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Summary of Ph.D. thesis

Investigation of the Feasibility and Efficiency of Cyclodextrin Complexation via Solvent-Free Co-Grinding with Different Active Substances

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1. INTRODUCTION

The field of green chemistry strives to create chemical products in a sustainable and cost-effective manner, with the dual objective of meeting environmental and economic objectives. It is an innovative area that seeks to balance these two goals. One of the major highlights of green chemistry is minimizing or eliminating the use of solvents, as a significant amount of industrial waste generated from chemical processes is linked to solvent usage. One of the green technologies that can be easily implemented is co-grinding (CG), where mechanical force is communicated to the system using various means. Most of the publications deal with organic chemical syntheses, however, pharmaceutical intermediates from existing active pharmaceutical ingredients (APIs) have also been produced in many cases.

Cyclodextrin (CD) complexation is an intermediate preparation method, that can enhance the physicochemical properties of hydrophobic molecules, making them suitable for a diverse range of applications. Often, the aim is to increase the solubility, dissolution rate, and stability of poorly soluble APIs. Incorporating an auxiliary component during the preparation of CD complexes is an effective approach to improve complexation efficiency and enhance the therapeutic potential of various APIs. While various auxiliary components affect the mechanisms of CDs differently, the use of water-soluble polymers has been demonstrated as the most efficient method to enhance the solubilizing effect of CDs.

Traditional methods for forming molecular complexes with CDs involve using organic solvents, which can lead to additional expenses and disposal issues. Solvent-free methods are becoming increasingly important in the pharmaceutical industry, and various "green" technologies, such as microwave irradiation and mechanochemistry, are being used to create CD complexes. These methods have advantages such as reduced costs and environmental impact. Moreover, recent studies have explored solvent-free methods such as CG, which uses mechanical force to form complexes. This method can result in amorphous or partially crystalline products, depending on the grinding parameters and raw material properties. CG is

Abbreviation: *API – Active Pharmaceutical Ingredient (API); CD – Cyclodextrin; FEN – Fenofibrate; TER – Terbinafine hydrochloride; DIMEB – Heptakis-(2,6-di-O-methyl)-β-CD; HPBCD – (2-hydroxypropyl)-β-CD; SBEBCD – Sulfobutylether-β-CD; XRPD – X-ray powder diffraction; HOT-XRPD – XRPD at variable temperature; DSC – Differential scanning calorimetry; TG – Thermogravimetry; FT-IR – Fourier-transform infrared;; Ph. Eur. – European Pharmacopoeia; HPMC – Hydroxypropyl methylcellulose; PVP – Polyvinylpyrrolidone K-90; SIF – Simulated intestinal fluid; SGF – Simulated gastric fluid; CG – Co-grinding; KN – Kneading; SE – Solvent evaporation; ATR – Attenuated total reflectance; DE – Dissolution efficiency; MDT – Mean dissolution time; PM – Physical mixture; TS – TER:SBEBCD product; TSP – TER:SBEBCD:PVP product; TSH – TER:SBEBCD:HPMC product*

considered a simple and economically desirable method for obtaining CD inclusion complexes without the need for solvents.

A comprehensive evaluation of CD complex production requires a multidisciplinary approach that incorporates a variety of analytical methods to guarantee the successful development and enduring stability of these pharmaceutical products.

2. AIMS

This Ph.D. work aims to prepare CD complexes with different model drugs by a novel, eco-friendly method, characterize this process, and compare it to conventional methods. This research was planned and carried out by the following points:

I) An extensive literature review was carried out on CD complexation especially eco-friendly, solventless preparation methods, which aimed to find less-explored alternative routes of complexation methods. Also, the literature background of different drugs was reviewed to find model drugs that were already proven to be suitable for complexation via conventional methods. Based on this review CG as a preparation method, two model drugs and several CD derivatives were selected. The preparation of amorphous products was aimed for better solubility enhancement.

II) First, the CG of Fenofibrate (FEN) was evaluated. As CD derivative Heptakis-(2,6-di-O-methyl)-β-CD (DIMEB) was selected, because pre-experiments showed that using DIMEB had the best impact on the physicochemical properties of FEN. Physicochemical and in vitro characterization was planned to characterize the product and the whole process also. A comparison of other well-known methods was also applied.

III) Secondly, a similar process and characterization were carried out on CG of Terbinafine hydrochloride (TER) with different CD derivatives. DIMEB and (2-hydroxypropyl)-β-CD (HPBCD) were selected for complexation. TER has pH-dependent solubility, so two different dissolution media were applied during in vitro tests.

IV) Based on the poor results with TER we aimed to prepare complexes with other CD derivative and apply auxiliary components also, to further enhance the positive effects of CDs. Pre-experiments showed that the combination of sulfobutylether-β-CD (SBEBCD) and water-soluble polymers have the best effect on enhancing the properties of the drug. Cytotoxicity of these products was also carried out since per os toxicity of SBEBCD is not yet fully explored.

V) Finally, we aimed to characterize the stability of complexed drugs, because amorphous products tend to recrystallize during storage. Thermoanalytical and crystallographic changes were examined in normal and accelerated stability tests for 3 months.

3. MATERIALS AND METHODS

3.1 Materials

3.2 Active pharmaceutical ingredients

Terbinafine hydrochloride (TER) and Fenofibrate (FEN) was kindly donated by Gedeon Richter Plc. (Budapest, Hungary).

3.3 Excipients

Different type of CD-derivatives was used in the studies discussed below. Heptakis-(2,6-di-O-methyl)-β-CD (DIMEB) was kindly donated by Cyclolab R&D Laboratory Ltd. (Budapest, Hungary). Sulfobutylether-β-CD (SBEBCD) was provided by Sanofi-Aventis Ltd. (Paris, France). (2-hydroxypropyl)-β-CD (HPBCD) was purchased from Wacker Chemie AG (Munich, Germany).

Hydroxypropyl methylcellulose (HPMC) was kindly supplied by Colorcon (Dartford, UK). Polyvinylpyrrolidone K-90 (PVP) molecular weight: 1300000 was received as a gift from Gedeon Richter Plc. (Budapest, Hungary).

For buffer preparation simulated intestinal fluid (SIF) and simulated gastric fluid (SGF) without enzymes were prepared based on Chapter 5.17.1 of the Ph.Eur. (10th edition). For the preparation of SIF (pH 6.8) 77.0 mL of 0.2 M NaOH, 250.0 mL of an aqueous solution containing 6.8 g of KH_2PO_4, and 500 mL of purified water were mixed, pH was adjusted to pH 6.8 and diluted to 1000 mL with purified water. SGF (pH 1.2) was prepared as follows: 1 g of NaCl was dissolved in purified water and 80 mL of 1 M HCl was added; finally, the solution was diluted to 500 mL with purified water. To prepare 1 L of phosphate buffer solution (PBS) 1.44 g of disodium phosphate dihydrate ($Na_2HPO_4 \times 2\ H_2O$), 0.12g potassium dihydrogen phosphate (KH_2PO_4), 8.00 g sodium chloride (NaCl), and 0.20 g potassium chloride (KCl) were dissolved and diluted to 1000 mL with distilled water. All materials used for these solutions were purchased from Sigma-Aldrich (Budapest, Hungary).

3.4 Methods

3.4.1. Product preparation methods

The first critical issue regarding the design of the product preparation reported in this thesis was the determination of the API to CD molecular ratio of the complexes for substances to be investigated. Based on the literature and our own preliminary experiments, complexes with a molecular ratio of 1:1 are formed with both APIs and the CD used. To accurately calculate the substances to be measured, water content was determined for each CD derivative. The moisture contents of the CDs were measured at 105 °C using a Mettler-Toledo HR73 (Mettler-Toledo Ltd., Budapest, Hungary) halogen moisture analyzer.

Each time, after determining the exact materials to be measured, a physical mixture (PM) was prepared, and samples of the PM were taken for later analysis. Different solvent and solvent-free complexation methods were then performed based on these PMs.

To achieve a lightweight and uniform product, an agate mortar was utilized for CG. The time of CG process was determined through preliminary tests. At intervals of 5 minutes, suitable sample quantities were collected for physicochemical analysis. During CG, samples were taken every 5 minutes and XRPD tests were performed simultaneously. Grinding was continued until an amorphous product was obtained. These grinding times varied depending on API and CD, but the final products always had amorphous characteristics. Grinding amorphous materials below their glass transition temperature can produce an amorphous phase while grinding at higher temperatures is more likely to lead to metastable polymorphic forms. Therefore, the CG preparation method was carried out at room temperature and pauses were included at fixed intervals to avoid heating due to friction. The grinding times mentioned in the thesis do not include these pauses, only the time spent on effective grinding.

For the preparation tool of the inclusion complex using the kneading (KN) method mortar was used. However, this time a minimal amount of 50% (v/v) ethanol-water mixture was employed. Once the liquid phase was added, the suspension system was continuously stirred until a significant portion of the liquid had evaporated. The resulting product was subsequently dried in a vacuum drier at room temperature for 24 hours and then finely powdered with care. The solvent evaporation (SE) products were prepared by completely dissolving the PM in 50%(v/v) ethanol-water mixture and evaporating the solvent in a vacuum drier at room temperature for 24 hours.

The production of ternary systems was similar. The only difference was in the calculation of the PM composition. Here, the predetermined amount to be prepared had a given polymer (PVP or HPMC) content of either 5 or 15 w/w%. The remaining 95 or 85 w/w% also contained API:CD in a 1:1 molar ratio, also taking into account the water content.

3.4.2. Differential scanning calorimetry (DSC)

The DSC analysis was performed with a Mettler-Toledo DSC 821e instrument (Greifensee, Switzerland), the heating rate was 5 °C min^{-1}, in the temperature interval of 25–300 °C, argon was used as a carrier gas (10 L h^{-1}). All samples and raw materials were characterized with these parameters, the investigated quantity was in the range of 2–5 mg, and examinations were performed in sealed Al pans of 40 µL with three leaks.

3.4.3. Thermal gravimetric analysis (TG)

Thermogravimetric analysis (TG) was performed with Mettler–Toledo TGA/DSC1 (Mettler–Toledo GmbH, Switzerland) instrument. The weight of the samples ranged from 5.0 to 5.5 mg. Samples were heated from 25 to 300 °C with a heating rate of 10 °C min^{-1}. The evaluation of all measurements was performed with STARe VER 9.30 software for both DSC and TG experiments.

3.4.4. X-ray powder diffractometry (XRPD)

For XRPD measurements a Bruker D8 Advance (Bruker, Brillecia, USA) diffractometer was used. The measurements were performed with Cu-KαI radiation at a wavelength of 1.5406 Å, X-ray tube voltage was 40 kV and 40 mA. Diffractograms were recorded in the angular range of 3-40° (2θ) with a pitch of 0.007° and a time constant of 0.1 s. The obtained data were evaluated with the Bruker DiffracPlus Eva software.

3.4.5. Fourier-transform infrared spectroscopy (FT-IR)

Spectra were recorded on different types of spectrophotometer equipped with attenuated total reflectance (ATR) devices. Measurements were performed between 4000 and 400 cm^{-1}, at an optical resolution of 4 cm^{-1}, and 256 scans were averaged to increase the signal-to-noise ratio. Spectral manipulations were performed by using Thermo Scientific GRAMS/AI Suite software (version 9.0) and Spectragryph - optical spectroscopy software.

3.4.6. In vitro dissolution rate studies

Dissolution rate studies of pure drugs and prepared solid inclusion complexes, and marketed products were carried out. According to European Pharmacopeia dissolution studies

were performed in a dissolution apparatus with a paddle method at 37 °C by applying 100 rpm but in a reduced volume of medium (50 mL). Aliquots were withdrawn and replaced with fresh dissolution medium at given times (5, 10, 20, 30, 60, 90, and 120 min) and immediately filtered (syringe membrane filter with a pore size of 0.22 μm). After proper dilution with the dissolution medium, the concentration of the dissolved drug was determined using a Unicam UV/VIS spectrometer (Thermo Fisher Scientific, Waltham, MA, USA).

Cumulative dilution caused by the medium replaced during sampling has been considered. To quantify the dissolution curves, two metrics were used, dissolution efficiency (DE) and mean dissolution time (MDT), calculated according to the following equations. DE represents the area under the dissolution curve up to a specified time and it is expressed as a percentage of the rectangle area and can be calculated using the equation:

$$\mathrm{DE} = \frac{\int_0^t \mathrm{y}\,\mathrm{dt}}{\mathrm{y}_{100}} 100\% \tag{2}$$

MDT is used to characterize the drug release rate of the APIs and the products, using the following equation:

$$MDT = \frac{\sum_{i=1}^{n} t_{mid} \Delta M}{\sum_{i=1}^{n} \Delta M} \tag{3}$$

3.4.7. Stability tests

The stability of the products was investigated under different conditions because of the expected instability of the amorphous materials. They were investigated at room temperature and for accelerated stability tests: 40 °C and relative humidity of 75% in a KKS TOP+ climate chamber (POL-EKO-APARATURA, Wodzisław Śląski, Poland) for 3 months. Stored products were evaluated by XRPD and DSC methods to reveal changes in the crystal structure, thermal behaviour, and intermolecular interactions.

4. RESULTS AND DISCUSSION

4.1 Complexation of Fenofibrate

The complexation of FEN was experimented with DIMEB and studies were carried out to investigate the complexation effect.

4.1.1. Thermoanalytical evaluation of Fenofibrate products

FEN thermogram contains a sharp peak around 80.5 °C indicating the melting point of the API. The test does not indicate any decomposition of the melted substance at higher

temperatures. On the DIMEB thermogram, a broad and flat drop in temperature range between 25 and 70 °C is observed, which is due to the presence of moisture in the material (Figure 5.a). The thermogram of the PM (0 min grinding) of FEN and DIMEB shows the endothermic peak of the drug's melting point. The presence of the complexing component widens the peak, and the area beneath the curve decreases proportionally to the weight ratio of the two-component product. As the grinding time progresses, the area under the curve corresponding to the drug's melting point diminishes, indicating a gradual reduction in the amount of crystalline drug. After 60 minutes of CG, it becomes challenging to detect the presence of crystalline API (Figure 5.b).

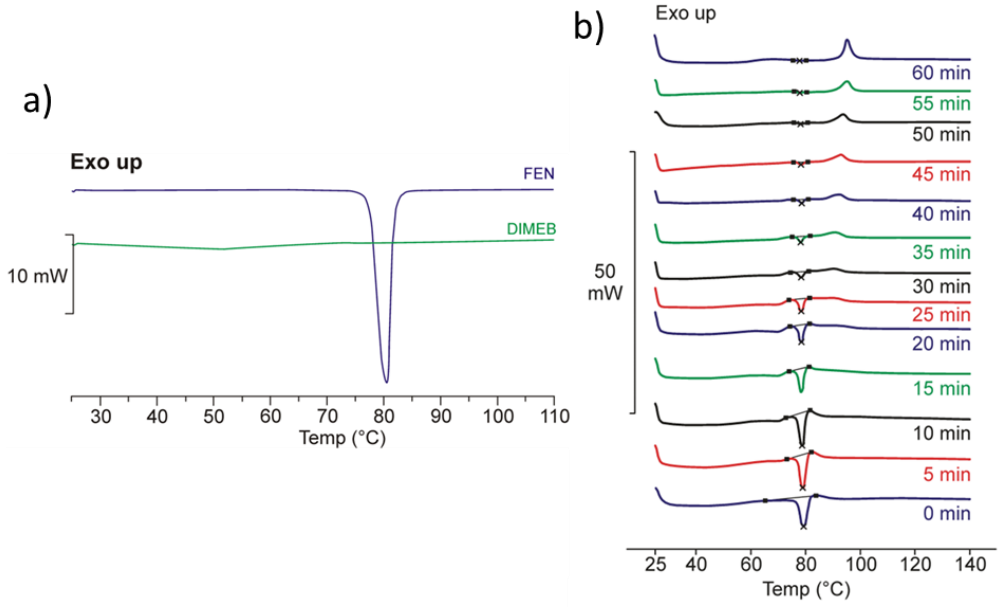

Figure 5. DSC diffractograms of starting materials (a) and CG samples from 0 to 60 min (b).

4.1.2. Crystallographic characterization of Fenofibrate products

XRPD diffractograms were obtained for both the PM and the CG products. FEN exhibited well-defined characteristic peaks, indicating its crystalline nature and belonging to polymorph I. On the other hand, DIMEB displayed a completely amorphous structure with no sharp peaks. The PM of FEN and DIMEB exhibits distinct peaks characteristic of the drug, which overlay the amorphous diffractogram of CD. Through 60 minutes of CG, the intensity of these peaks gradually decreasing, leading to the amorphous nature of the product (Figure 6.). This finding aligns with the continuous decrease and eventual absence of the endothermic peak at the melting point of FEN observed in the DSC studies. The KN method did not succeed in obtaining a completely amorphous product, but a partially crystalline product. (data not shown).

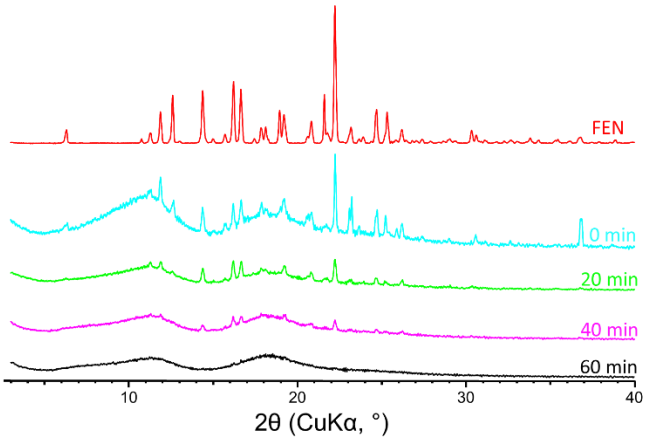

Figure 6. XRPD diffractograms of FEN (polymer I.), PM (0 min), and CG samples are shown every 20 minutes until complete amorphization at 60 minutes.

4.1.3. Vibrational spectroscopic evaluation of Fenofibrate products

The grinding process led to complex changes in the FTIR spectra of the samples, making it difficult to interpret the results using simple methods such as spectral subtraction or more advanced techniques like Fourier deconvolution or peak fitting. However, by constructing a dynamic surface using normalized spectra, it became apparent that the grinding process could be examined in three distinct 20-minute intervals.

The changes in intensity indicated that the behaviour of the peaks could be grouped into three categories based on the grinding time. Some peaks exhibited increasing intensities, while others showed decreasing intensities. Additionally, some peaks reached their maximum intensity around the middle of the grinding time. During the grinding process, it appeared that the first and last 20 minutes were primarily influenced by a single dominant process. In contrast, the middle 20 minutes indicated a combination of multiple processes occurring simultaneously.

4.1.4. In vitro studies of Fenofibrate products

Dissolution studies were carried out using modified pharmacopeia methods in SIF. Figure 8 illustrates the dissolution curves of the pure drug and its equimolar products. FEN on its own demonstrated minimal solubility in this aqueous solution. All binary systems exhibited improved dissolution properties compared to the pure drug. The PM showed enhanced solubility compared to the API alone, attributed to the presence of a small concentration of dissolved CD in the medium. Nevertheless, this improvement was limited. In contrast, the KN

and CG products achieved a much greater increase in solubility. The CG product exhibited a faster dissolution rate and slightly better solubility compared to the kneaded product.

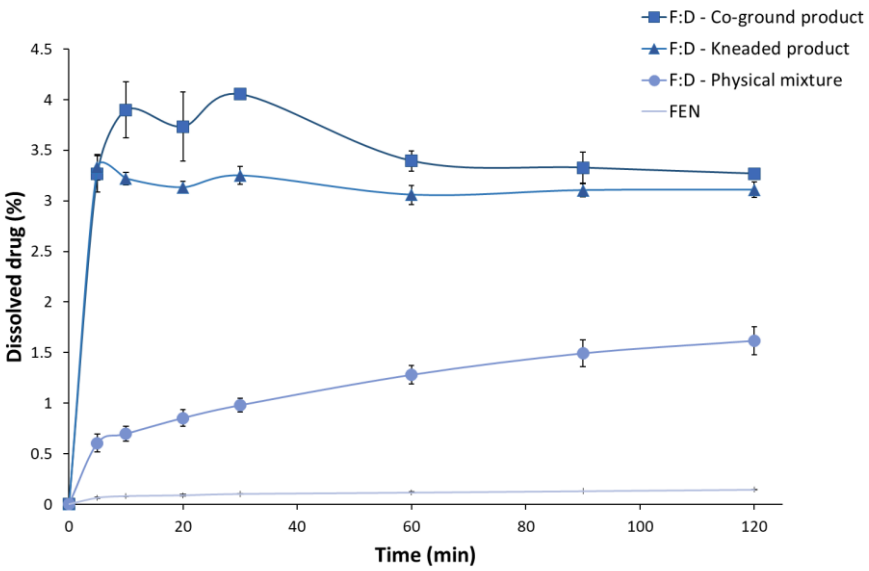

Figure 8. Dissolution curves of FEN, PM, KN, and CG products in SIF.

4.2 Binary systems of Terbinafine HCl with cyclodextrins

This part of the thesis aimed to prepare CG products and follow through the preparation process of the CG method with analytical tools in the case of TER with HPBCD and DIMEB.

4.2.1. Thermoanalytical characterization of binary systems of Terbinafine HCl

Figure 9. displays the DSC thermograms of TER, DIMEB, HPBCD and the samples obtained after 105 minutes CG process with these materials. At lower temperatures, both CDs exhibit a broad endothermic signal (25–85 °C), indicating the presence of water in the CD derivative. DIMEB also exhibits an exothermic peak at 187 °C, indicating a crystallographic phase transition. The DSC data reveals a distinct peak corresponding to the melting point of TER near 209 °C, TER degradation at higher temperature. Products containing DIMEB show a complex thermoanalytical signal below the melting point of the API. This peak broadens with longer grinding time and can be detected in the final product at 150–190 °C. In the case of HPBCD, this phenomenon is absent, but a broad complex endothermic peak appears between 220 and 260 °C, likely associated with the degradation of the API.

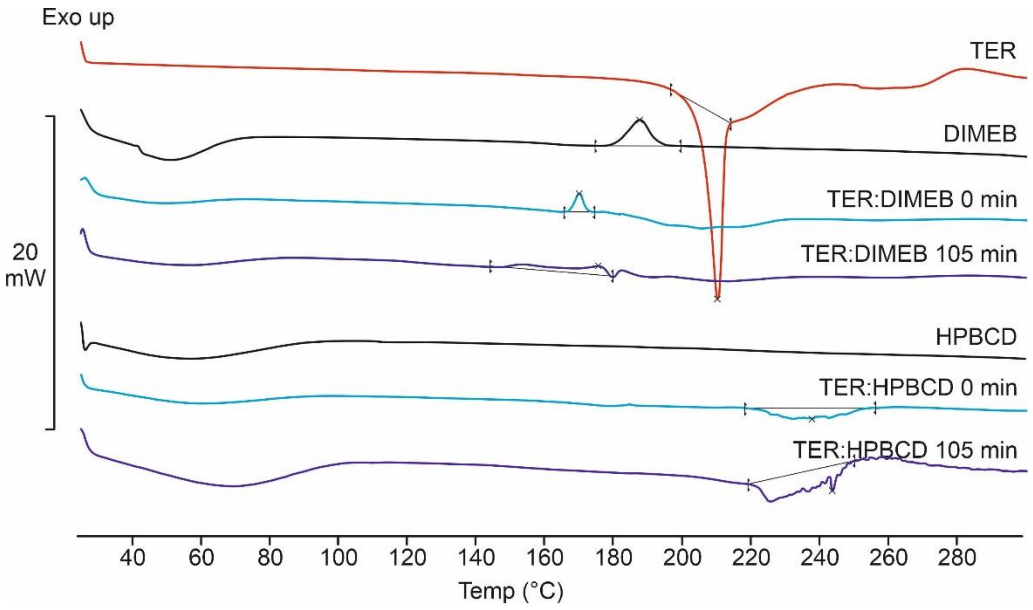

Figure 9. DSC thermograms of raw materials, PM (0 min), and ground products containing DIMEB and HPBCD.

4.2.2. Crystallographic characterization of binary systems of Terbinafine HCl

Only the XRPD diffractogram of TER exhibited distinct peaks, indicating crystallinity, while CDs, being amorphous materials, lacked well-defined peaks in their diffractograms. The peak intensity seen in the diffractogram of the PMs decreased as the grinding time progressed, and with 105 minutes of the process, the diffractograms exhibited the absence of well-defined peaks. The formation of amorphous character was observed with both CDs (Figure 10).

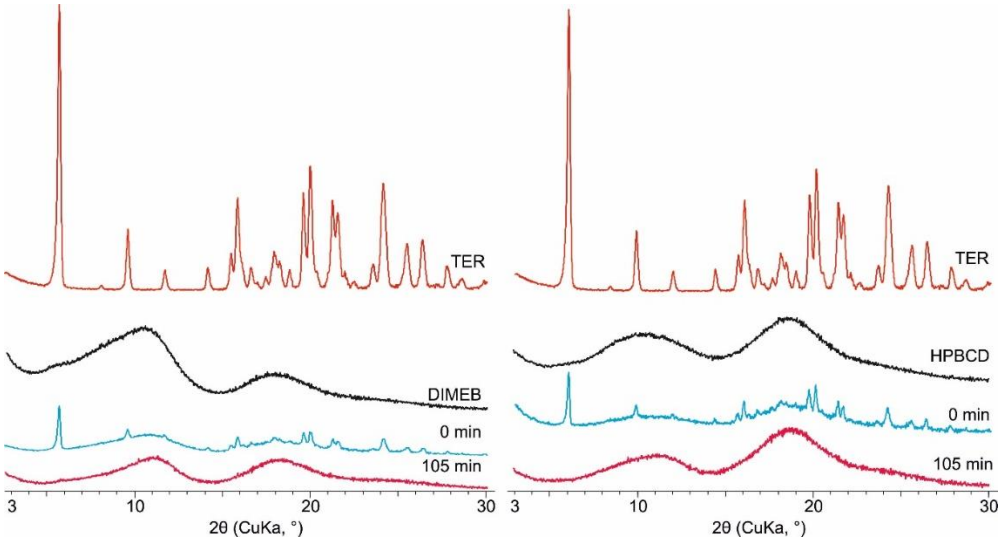

Figure 10. XRPD diffractograms of raw materials, PMs, and final CG products (105 min) containing DIMEB (a) and HPBCD (b).

4.2.3. Vibrational spectroscopic evaluation of binary systems of Terbinafine HCl

The infrared spectra of the samples, obtained at different grinding times, showed noticeable changes. The broad N-H stretching band of the ammonium group gradually decreased and became indistinguishable from the baseline after 30 minutes. Moreover, the characteristic bands of TER mostly vanished, became broader, and merged with the broad bands of the product, regardless of whether DIMEB or HPBCD was used. Among the spectral regions, the progress of product formation could be tracked for a longer duration in the out-of-plane vibrations of the aromatic rings between 820-675 cm^{-1} (Figure 11.).

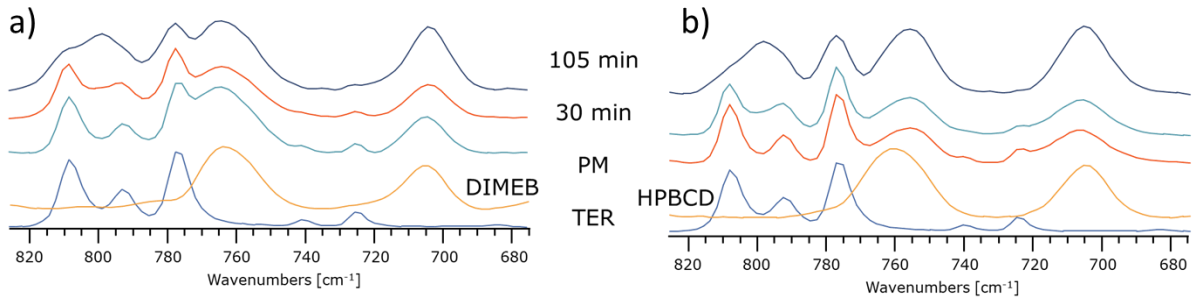

Figure 11. Selected spectra between 820-675 cm^{-1} FTIR spectra of the starting materials raw materials, PMs, intermediate (30 min), and final CG products (105 min).

4.2.4. In vitro studies of binary systems of Terbinafine HCl

The solubility of the TER is highly pH-dependent, with lower solubility observed at higher pH levels, resulting in incomplete dissolution or precipitation and reducing bioavailability. This can be a concern for TER, particularly at high doses. In SIF, the solubility of TER is below 1%. The use of CDs demonstrated a solubility-enhancing effect on TER. However, the extent of this enhancement varied depending on the CD used. The TER product containing DIMEB showed a significant increase in DE to 4.81±0.09% by 120 minutes, and a higher dissolution rate indicated by the MDT value. On the other hand, the HPBCD product exhibited a lesser increase in DE to 1.70±0.02%, accompanied by a lower dissolution rate compared to the pure drug (Table 5). In SGF, the solubility of pure TER reached nearly 100%. Both CD had a positive impact in this scenario, enhancing the dissolution rate of the API as evidenced by the decreased MDT values (Table 5).

Table 5. DE and MDT values in SIF and SGF.

	Simulated intestinal medium			Simulated gastric medium		
	DE_{60min}	**DE_{120min}**	**MDT**	**DE_{60min}**	**DE_{120min}**	**MDT**
TER	0.85±0.05	0.90±0.04	18.88±7.49	68.57±6.91	80.45±5.19	18.35±2.69
TER:DIMEB	4.75±0.09	4.81±0.09	11.02±2.00	88.68±1.50	91.54±1.02	12.54±6.08
TER:HPBCD	1.40±0.04	1.70±0.02	28.86±1.63	88.80±0.84	88.54±1.32	7.69±5.68

4.3 Ternary systems of Terbinafine HCl with cyclodextrins and water-soluble polymers

The TER:SBEBCD:PVP (TSP) and the TER:SBEBCD:HPMC (TSH) products obtained by different methods have been prepared and evaluated in this part. The 2 polymers were used in 5 and 15 w/w%, and the 3 methods were applied for all compositions. As a combination of these compositions and methods, 15 products were prepared. Abbreviation of these products in the thesis contains the materials (TSP, TSH), preparation method (SE, KN, CG) and percentage of applied polymer (5 or 15%)

4.3.1. Thermoanalytical characterization of ternary systems of Terbinafine HCl

DSC data (Figure 13) displayed a distinct peak at approximately 209 °C for TER, followed by decomposition at higher temperatures. The DSC curve of SBEBCD demonstrated the amorphous nature of the CD derivative. PVP exhibited a significant endotherm peak between 40 and 110 °C, indicating water loss. HPMC exhibited a similar thermal profile, with a broad endothermic transition around 60 °C. The disappearance of the melting point of the crystalline drug in the DSC curves of the presumed complexes provides (one example showed in Figure 13.) evidence of the drug molecule being incorporated into the CD cavity.

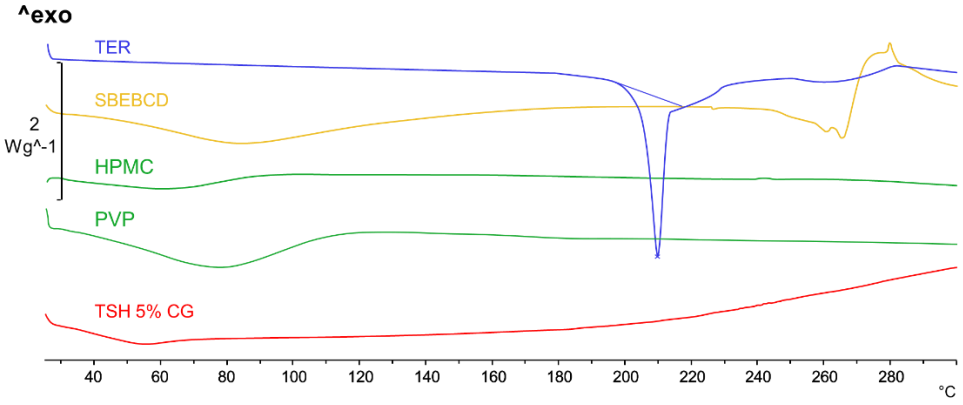

Figure 13. DSC thermograms of starting materials and TSH CG 5% as a representation of all amorphous products.

4.3.2. Crystallographic characterization of ternary systems of Terbinafine HCl

XRPD patterns on the powder of the individual components and the products were compared. The preparation methods of the complexes using SE and CG resulted in amorphous products with both polymers. However, the obtained products using the KN exhibited characteristic peaks of TER, indicating only partial loss of crystallinity. Generally, the percentage of polymer used did not influence the crystalline nature of the products.

Amorphization of the products was achieved through the continuous energy supply during the grinding process. Increasing the grinding time led to a greater loss of crystallinity, with the characteristic peaks of the drug showing decreased intensity. The time required for amorphization was different. For example, the product containing 5 w/w% PVP required 70 minutes of grinding for complete amorphization, while all other products achieved it in a shorter time of 30 minutes. This behavior was observed across all systems, regardless of the polymer used in the preparation of the ternary system (Figure 14).

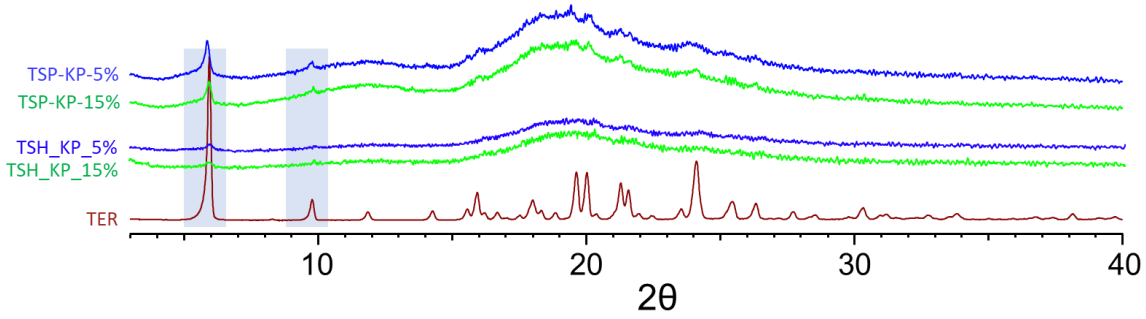

Figure 14. XRPD diffractograms of TER and KN products with highlighted crystalline peaks.

4.3.3. Vibrational spectroscopic evaluation of ternary systems of Terbinafine HCl

The FTIR analysis provided additional insights into the intermolecular interactions between TER and the excipients. In the CG products, a broad band corresponding to the amine group at 2446 cm^{-1} was present, which disappeared in SE and KP products. The aromatic out-of-plane bands (in the range of 820-760 cm^{-1}) and the aromatic C=C bond band (1514 cm^{-1}) did not exhibit any shifting or decrease in intensity. The band related to the aliphatic C=C group (1631 cm^{-1}) was not detectable in the products due to the presence of a broad band attributed to the CD in the same region. The trans-substituted olefin group band at 958 cm^{-1} was only detected in the CG products, although reduced and shifted. Other C=C bands associated with stretching vibration at 1450, 1318, and 1248 cm^{-1} either completely disappeared or, in the case of CG products with HPMC, significantly reduced and shifted (Figure 17.c). However, for PVP, interpreting interactions using the band at 1318 cm^{-1} was challenging due to the presence of a broad band in this spectral range associated with PVP itself.

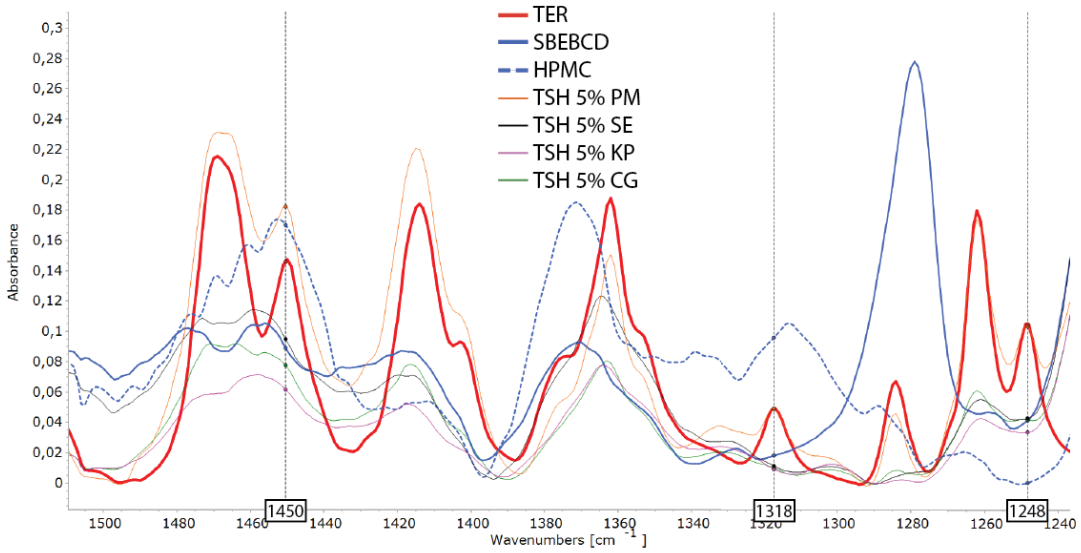

Figure 17. FTIR spectra of TSH products and corresponding raw materials and their PM. C=C bands associated with stretching vibration at 1450, 1318, and 1248 cm^{-1}.

There are some differences in spectral changes depending on the polymer used. For aromatic C-H deformation bands referring to the methyl and methylene groups at 2968 cm^{-1} disappearance of the peak can be seen in the spectra of TSP SE products and shifting in TSP KP and CG products, while there were no changes in the spectra of HPMC-products. Similarly, shifting of t-butyl axial deformation (1361 cm^{-1}) can be detected in all PVP products, in HPMC

KP and CG products no changes occurred, except for shifting bands of TSH SE products. Overall, the amount of polymers used did not affect the interactions that occurred, so for better transparency, only products containing 5% of polymer are shown in Figure 17.

Physicochemical characterization showed no difference between products with 5% and 15% polymers, so further tests were carried out with 5% polymer-containing products.

4.3.4. In vitro studies of ternary systems of Terbinafine HCl

In vitro dissolution studies were performed to assess the dissolution performance of the formulations in comparison to the pure drug, binary system, and pulverised commercial product Terbisil® tablet. Various pH conditions were tested in the dissolution studies to comprehensively understand the release profiles of TER. In the SIM dissolution experiments, the DE was calculated at 120 minutes (Figure 18.). Pure drugs and Terbisil® exhibited DE of less than 1%, which increased to around 8% when using SBEBCD alone. PVP products did not further enhance the DE, indicating no impact of this polymer on solubility improvement. However, HPMC products approximately doubled the DE, with variations among the different products. Among them, CG products showed the highest DE value, reaching approximately 20%.

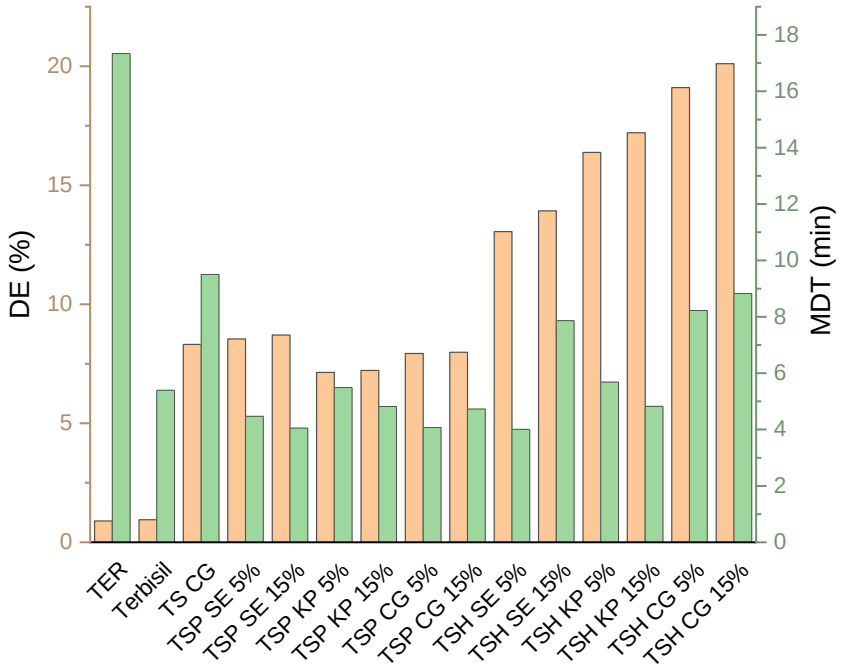

Figure 18. DE values at 120 minutes were measured in SGF and MDT values were measured in SIF.

In lower pH, in the SGM dissolution of TER results in increased dissolution. Over 90% of the pure drug dissolves within 60 minutes. However, the products achieve this level of dissolution in a shorter time, indicating a higher dissolution rate. To measure this phenomenon, the MDT was calculated (Figure 18.). A lower value of MDT means a faster dissolution rate. The MDT reveals a longer dissolution time for TER compared to each product, including the pulverised marketed product. However, none of the products exhibit a significantly lower MDT than the binary system.

4.4 Stability tests of prepared co-ground products

We have shown above that inclusion complexes were formed during CG with the applied substances and that the properties of the APIs were modified. One of the most important of these changes was the formation of amorphous products from crystalline APIs. Since such products are prone to recrystallization over time, stability studies were performed. DIMEB complexes of the two tested drugs were selected from the previous products prepared by CD grinding. Stability studies were performed for 3 months under normal conditions at room temperature and accelerated stability studies at higher temperatures and humidity. The amorphous properties were most effectively monitored by XRPD measurements.

4.4.1. Crystallographic characterization during stability tests

The Figure 21. shows the diffractograms of the APIs used, the freshly prepared CG products, and the samples of 3-month accelerated stability tests.

In the case of TER, we also found during XRPD tests that the physicochemical properties of the product did not change either under normal or elevated conditions during 3 months. The diffractograms of the product show that the products remained amorphous.

The stable amorphous form was also characteristic of the FEN product during the tests carried out under normal conditions. However, during accelerated tests, the initially amorphous product showed crystalline peaks that were different from those of the original FEN and every known polymorph of the API. 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. We can conclude that based on both DSC and XRPD measurements the TER product has stable amorphous properties, while the FEN products are less stable at high temperatures and humidity due to the low-temperature crystallization process.

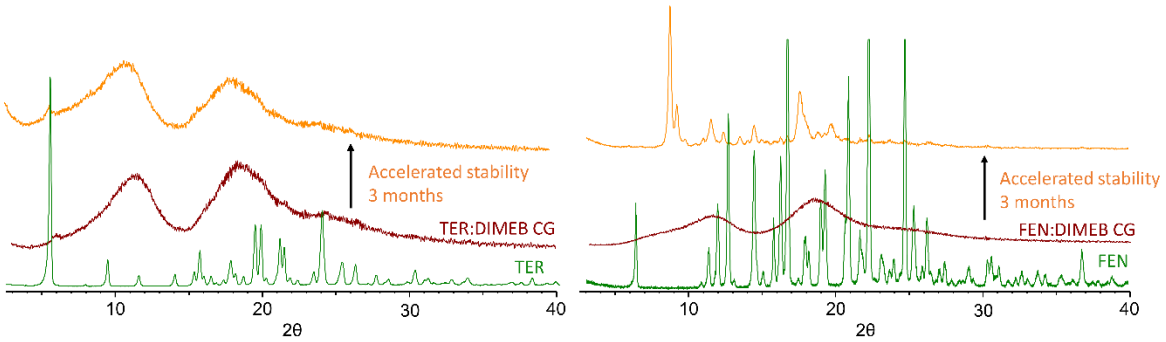

Figure 21. Diffractograms of the APIs (TER: left, FEN: right) used the freshly prepared CG products and the samples of 3-month accelerated stability tests.

5. CONCLUSION

The novel results of this Ph.D. work aim to prepare CD complexes of the applied APIs in the novel, solventless method and compare conventional methods, that is summarized in the following points:

I) During this work co-ground CD complexes were prepared using FEN and TER. CD complexation is a widely studied area of pharmaceutical technology. However, despite the advantages of the method, there are only a few studies dealing with solventless, CG complexation.

II) FEN complexes were prepared with DIMEB. Based on the DSC thermograms, the disappearance of the melting point associated with the API indicates the presence of amorphous properties. This finding was further supported by the XRPD measurements, which revealed the formation of amorphous products via grinding and solvent method, while KN produced a crystalline and amorphous mixture. Changes in the FTIR spectra indicated a three-stage process, proving the fact of complexation. Dissolution studies showed increased DE for the products. Based on these findings, XRPD and DSC studies can be useful in monitoring the degree of complexation during grinding when intermolecular interactions between materials have been characterized using other methods.

III) TER-containing CD complexes were successfully prepared by grinding API and two kinds of CD derivatives. The DSC thermograms do not show the characteristic melting point of the drug, suggesting interactions between the components. The XRPD measurements also showed that the formation of presumed complexes is a time-consuming process leading to

practically amorphous products. FTIR results confirmed that TER with DIMEB or HPBCD formed an inclusion complex through the unsaturated alkyl-chain of TER by simple grinding. Dissolution studies showed that each product has a higher dissolution rate in both applied mediums and that each product has a higher solubility than the API in SIF.

IV) Complexation of TER with SBEBCD and the modifying effects of two different polymers, PVP and HPMC were investigated. Two concentrations of the polymers, 5, and 15 w/w%, were used. The ternary complexes were compared with the binary complex, drug alone, and commercial tablet formulation of TER. FTIR measurements showed that different methods of obtaining ternary complexes and different polymer excipients had varying effects on the non-covalent bond formation between the drug and CD. These interactions influenced thermoanalytical and in vitro behaviors. Overall, both polymers positively modified the complex properties, with no significant difference observed between the polymer concentrations used. The 5 w/w% systems exhibited similar synergistic effects and did not affect the trend of crystallinity decrease compared to the 15 w/w% systems. This suggests that a lower polymer concentration is sufficient to achieve the same effect. Additionally, the 15 w/w% polymer product contained a lower CD concentration, which has economic advantages. By adding a larger amount of cheaper polymer, the relatively expensive CD derivative, and API can be reduced. These findings indicate that polymers can enhance bioavailability and should be considered in a future product design of these materials, taking into account the lower material cost associated with producing complexes.

V) During real-time stability studies no changes were observed in the crystalline properties of the products. Similar phenomena were observed for TER in the accelerated stability tests. However, the FEN products showed crystalline properties under these conditions. However, this crystallized material did not match the initial FEN or any polymorphs of FEN.

The main new findings and practical aspects of the work:

1. FEN complexes with DIMEB were prepared via CG for the very first time, with a comprehensive analytical evaluation.
2. For the first time, binary complexes of TER with DIMEB and HPBCD were prepared using the CG technique, and a comprehensive analytical evaluation was performed.
3. Using the CG technique, ternary complexes of TER with SBEBCD and polymers (PVP and HPMC) were successfully prepared for the first time, followed by a comprehensive analytical evaluation.

4. We utilized a range of analytical methods to assess the CG method and establish a rapid screening protocol essential for the CG procedure with CD binary and ternary systems.
5. However, applied APIs were model drugs, with improved physicochemical properties suggesting possible “Supergenerics” can be formulated in the future.

PUBLICATIONS

(1) **Kondoros, B. A.**; Jójárt-Laczkovich, O.; Berkesi, O.; Szabó-Révész, P.; Csóka, I.; Ambrus, R.; Aigner, Z. Development of Solvent-Free Co-Ground Method to Produce Terbinafine Hydrochloride Cyclodextrin Binary Systems; Structural and In Vitro Characterizations. Pharmaceutics 2022, 14 (4), 744. https://doi.org/10.3390/pharmaceutics14040744. **Q1 IF = 5.4 (2022)**

(2) **Kondoros, B. A.**; Berkesi, O.; Tóth, Z.; Aigner, Z.; Ambrus, R.; Csóka, I. Cyclodextrin Complexation of Fenofibrate by Co-Grinding Method and Monitoring the Process Using Complementary Analytical Tools. Pharmaceutics 2022, 14 (7), 1329. https://doi.org/10.3390/pharmaceutics14071329. **Q1 IF = 5.4 (2022)**

(3) **Kondoros, B. A.**; Kókai, D.; Burián, K; Sorrenti, M.; Catenacci, L.; Csóka, I.; Ambrus, R. Ternary cyclodextrin systems of terbinafine hydrochloride inclusion complexes: Solventless preparation, solid-state, and in vitro characterization. Heliyon 2023. https://doi.org/10.1016/j.heliyon.2023.e21416 **Q1 IF = 4.0 (2023)**

(4) **Kondoros, B. A.**; Csóka, I.; Ambrus, R. Short-term stability studies of amorphous cyclodextrin complexes prepared by co-grinding. Acta Pharmaceutica Hungarica 2023. Accepted for publication. **Q4 IF = -**

PRESENTATIONS

<u>Oral presentations</u>

1. **Kondoros, B. A.**; Aigner, Z. – Physicochemical characterization and dissolution studies of terbinafine hydrochloride–cyclodextrin complexes prepared by solvent-free co-grinding. II. Symposium of Young Researchers on Pharmaceutical Technology, Biotechnology and Regulatory Science, Szeged, Jan 2020

2. **Kondoros, B.A.**; Laczkovich, O.; Berkesi, O.; Aigner, Z. – Analytical investigation of organic solvent-free co-grinding technique in terbinafine hydrochloride cyclodextrin complexation. Congressus Pharmaceuticus Hungaricus XVI., Debrecen, Sept 2020

3. **Kondoros, B. A.**; Aigner, Z. – Evaluation of fenofibrate-cyclodextrin complexes prepared by co-grinding method. III. Symposium of Young Researchers on Pharmaceutical Technology, Biotechnology and Regulatory Science, Szeged, Jan 2021

4. **Kondoros, B.A.**; Laczkovich, O.; Berkesi, O.; Aigner, Z. Analytical Investigation of Cyclodextrin Complexation Using the Co-Grinding Technique in the Case of Terbinafine Hydrochloride. 1st International Electronic Conference on Pharmaceutics Proceedings 2021, 78, 19. https://doi.org/10.3390/IECP2020-08714

5. **Kondoros, B. A.**; Berkesi, Ottó, Aigner, Z. – Fenofibrát tartalmú ciklodextrin-komplexek előállítása oldószer mentes technológiával és a folyamat analitikai jellemzése. Szent-Györgyi Albert Konferencia, Budapest, Apr 2021

6. **Kondoros, B. A.**; Aigner, Z.; Csóka, I.; Ambrus, R. – Együtt-őrléssel előállított fenofibrát tartalmú ciklodextrin-komplexek vizsgálata. Magyar Kémikusok Egyesülete - Kerekasztal Konferencia, Balatonszemes, Dec 2021

7. **Kondoros, B.A.**; Sanzeri, G.; Bonferoni, C.; Sorrenti, M.; Csóka, I.; Ambrus, R. – Ternary Systems of Terbinafine Hydrochloride Inclusion Complexes: Preparation, Solid State Characterization, Dissolution Studies. IV. Symposium of Young Researchers on Pharmaceutical Technology, Biotechnology and Regulatory Science, Szeged, Jan 2022

8. **Kondoros, B.A.**; Sorrenti, M.; Csóka, I.; Ambrus, R. – Solid-state and in vitro characterization of co-ground ternary systems of Terbinafine hydrochloride. International Cyclodextrin Symposium, Giardini Naxos (Italy) Jun 2022

9. **Kondoros, B. A.**; Szabó-Révész, P.; Csóka, I.; Ambrus, R. – Háromkomponensű, hatóanyagtartalmú ciklodextrin-zárványkomplexek előállítása és jellemzése fizikai-kémiai és in vitro módszerekkel. Gyógyszerkémiai és Gyógyszertechnológiai Szimpózium, Herceghalom, Sept 2022

10. **Kondoros, B.A.**; Csóka, I.; Ambrus, R. – Investigation of the Feasibility and Efficiency of Solvent-Free Co-Grinding with Different Active Substances. V. Symposium of Young Researchers on Pharmaceutical Technology, Biotechnology and Regulatory Science, Szeged, Jan 2023

11. **Kondoros, B. A.**; Csóka, I.; Ambrus, R. – Különböző hatóanyagok ciklodextrines komplexeinek oldószermentes ko-őrléses előállítása és vizsgálata. Magyar Kémikusok Egyesülete - Kerekasztal Konferencia, Balatonszemes, Jun 2023

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