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Analytical Investigation of Cyclodextrin Complexation using the co-grinding Technique in the case of Terbinafine Hydrochloride

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Abstract: Recent scientific publications have already demonstrated that co-grinding appears as an efficient, solvent-free technique for preparing cyclodextrin (CD) complexes and improving physicochemical properties (solubility, stability, etc.) of active ingredients. The improvement of solubility and dissolution rate could enhance the biopharmaceutical properties of the active ingredients in pharmaceutical products. In this study terbinafine hydrochloride (TER), an antifungal BCS II drug was chosen as a model drug. The aim of this study was the follow-through of inclusion complex preparation by co-grinding using several analytical methods. TER and two amorphous cyclodextrin derivatives (hydroxypropyl-β-cyclodextrin (HPBCD); heptakis-(2,6-di-O-methyl)-β-cyclodextrin (DIMEB)) were used for the preparation of products. Products were analyzed by differential scanning calorimetry (DSC), X-ray powder diffractometry (XRPD), hot-humidity stage X-ray powder diffractometry (HOT-XRPD), Raman spectroscopy, and Fourier transform infrared spectroscopy (FT-IR). Dissolution studies of TER and products were also performed out. DSC and XRPD studies suggested that the crystallinity of products gradually decreased by the increasing grinding time, and after 75 min of co-grinding the products were completely amorphous. HOT-XRPD studies revealed that the amorphous product containing HPBCD did not change with increasing temperature. However, in the same process, the DIMEB containing product recrystallized in a new crystalline phase. Raman and FT-IR spectroscopy confirmed the molecular interactions between the components. Dissolution studies showed that the dissolution rate of complexes improved, and the solubility of TER increased both in simulated gastric and intestinal fluid, depending on the pH of the dissolution medium.

Keywords: cyclodextrin; co-grinding; terbinafine hydrochloride; solvent-free method

1. Introduction

CD-s are used widely in the pharmaceutical industry and other industrial sectors. The shape of CD-s is a truncated cone or torus, due to the chair conformation of the glucopyranose units. The hydroxyl groups are orientated to the cone exterior with the primary hydroxyl groups of the sugar residues at the narrow edge of the cone and the secondary hydroxyl groups at the wider edge. The central cavity of the CD molecule is lined with skeletal carbons and ethereal oxygens of the glucose residue, which gives it a relatively lipophilic character [1]. As a result of this structure, cyclodextrins

can form inclusion complexes with a wide variety of hydrophobic guest molecules. The mechanism for this complexation is based on non-covalent dynamic inclusion complex-formation.

CD-s have received considerable interest due to their ability to form inclusion complexes thereby improving the solubility, stability, and bioavailability of various drugs [2]. The frequently used complexation methods (kneading, spray-drying, precipitation, etc.) require the application of a certain amount of solvent [3]. Several recent studies prove the applicability of solvent-free co-grinding in the case of numerous drugs and CD-s.

Drugs and CD-s subjected to grinding often lead to amorphous products or products containing a relatively low amount of crystalline material. This depends on the grinding parameters (time and intensity) and on the physicochemical properties of raw materials. Generally, complete product amorphization requires longer grinding time and intensity, but crystalline CD-s lead to only partial drug complexation [4].

2. Experiments

2.1. Materials

TER: (E)-N,6,6-trimethyl-N-(naphthalene-1-ylmethyl)hept-2-en-4-yn-1-amine; hydrochloride was kindly donated by Gedeon Richter Plc. (Budapest, Hungary). DIMEB (degree of substitution: 14.00; molecular weight: 1331.0 g mol^{-1}) was obtained from Cyclolab R&D Laboratory Ltd. (Budapest, Hungary). HPBCD (degree of substitution: 4.50; molecular weight: 1500.9 g mol^{-1}) was purchased from Wacker Chemie AG (Munich, Germany).

2.2. Methods

Preparation of co-ground mixtures

Co-ground mixtures of TER and CD derivatives in 1:1 molar ratio were prepared using agate mortar and pestle for 105 min. The actual moisture content of CD-s was considered during the calculation. Suitable quantities of samples were removed at prescribed intervals (15, 30, 45, 60, and 75 min) for further physicochemical evaluation.

Differential scanning calorimetry

The DSC analysis was performed with a Mettler Toledo STAR^e thermal analysis system, version 9.30 DSC 821^e (Greifensee, Switzerland), the heating rate was 5 °C min^{-1}, in the temperature interval 25 – 300 °C, argon was used as a carrier gas (10 L h^{-1}). The sample quantity was in the range 2 – 5 mg and examinations were performed in sealed 40 μL of pans with three leaks.

X-ray powder diffractometry

XRPD diffractograms were recorded with a BRUKER D8 Advance diffractometer (Karlsruhe, Germany) system with Cu K_α radiation (λ = 1.5406 Å) installed with a common sample changer or an MRI Basic hot-humidity stage and a VÅNTEC-1 detector. The samples were scanned at 40 kV and 40 mA, in the interval of 3–40 2θ. All manipulations ($K_{\alpha 2}$-stripping, background removal, and smoothing) were performed with DIFFRACplus EVA software.

Fourier-transform infrared spectroscopy

Spectra were recorded on a Bio-Rad Digilab Division FTS-65A/896 FT-IR spectrometer, using Harrick's Meridian SplitPea single reflection, diamond, ATR accessory. Measurements were performed between 4000 and 400 cm^{-1}, at an optical resolution of 4 cm^{-1}, 256 scans were averaged to increase the signal-to-noise ratio.

Spectral manipulations were performed by using Thermo Scientific GRAMS/AI Suite software. Curve fitting algorithm was applied with Gaussian–Lorentzian function. The best curve-fitting

procedure was achieved by iterative fits toward a minimum standard error. Subtracting atmospheric water vapor superimposed on the sample spectrum was performed using a measured water vapor spectrum.

Raman spectroscopy

Raman spectra were obtained with a Thermo Fisher DXR Dispersive Raman (Waltham USA) equipped with a CCD camera and a diode laser (λ=780 nm). Raman measurements were carried out with a laser power of 12 and 24 mW at 25 μm slit aperture size, using 6 sec of exposure time. The data were collected in the spectral range of 3300 – 200 cm^{-1} using automated fluorescence corrections. OMNIC 8 software was used for data collection, averaging a total of 20 scans. For the removal of cosmic rays, a convolution filter was applied to the original spectrum using Gaussian kernel.

Dissolution rate studies

Studies were performed in simulated intestinal fluid and simulated gastric fluid using modified rotating paddle method. 27.77 mg of TER, or product containing 27.77 mg of TER was measured and added to 100 mL of each medium. Aliquots were withdrawn at 5, 10, 20, 30, 60, 90 and 120 min and immediately filtered (0.22 μm pore size syringe membrane filter). At each sampling time, an equal volume of fresh medium was added, and the correction for the cumulative dilution was calculated. After suitable dilution, the concentration of TER was measured by Unicam UV/VIS spectrometer at 284 nm. There was no absorption of CD-s at the absorption maximum of the TER.

3. Results and Discussion

3.1. Differential scanning calorimetry

DSC data show a sharp peak of TER melting point near 209 °C, also an endothermic peak of DIMEB. No characteristic melting point of the active substance can be detected in the co-ground products, which may indicate rapid complexation or amorphization. For products containing DIMEB, a complex thermoanalytical signal can be detected at a temperature range lower than the melting point of the active substance (150 – 190 °C). In the case of HPBCD, this phenomenon is not observed, but between 220 and 260 °C a broad complex endothermic peak appears.

3.2. X-ray powder diffractometry

The diffractogram of TER contains sharp peaks, suggesting crystalline properties. While amorphous materials, like the applied CD-s, has no well-defined peaks. Physical mixtures prepared without milling show the characteristic peaks of TER, where the intensity corresponds to the weight ratio of the mixture. These peaks are decreasing with the increasing grinding time, and after 75 min of the process, the products are showing completely amorphous properties (Figure 1).

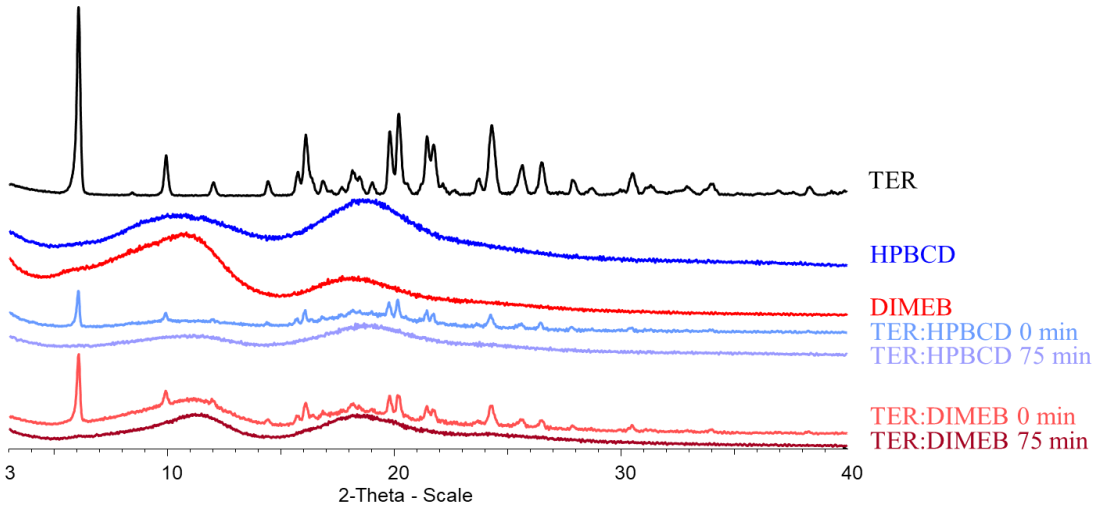


Figure 1. XRPD diffractograms of TER and products.

3.3. Hot-humidity X-ray powder diffractometry

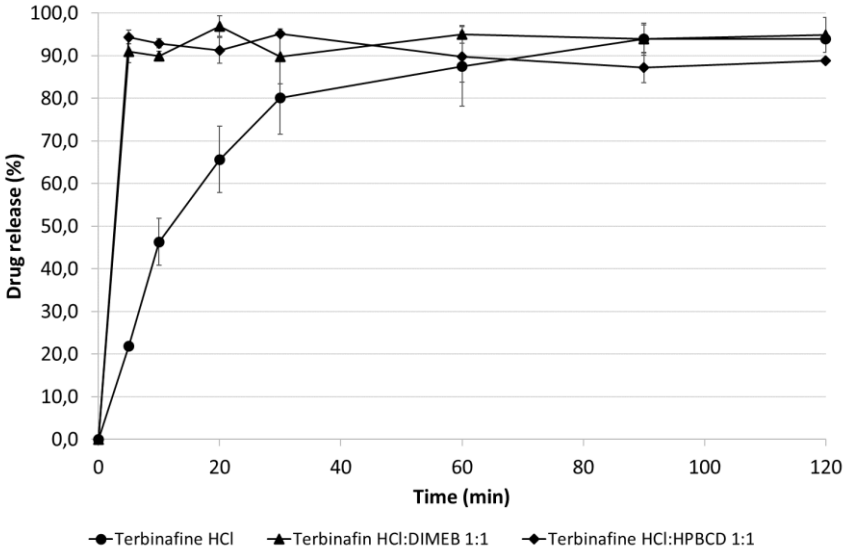
To enlighten the phenomenon shown on the DSC thermograms, XRPD measurements were carried out in a wide temperature range, in hot-humidity chamber. As we have seen, at room temperature products are amorphous, but after heating the DIMEB containing products, sharp peaks showing up at about 135 °C. The characteristic peaks that appeared were not identical to those of TER, which confirmed the appearance of a new crystalline phase. Further heating melts the product completely at about 240 °C. In the case of HPBCD, no such phenomena can be detected. Heating the product and cooling it back down, does not change the amorphous property.

3.4. Fourier-transform infrared spectroscopy

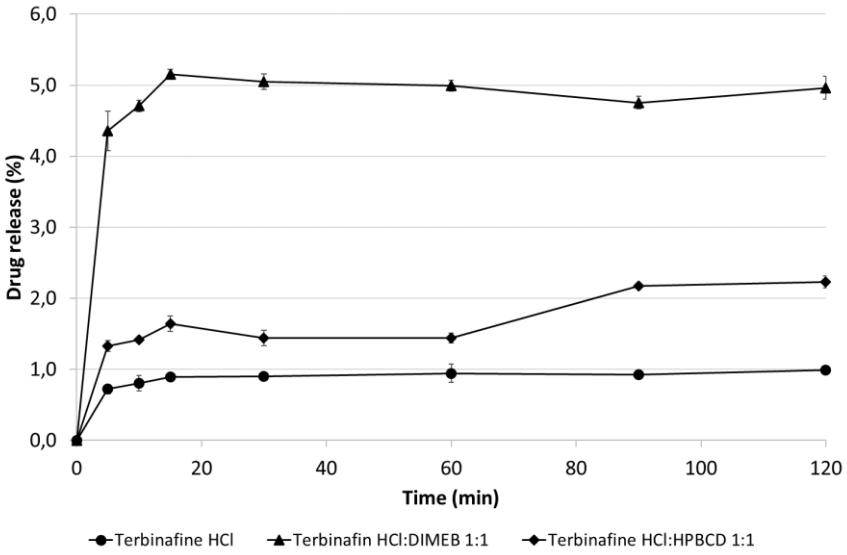
DSC and XRPD results suggested the possibility of inclusion complex formation, in order to verify molecular interactions in detail Raman and FT-IR studies were performed. In this case, we assumed, that FT-IR spectra carry more valuable data, so further analysis and calculations were performed on FT-IR spectra. In order to decompose envelopes to sub-bands, mathematical processes were carried out, like Fourier-transform self-deconvolution and peak fitting. An interesting change happening in the low spectral range. This chosen region refers to the out of plane deformation of the TER aromatic system. In both products, the same two peaks of TER constantly decreasing with the increasing grinding time, and a new band appears between them. The process could be described by a linear equation up to 75 min of co-grinding, after that the relative area of peaks did not change with further increase of the grinding time. Based on these observations, we assume grinding causes complexation between TER aromatic rings and both CD-s via H-bonding. Raman spectra confirmed those observations.

3.5. Dissolution studies

Dissolution studies were carried out based on pharmacopeia methods. In simulated gastric fluid only increasing dissolution rate was achieved, but in intestinal fluid, solubility increased too (Figure 2).



(a)



(b)

Figure 2. *In vitro* dissolution studies of TER and products in (a) simulated gastric fluid and in (b) simulated intestinal fluid.

4. Conclusions

In summary, we have presented a solvent-free technology and the characterization of the process in the case of TER with two CD derivatives. The thermoanalytical behavior was analyzed by DSC and showed that the melting point of the API was not present in the products. For products containing DIMEB, a complex thermoanalytical signal can be detected at a temperature range lower than the melting point of the active substance. Thermograms of the HPBCD containing products did not show these types of peaks. The co-grinding occurred changes in crystalline properties were investigated by XRPD, revealing that the crystallinity of both products constantly decreased with the increasing grinding time. To enlighten these phenomena HOT-XRPD measurements were performed, which revealed recrystallization in case of DIMEB. Chemical interactions between the components were analyzed by Raman spectroscopy and FT-IR. A tendency was observed, which indicated secondary bond formation between components. Dissolution studies of TER and products

were also carried out, proving increased dissolution rate and increased solubility in some cases. Co-grinding has been shown to be useful in improving the dissolution rate of TER in amorphous products containing CD derivatives in pharmaceutical formulation.

Abbreviations

The following abbreviations are used in this manuscript:

CD	cyclodextrin
TER	terbinafine hydrochloride
HPBCD	hydroxypropyl-β-cyclodextrin
DIMEB	heptakis(2,6-di-O-methyl)-β-cyclodextrin
DSC	differential scanning calorimetry
XRPD	X-ray powder diffractometry
HOT-XRPD	hot-humidity stage X-ray powder diffractometry
FT-IR	Fourier transform infrared spectroscopy

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Conflicts of Interest: The authors declare no conflict of interest.

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