Characterization of Pharmaceutical Acetylsalicylic Acid - theophylline Cocrystal Obtained by Slurry Method Under Microwave Irradiation

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A solvent-assisted slurry grinding combined method with microwave irradiation (MW) was used to investigate the cocrystal formation between acetylsalicylic acid (ASA) and theophylline (THE). Three instrumental techniques were used, namely MW irradiation for the synthesis of cocrystal, respectively thermal analysis (TG/DTG/HF) and UATR-FTIR spectroscopy for evaluation of cocrystal formation. This study indicates that a cocrystal consisting in 1:1 (molar ratio) ASA:THE was obtained and was characterized by the above-mentioned thermal and spectroscopic techniques. The Heat Flow data for the two active substances (ASA and THE) presents two endothermic peaks at 141 °C and 272 °C which corresponds to the melting processes of these cocrystal formers. On the HF curve of the obtained cocrystal, it was observed only one endothermic sharp peak at 129.3 °C, due to the fusion point of ASA-THE cocrystal. The results obtained by thermal analysis were corroborated with the ones from UATR-FTIR spectroscopy.

Keywords: cocrystal, slurry method, theophylline, acetylsalicylic acid, microwave irradiation

Nowadays, the cocrystallization of an active pharmaceutical ingredient (API) with a cocrystal former, or the cocrystallization of two bioactive substances are an increasingly technique to design and obtain new solid forms with modified physico-chemical properties such as dissolution rate, melting behaviour, wetting properties, stability and especially bioavailability without being necessary to perform a chemical modification of API’s structure [1-3], which is known to be a more expensive and time consuming process.

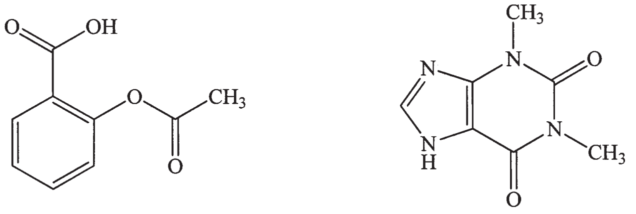
Cocrystals can be usually prepared by crystallization following solvent evaporation [4-5], slurry technique [6-8], co-grinding technique [9], crystallization from melted state [10-11] and ultrasound-assisted cocrystallization from solution [12].

Microwave irradiation is considered an alternative form of energy transfer that gained great attention mainly for the fact that it reduces significantly the time necessary for completing a chemical reaction or a physical process (melting, heating, vaporization etc.), even if strongly depend on the nature of the substance(s) involved. If in the case of designed process(es), polar compounds are involved, a considerable reduced time –from hours to few minutes – is necessary for achieving the desired experimental conditions comparative to conventional energy transfer methods [13-15].

Aspirin (acetylsalicylic acid, ASA) is one of the most common over the counter drug used in numerous pharmaceutical formulations for the treatment of pain, aches and fever [16], inflammatory diseases, such as pericarditis [17] and rheumatoid arthritis [18], and in secondary prevention following transient ischemic attack or stroke [19]. Moreover, several studies were focused on the evaluation the physicochemical profile and behaviour of acetylsalicylic acid [20-22] in solution and solid-state.

Theophylline (1,3-dimethyl-7*H*-purine-2,6-dione, THE), generally known as 1,3-dimethylxanthine, is a bioactive derivative mainly used in therapy of respiratory problems such as chronic obstructive pulmonary disease [23] and asthma [24], in the blocking of the action of adenosine, an inhibitor neurotransmitter that induces sleep [25-26] and as well in the recovery of atrioventricular block [27].

The structures of acetylsalicylic acid (ASA) and theophylline (THE) are presented in figure 1.



a) ASA b) THE

Fig. 1. Structures of ASA and THE

Even if microwave irradiation has become a convenient tool in sample preparation, including extraction protocols [28-30], the current use for derivatization and functionalization purposes is not extensively developed, even though several papers and reviews are published in this domain [31-32].

In our previous studies [33-36], we described the importance of the hyphenated thermal techniques TG-DTG-HF combined with kinetic analysis to the thermal stability of pharmaceuticals and compatibility studies of APIs with

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different compounds used as vehicles or diluents for drugs [37-40] in current pharmaceutical formulations.

According to this, we set our goal in the synthesis and characterization of a new pharmaceutical cocrystal containing two crystal formers, namely two bioactive molecules, acetylsalicylic acid (ASA) and theophylline (THE), obtained by slurry method and under microwave irradiation, followed by the solid-state characterization precursors (THE and ASA) and their 1:1 cocrystal by the use of thermoanalytical methods (TG/DTG/HF) and FTIR spectroscopy.

Experimental part

Materials and methods

The reagents were commercial products: acetylsalicylic acid (crystalline, purity >99%, Sigma-Aldrich, A5376) and theophylline (anhydrous powder, purity ≥ 99%, Sigma-Aldrich, T1633) and used as received, without further purification. Absolute ethanol (Chimreactiv S.A. Bucharest) was used. “Solvent free” chemical synthesis was carried out in a glass vial that was inserted in a sealed Teflon flask that was subject to microwave irradiation in an Elta domestic oven at 600W for an optimized time.

Thermal analysis TG-DTG-HF

Thermoanalytical measurements (TG/DTG/HF) was carried out by means of a Perkin-Elmer DIAMOND thermobalance, the sample was heated in the range of 30-550 °C with a heating rate β = 10 $°C{\cdot}min^{-1}$. The curves were obtained in dynamic air atmosphere with a flow of 100 $mL{\cdot}min^{-1}$, using approx. 7 mg of sample, which was weighed 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 acetylsalicylic 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. Spectra were built up after a number of 16 acquisitions.

Microwave-induced synthesis of cocrystal

In an agate mortar with pestle, 0.1802 g (0.001 mol) of ASA was mixed with 0.1802 g (0.001 mol) of THE. The molar ratio 1:1 is equal in this case with the massic ratio, due to the fact that both bioactive compounds have the same molar mass (180.2 g/mol).

The mixture was homogenised for 5 min in the presence of 0.5 mL absolute ethanol, until the slurry mixture turned into powder. A new quantity of absolute ethanol was added (0.5 mL), and the suspension was placed in a glass vial closed with a ground glass stopper having a 0.5 mm pierce (in order to avoid the pressure increasing in the vial during heating).

The vial was inserted in a Teflon flask and subject to microwave irradiation at 600W for 6 min. After irradiation stopped, the white-powdery vial content was kept at 60 °C for 1h and then placed in a desiccator until the analyses were carried out.

Results and discussions

Thermal analysis

The Heat Flow curves of the active substances (THE and ASA) and co-crystalization compound obtained are presented in figure 2.

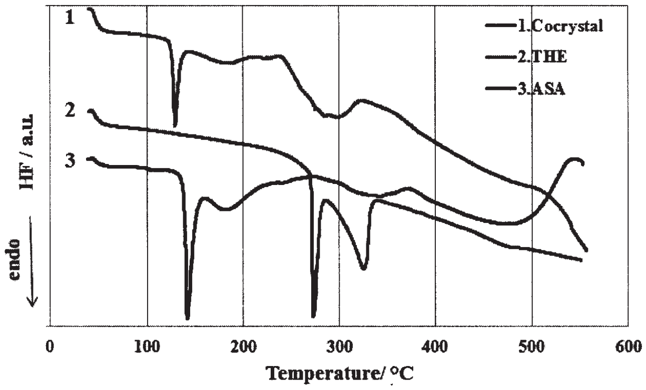

Fig. 2. HF curves of theophylline (THE), aspirin (ASA) and cogrinding THE-ASA cocrystal

The HF curve for pure THE revealed a narrow and prominent endothermic peak in 261–290 °C temperature range, with a maximum at 272.9 °C and $\Delta H_{process}$ = 117.4 $J{\cdot}g^{-1}$ due to the melting with decomposition of THE, followed by a broad endothermic event in 273–334 °C domain, corresponding to the advanced degradation of the theophylline’s skeleton.

In the case of ASA, the HF curve presents a narrow endothermic peak in the 127.8–157.4 °C temperature range, with $T_{peak\ HF}$ = 140.2 °C and ΔH_{fusion} = 108.7 $J{\cdot}g^{-1}$ corresponding to the melting point [41], followed by two endothermic degradative events with maximum peaks at 174.1 and 348.7 °C, respectively. These processes identified in the Heat Flow curve correspondent also on the DTG curve of pure ASA.

The HF curve of new cocrystal of THE and ASA presents three important peaks. The first peak is a sharp endothermic one at T_{HF} =129.3 °C ($T_{onset\ HF}$= 119.2 °C and ΔH_{fusion}= 59.3 $J{\cdot}g^{-1}$) due to the sample melting followed by two broad endothermic events with maximum at 171.8 °C and 280.3 °C respectively, corresponding to the thermal degradation of the cocrystal.

The thermal behaviour of two pure active substances and solid cocrystal obtained by cogrinding has been also analyzed by thermogravimetric (TG) and derivative thermogravimetric analysis (DTG).

Thermal decomposition of THE in synthetic air atmosphere occurs in two events. The TG curves of theophylline indicate a mass loss of 11.38 % in the 205-273 °C temperature range, with $T_{peak\ DTG}$= 266 °C associated with a sharp melting endothermic peak on HF curve, followed by a continuous process of degradation with a mass loss of 88 % in the 273-332 °C temperature range. This process can be associated with the breakdown of the THE molecular structure (figs.3 and 4).

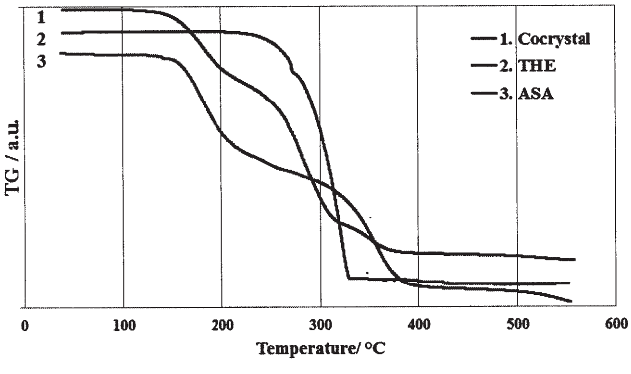

Fig. 3. TG curves of theophylline (THE), aspirin (ASA) and cogrinding THE-ASA cocrystal

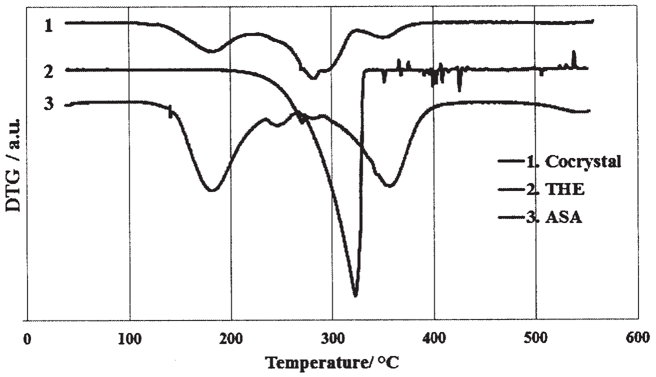

Fig. 4. DTG curves of theophylline (THE), aspirin (ASA) and cogrinding THE-ASA cocrystal

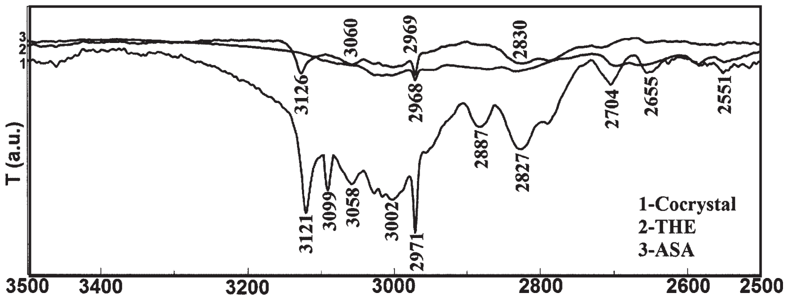

Fig. 5. UATR-FTIR spectra of cocrystal (1), THE (2) and ASA (3) for spectral domain 3500-2500 cm^{-1}

The TG-DTG curves for the pure acetylsalicylic acid shows a 39.6 % mass loss between 115-227 °C with $T_{peak\ DTG}$= 174°C. In the 227-400°C temperature range, a significant continuous mass loss was observed, representing a Δm=59 %, associated with three maximum on the DTG curve ($T_{peak\ DTG}$= 240 °C; 274 °C and 350 °C). This continuous degradation process is accompanied by a mass loss of the ASA sample by the formation of volatile compounds evolved from the destruction of molecular edifice.

The TG-DTG curves of new cocrystal shows a 27.87 % mass loss between 121 and 218 °C, corresponding on the DTG curves to a broad peak with maximum at 173 °C. In the range of 220-318 °C, an intense DTG peak appears with maximum at 277 °C, corresponding to a 55.59 % mass loss, followed by a broad and small DTG peak in the 318-384 °C domain, with maximum of temperature at 347 °C when the 11.94 % mass loss occurs. The thermal profile of the sample obtained from 1:1 ASA:THE is clearly different from the one of pure precursors, suggesting that the cocrystal formation occur.

Spectroscopic analysis

The FTIR spectra of raw materials and solvent-assisted ground mixtures of cocrystal formers are presented in figures 5 and 6. Literature [42] mention that aspirin exhibit two polymorphs, namely forms I and II, the first being thermodynamically more stable.

The broad absorption peak between 3200 and 2500 cm^{-1} (fig. 5) belongs to ν_{OH} stretching vibration of COOH group in the ASA and indicate the strong intermolecular interactions by H-bonding(s). The analysis of 1900–700 cm^{-1} spectral region (fig. 6) reveals peaks at 1750 cm^{-1} corresponding to $\nu_{C=O}$ stretching mode of acetoxy group and the maximum at 1681 cm^{-1} corresponds to $\nu_{C=O}$ stretching mode of from carboxyl moiety. These two peaks presents shoulders, which appeared due to the effect of crystal field splitting. The sharp intense peak with the maximum at 1605 cm^{-1} corresponds to the skeletal in-plane vibration of aromatic ring [42].

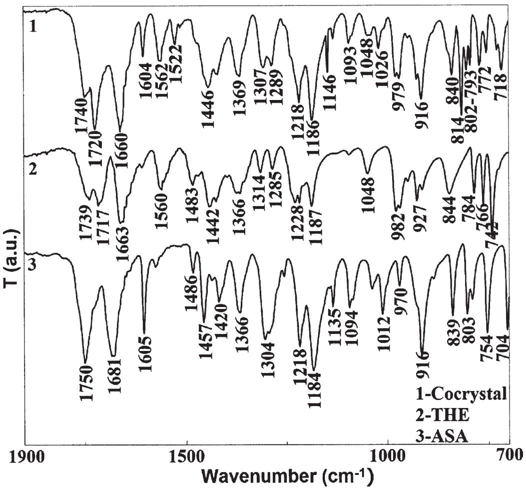

Fig. 6. UATR-FTIR spectra of cocrystal (1), THE (2) and ASA (3) for spectral domain 1900-700 cm^{-1}

By the presence of characteristic bands from FTIR spectra of ASA [42], it is a clear indication that the cocrystal precursor is the polymorph Form I. The shifting of maximum peaks to lower/higher wavenumbers, appearance and/or disappearing of certain FTIR bands offer information regarding the modification of structure and/or packaging of ASA and THE molecules in cocrystal comparative to the packaging properties in the pure form of active substances. These observations can be considered indicators for the estimation of interaction between ASA and THE. The FTIR analysis in both spectral windows (3500-2500 cm^{-1} and 1900-700 cm^{-1}, respectively) exhibited several different IR peaks in the IR spectra of the solvent-assisted ground mixture, implying that the interaction took place between acetylsalicylic acid and theophylline.

Due to the fact the formation of cocrystal took place with the maintenance of covalent bonds from the structure of formers, it is expected that the FTIR spectra reveal numerous bands that are present in both ASA and THE spectra. However, if any interactions occur between precursors, differences are expected to be noticed in the spectrum of mixture. The main important shiftings are observed in the 1600-1750 cm^{-1} spectral region around and can be assigned to the involvement of carboxylic oxygens in intermolecular H-bondings with heterocyclic nitrogens

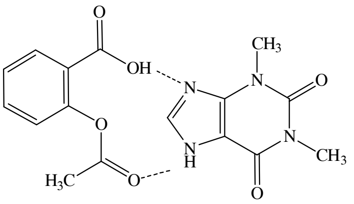

Fig. 7. A proposed structure for H-bonding interaction between ASA and THE

from THE structure. According to the observed shiftings of characteristic bands corresponding to C=O and N-H groups from ASA and THE molecules, a tentatively interaction that took place is presented in figure 7.

Once the cocrystal was formed, numerous significant IR spectral differences were also observed for the cocrystal comparatively to ASA and THE. Several unique peaks at 3099, 3002, 2887, 2704, 2655, 2551, 1522, 1146, 1026, 814, 802, 793, 772, 718 cm^{-1} were observed in the FTIR spectra of the cocrystal. The appearance of these new FTIR peaks in the spectrum of microwaved-solvent-assisted ground ASA-THE samples are due to the solid-state cocrystal formation via intermolecular interactions between the components.

Conclusions

By corroboration of results obtained from thermal analysis and FTIR spectroscopy, the microwaved-solvent-assisted ground ASA-THE sample can be identified as a cocrystal. The microwave assisted irradiation of grinded mixture accelerates the formation of cocrystal between the two precursors, which was not obtained when a simplex mixing of ASA and THE, in the lack of MW irradiation, both in presence or absence of absolute ethanol as solvent. It was shown that the cocrystal formation took place in a short period of time (6 min) for the irradiation of a slurry mixture of same amount of precursors in the absolute ethanol medium. The thermal and spectroscopic analysis carried out on the precursors and cocrystal confirm a different thermal behavior of mixture, and as well a different FTIR spectra. However, it was shown that the identity of precursors is not alliterated by the process, by the presence of characteristic FTIR bands even in the spectrum of cocrystal.

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