2.4. Stability tests

Stability tests were carried out over 3 months of real-time stability studies (at room temperature), and more rapid, so-called accelerated stability evaluations at elevated temperatures and humidity levels (40 °C and 75% relative humidity). The amorphous characteristics were observed through measurements using DSC and XRPD studies.

3. Results and Discussion

3.1. Thermoanalytical analysis

In the case of freshly prepared samples, the DSC thermograms lacked the characteristic melting point associated with the APIs for both drugs (Figure 1a and 1b TER and FEN, respectively). However, they both exhibited a broad endothermic peak at lower temperatures caused by the presence of water content in the product. Additionally, both thermograms displayed an exothermic peak that showed the recrystallization of a substance. These exothermic peaks appeared at distinct temperatures (151 °C and 100 °C for TER:DIMEB and FEN:DIMEB products, respectively). The materials that had undergone recrystallization melted within a temperature range that was closely aligned (195 °C and 185 °C for TER:DIMEB and FEN:DIMEB products, respec-

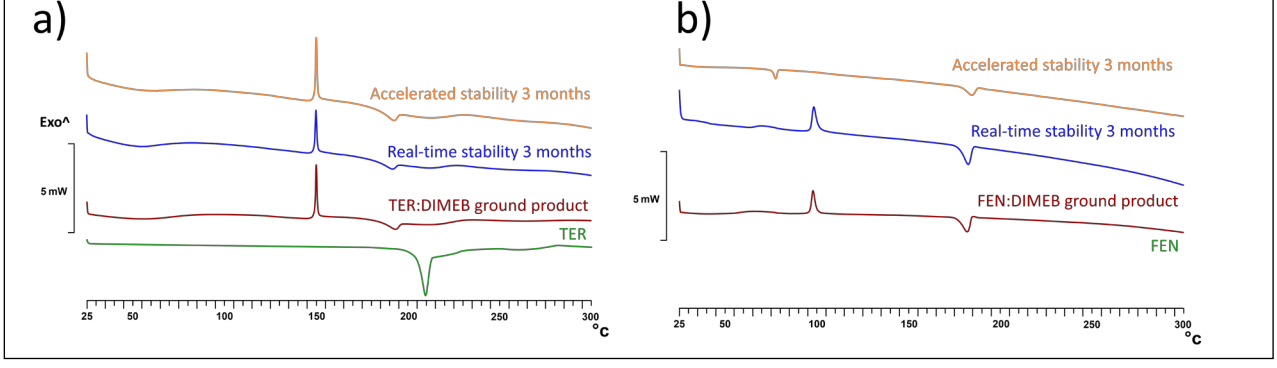

Figure 1 *DSC thermograms of APIs, prepared products, and samples of real-time and accelerated stability tests.*

tively). This suggests that the same crystal structure was formed during the previous recrystallization, at varying temperatures depending on the API content.

The thermal properties of the TER product remained consistent in both real-time and accelerated stability tests. Based on this data, it seems to be a stable amorphous product. The only noticeable change was a slight shift in the melting point of the recrystallized material. In the case of FEN, there were no changes in the real-time stability tests, the thermograms after 3 months were similar of the freshly prepared sample. However, under accelerated conditions, after 3 months the FEN-containing product no longer retained its original thermal characteristics. A melting point around 80 °C, characteristic of FEN, was identified, suggesting that the original polymorph of FEN existed in crystalline form to some extent. Moreover, the previously observed exothermic peak was absent. The melting point that indicated the crystallized unknown product remained at the same temperature. To explore this apparent contradiction, XRPD studies were conducted at room temperature, gradually progressing to higher temperatures.

3.2. Crystallographic analysis

Figure 2 illustrates the diffractograms of the APIs, the freshly prepared ground products, and samples from the 3-month real-time and accelerated stability tests. Additionally, it presents selected diffractograms obtained through HOT-XRPD measurements at varying temperatures for the two products, corresponding to the exothermic peak temperatures noted in the DSC thermograms. In the case of TER (Figure 2a), XRPD examinations revealed that the physicochemical attributes of the product remained unchanged during both real-time and accelerated stability tests over 3 months. The diffractograms of the product indicate its amorphous nature, and this attribute remains stable through the stability tests. HOT-XRPD measurements shows the crystallization of a substance at 175 °C, which is different from the original TER crystal structure as well as all known polymorphic crystal structures of TER.

The FEN product also maintained its stable amorphous form during tests conducted under standard conditions (Figure 2b). However, during accelerated tests, the initially amorphous product exhibited distinct crystalline peaks that were different from those of the original FEN and all known polymorphs. The peaks characteristic of FEN can only be observed to a small extent, but the determination of this is disturbed by the presence of the unknown, recrystallized material.

In alignment with the exothermic peak observed in the DSC thermograms, a similar phenomenon occurred during HOT-XRPD measurements at around 105 °C. Crystalline material emerged, displaying the same peaks as those identified in the accelerated stability test of the FEN product. This resolves the apparent contradiction raised by DSC measurements. The unknown product did not recrystallize during DSC measurements, as it was already present in the sample before the DSC measurement. Additionally, it is evi-

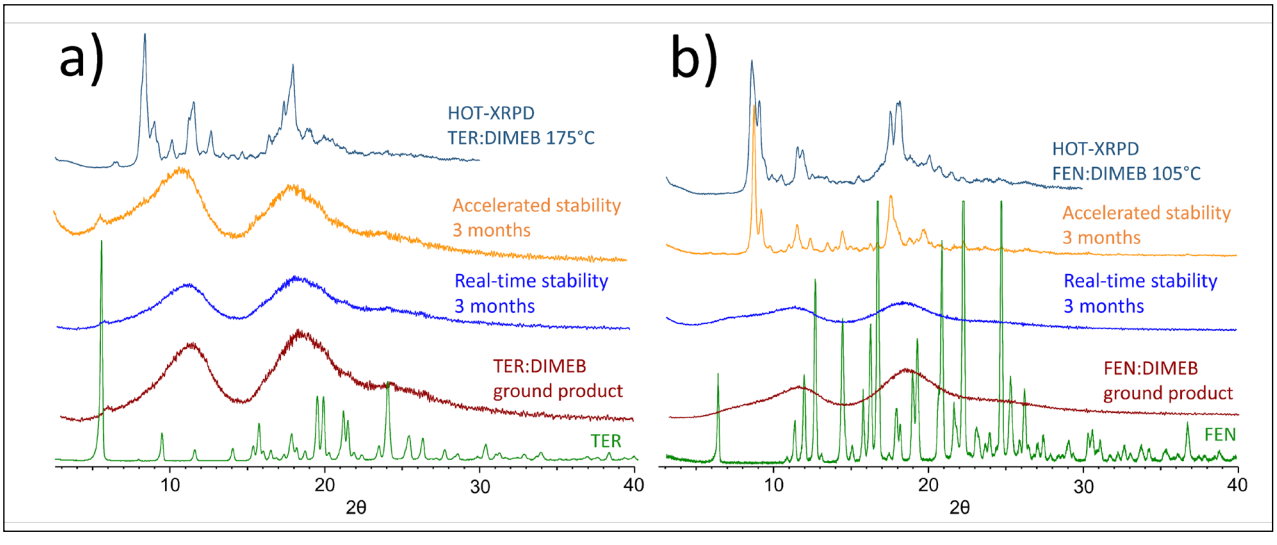

Figure 2 *XRPD diffractograms of APIs, prepared products, samples of real-time and accelerated stability tests, and results of -HOT-XRPD measurements at different temperature points for the different drugs.*

dent that the crystallized product in the case of TER also manifests the same peaks.

The differences observed in the accelerated stability tests of the two products could originate from the fact that the temperature required for FEN product recrystallization closely approaches the temperature employed in the accelerated stability test. Given the prolonged exposure to this slightly lower temperature during the stability test compared to DSC measurements, the product underwent greater thermal stress, prompting recrystallization even at a lower temperature. However, this temperature was not sufficient for achieving recrystallization at a temperature surpassing that of TER during the test.

4. Conclusion

The grinding procedure successfully produced amorphous products with both active compounds. No changes in the thermoanalytical and crystalline properties of the products were observed in the real-time stability studies. The same was observed for TER in the forced stability studies. However, under these conditions, FEN already showed crystalline properties, but the crystallized material did not match the original drug. Thus, the stability of the products prepared with the different active ingredients were different, depending on the API content. This unstable amorphous characteristic may cause changes in dissolution properties and bioavailability during storage at higher temperature and humidity.

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