Synthesis and Physico-chemical Characterization of Theophylline-succinic Acid Binary Adduct

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Solubility and bioavailability of active pharmaceuticals can be modified by cocrystal formation. Theophylline is known as a co-formator in a usual reaction conditions. The purpose of this paper was the synthesis and physico-chemical characterization of a new cocrystal formed between theophylline and succinic acid.

Keywords: co-crystal; theophylline; succinic acid; thermal analysis; Heat Flow

The efficiency of an active pharmaceutical ingredient (API) is dictated by its properties, such as physicochemical properties, dissolution rate and bioavailability [1]. Considering the fact that currently only about 8% of the molecules tested to enter the market have both high permeability and solubility and more than 40-60% of the APIs have been reported to have water solubility issues, improving this properties is crucial in the pharmaceutical field [2]. Therefore, one of the main focus points in the research area nowadays is finding and formulating a thermodynamically-stable crystalline form of APIs that has good physiochemical properties, but also an enhanced water solubility [2].

In order to successfully improve these properties, salt formation and cocrystallization were highly studied up to the date. While salts can be defined as crystals that contain ionic interactions, co-crystals are formed by non-covalent interactions (hydrogen bonds, p-p packing and/or van der Waals intermolecular bonds) [1]. Due to the fact that acid-base pairing is not always a viable option, non-ionizable molecules being incapable of salt formation [3], studies were also focused on co-crystal formation. Co-crystals are crystalline structures that contain two or more different molecules in a predicted stoichiometric ratio [4], but in comparison with the "classical" salts, their formation depends on the mutual recognition between the API and the co-former [3].

The importance of pharmaceutical co-crystals relates not only with the enhancement of the APIs solubility and therefore in its bioavailability, but at the same time, they allow the pharmaceutical companies to address the intellectual property and new patent of APIs for extending their life cycle [4]. Therefore, a large number of research publications and patent applications have been brought to light in the last years, expanding the approaches and techniques used to successfully obtain these co-crystals.

There are a variety of methods used in the preparation process of co-crystals, such as slow evaporation from a solution, solvent-drop grinding, dry grinding or growth from the melt, but usually the final compound does not depend on the preparation technique. There are also a number of other procedures that can be used, including spontaneously forming under appropriate conditions [3].

Theophylline is a methyl-xanthine drug that belongs to the pharmacological class of bronchodilators used in therapy for respiratory diseases such as chronic obstructive pulmonary disease (COPD) and asthma. Alongside its bronchodilator action, a great importance is given to its anti-inflammatory and immunomodulatory properties. One of the most important molecule used for the treatment of asthma, theophylline is well known for its very poor water solubility (0.5%). Due to this disadvantage, and to the fact that its oral absorption rate is often influenced by food, theophylline has become one of the most studied molecules in the research field [5].

There are a number of techniques that were applied to achieve an increase in its physicochemical properties, manly its solubility. So far, good results were obtained using salts complexes such as aminophylline, a complex of theophylline with ethylenediamine in 2:1 ratio. However, the stability of this compound leaves a lot to be desired, due to the low alkalinity of theophylline (pK_a = 8.81) and the large number of patients that have accused a higher sensitivity to this complex in relation to the API. This led to a change in the research direction, transferring the focus from the salt complexes to a new domain for this substance, co-crystals [6,7].

There are several crystallographic structures of co-crystals of theophylline in the Cambridge Structural Database. Mainly, the co-formers are dicarboxylic acids with a chemical structure that allows the compound formation, while maintaining the new molecule pharmaceutically acceptable [6].

The main goal is that the resulting crystal form possesses a new physicochemical profile that leads to improvement of the solubility and/or physical stability of the API.

Considering the fact that there are quite few possible choices for the co-former molecule, a rational approach to co-crystal design is required to maximize experimental efficiency. Therefore, it is important to take into account the potential intermolecular interactions and, at the same time, the general hydrogen bonding rules [8] that may be involved in the formation of the new co-crystal.

Due to the great importance of theophylline in therapy nowadays, researchers have developed a number of co-

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crystals, all meant to improve solubility, drug stability, manufacturing processability, bioavailability, and acceptance by patients [6]: CSD reference codes CSATEO (with 5-chlorosalicylic acid); DUXZAX (with urea); SULTHE (with sulfathiazole); THOPBA (with phenobarbital); TOPPNP (with p-nitrophenol);WUYROX (with N-(2-aminoethyl)-carbamate); ZAYLOA (with 5-fluorouracil and water); ZEXTIF (with p-nitroaniline)] [8], but also with nicotinamide [3], citric acid [4] and a number of dicarboxylic acids (malonic, maleic and glutaric) [6].

Succinic acid (butanedioic acid) is a diprotic, dicarboxylic acid with chemical formula $C_4H_6O_4$ and structural formula HOOC-$(CH_2)_2$-COOH. It is a white, odorless solid, used in the food and beverage industry, primarily as an acidity regulator [9,10]. The chemical structures corresponding to the utilized active substances are presented in figure 1.

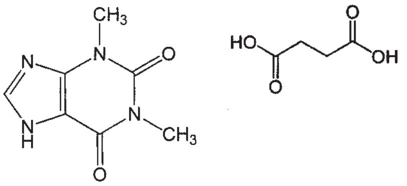

Fig. 1. Chemical structure of THE and SCA

The compatibility between the active pharmaceutical ingredient and the excipients used to manufacture a drug is one of the most important parts of the development process. The possible physical and chemical interactions between these components can have a negative effect on the bioavailability, stability, safety and potency of the final pharmaceutical form, transforming the results of these compatibility studies in the key of the decision making process regarding the appropriate drug formulation.

Considering these aspects, the type and nature of the chosen co-former can have multiple ramifications in both solid formulations, considering the physical and chemical interactions, and *in vivo*, where the effects are physiological. The lifetime and bioavailability of the resulting drug can be highly influenced by these excipients, thus making them one of the “key components” in the technological process today [11-13].

Thermal analysis is a frequently used technique in the characterization of active substances, with many applications in the study and prediction process of the pharmaceuticals stability [14-16]. This method plays an important part in the pre-formulation stage of the dosage forms development process due to the fact that it is used in the compatibility study between active substances and excipients.

The possible incompatibilities are analyzed comparing the thermal curves obtained for the pure substances with those obtained for their physical mixture [17-18]. Alongside with the thermoanalytical methods, FTIR spectroscopy, DSC and XRD are used in order to evaluate and assess the compatibility/incompatibility of active pharmaceutical ingredients with excipients [19] and the characterization of potential biological compounds [20-22].

Experimental part

Materials and methods

Materials and binary adduct preparation and storage

All the starting reagents were commercial products, used as received: theophylline (THE, anhydrous, purity ≥ 99%, Sigma-Aldrich, T1633) and succinic acid (SCA, crystalline, purity >99%, Sigma-Aldrich, 398055). Absolute ethanol (Chimopar S.A. Bucharest) was used. For the preparation of the binary adduct, THE and SCA were weighted in the molar ratio 1:1, placed and mixed in an agate mortar with a pestle in the presence of few drops of absolute ethanol for 5 min. The procedure was repeated several times. The sample was than dried out in an oven at 35 °C for 24h, placed in a seal tube and kept under ambient temperature until the analyses were carried out.

Thermal analysis TG-DTG-HF

Thermoanalytical data (TG/DTG/HF) were collected on a Perkin-Elmer DIAMOND thermo-balance, the samples were heated up to 500 °C with a heating rate $\beta = 10$ °C·min^{-1}. The curves were obtained in dynamic air atmosphere with a flow of 100 mL·min^{-1}, using approx. 5 mg of sample, which was weighted into an open aluminium crucible. For determining the thermal effects, the DTA data (μV) were converted in HF (Heat Flow) data (mW).

Infrared spectroscopic analysis

The FTIR spectra of pure active substances (theophylline and succinic acid) and of the obtained co-crystal were carried out using a Perkin Elmer SPECTRUM 100 device. The data was collected in the range of 4000-600 cm^{-1} on an UATR device. The samples were not prepared before analysis. Spectra were drawn up after a number of 32 co-added acquisitions, with a resolution of 4 cm^{-1}.

Results and discussion

Thermoanalytical profile

The three samples, pure starting materials and the binary adduct, were investigated by thermoanalytical methods using a heating rate of 10°C· min^{-1}, from ambient temperature up to 500°C. The HF profiles of pure pharmaceutical ingredients and for the co-crystal are presented in figure 2. These substances can be differentiated from each other on the basis of endothermic events which evidenced the melting behaviors, in good agreement with the data mentioned the literature [2, 23]. According to this, the two endothermal peaks at 272 and 321 °C identified in the HF curves were attributed to the melting process of anhydrous THE (fig. 2; curve 2) and SCA (fig. 2; curve 3), respectively. After melting, the HF curve of SCA reveals a broad endotherm event corresponding to the degradation of the compound. Analyzing the HF curve of the binary adduct (fig. 2; curve 1), three endothermic events were noticed with peaks at 153, 249 and 284 °C, respectively. The endothermic sharp peak at 153°C characterizes the fusion of THE-SCA co-crystal, since on the TG curve, no mass loss occurs (fig. 3, curve 1) at the same temperature.

Figure 3 presents the thermogravimetric curves obtained after the analysis of pure active substances and of obtained co-crystal. The thermal degradation of the new co-crystal

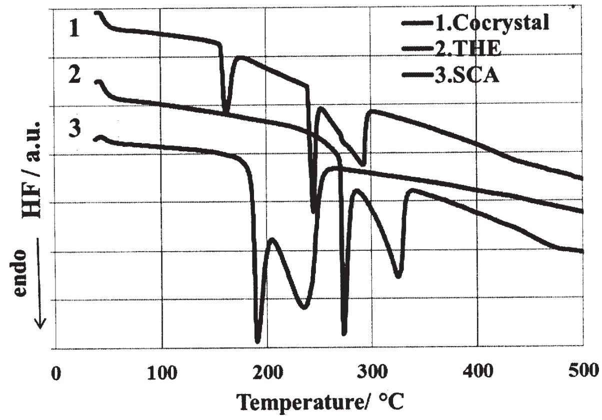

Fig. 2. HF curves for the analyzed samples: THE, SCA and co-crystal

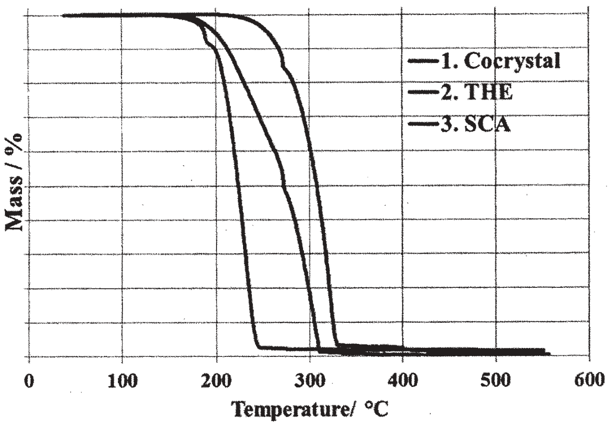

Fig. 3. Thermogravimetric curves of THE, SCA and co-crystal on 40–600 °C temperature range

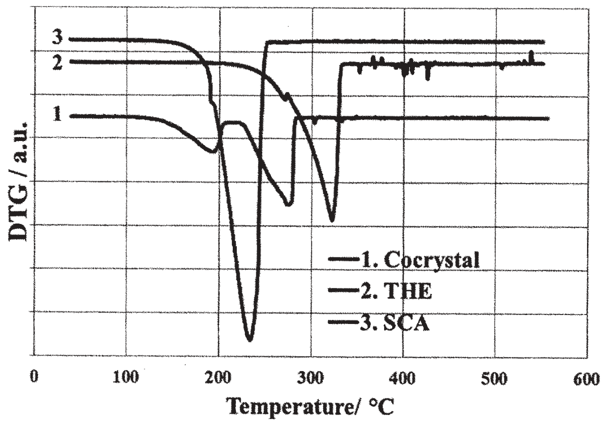

Fig. 4. DTG curves of THE, SCA and co-crystal on the analyzed temperature range

Table 1
THERMOANALYTICAL DATA OF NEW CO-CRYSTAL AND CO-FORMERS (THE AND SCA)

Samples	HF		ΔH_{fusion}/J g^{-1}	DTG		Δm/ %
	$T_{onset\ (fusion)}$/°C	$T_{peak\ (fusion)}$/°C		T_{onset} /°C	$T_{peak\ DTG}$/°C	
THE	255; 282	272; 321	98.4	217	319	99.9
SCA	165; 202	189; 229	102.1	144	230	99.9
Co-crystal	144; 229; 250	153; 249; 284	81.5	130; 216	186; 268	99.8

begins at 160 °C with a clear change in the allure of the TG curve compared with the other two thermograms. The decomposition of co-crystal took place with a continuous mass loss up to 305 °C, when the mass loss is complete ($\Delta m \approx 100$ %). None of thermoanalytical curves recorded for co-crystal overlaps with the curves which characterizes the pure components (THE and SCA).

Analyzing the DTG curves (fig.4), different maxima were identified in the case of co-crystal comparative with pure THE and SCA. This fact demonstrates the different pathway of degradation and can be considered a further proof for the synthesis of new co-crystal, since the DTG curve recorded for co-crystal is not an overlapping of the two DTG curves of starting materials.

XRPD analysis

The XRPD diffractograms for THE, SCA and THE–SCA co-crystal are presented in figure 5. The characteristic peaks of THE and SCA have been reported previously [12, 24]. The unique XRPD pattern of the new co-crystal was significantly different from both pure raw materials - theophyline and succinic acid. The formation of a new crystalline structure is sustained by the new peaks in diffractogram which are presented in table 2. Analysing the XRPD data, it can be observed that the characteristic peaks of pure compounds are alliterated in the case of adduct, suggesting the dramatic modification of crystalline structure.

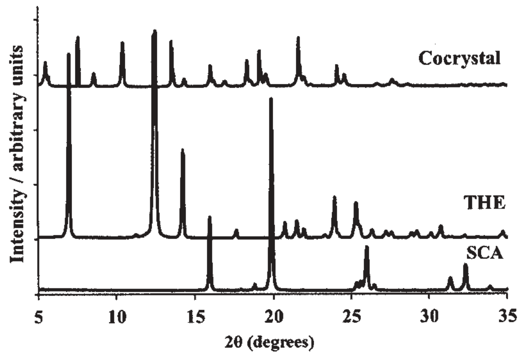

Fig. 5. Experimental diffraction patterns of pure THE, pure SCA and 1:1 THE-SCA co-crystal, respectively

Table 2
X-RAY DIFFRACTION DATA FOR CO-CRYSTAL, PURE THE AND PURE SCA

Co-crystal		THE		SA	
2θ	I%	2θ	I%	2θ	I%
5.45	51.6	7.0	88.7	15.936	23.36
7.55	80.5	12.5	100	19.879	100
8.55	29.3	14.25	42.3	25.332	3.43
10.4	89.4	17.55	4.4	25.993	20.81
13.55	91.4	20.7	8.0	31.365	6.18
14.25	17.8	21.5	8.8	32.315	12.32
16.3	9.3	23.95	20.1		
18.35	53.5	25.3	17.3		
19.15	71.7	26.35	4.4		
21.65	97.1	27.25	3.6		
24.1	41.7				

FTIR analysis

The FTIR spectra of the pure substances (THE and SCA) and of THE–SCA co-crystal prepared *via* solid-state grinding used in the study are presented in figure 6. Theophylline contains two carbonyl groups assimilated in the amidic group, which can be observed at 1668 cm^{-1} as peak in the FTIR spectrum due to the tertiary structure. Because to the N-H stretching vibration (secondary amine), the FTIR spectrum contains a single weak band at 3126 cm^{-1}.

By formation the co-crystal, this characteristic peak is shifted because the formation of the hydrogen bonding (ν_{N-H} = 3089 cm^{-1}).

The IR absorption bands from 3346-2748 cm^{-1} range with maxima at 2999 and 2989 cm^{-1} assigned to O-H stretching for SCA disappeared, this fact can explain the formation of co-crystal since no peaks belonging to carboxylate group could be found.

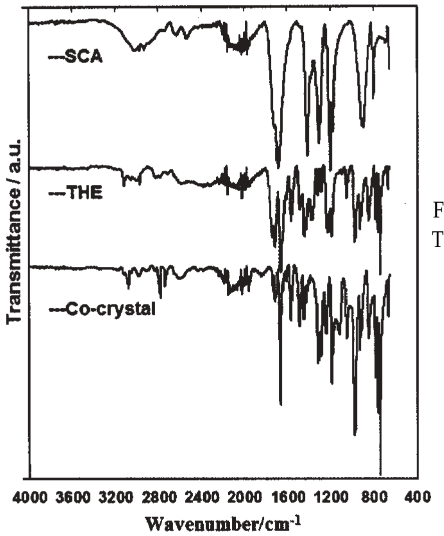

Fig. 6. FTIR spectra of THE, SCA and new co-crystal

Conclusions

This paper presents the formation of a new cocrystal of THE and SCA by employing a fast, simple and reproducible tool, without the necessity of employing special conditions. THE and SCA were used in 1:1 molar ratio and the physical mixture was prepared by wet grinding of the two components. This new binary adduct was analysed with thermoanalytical methods, X-ray powder diffraction and FTIR spectroscopy.

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