Novel solid forms of thiamine hydrochloride with succinic acid

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The present study reports on new solid forms obtained from thiamine hydrochloride with succinic acid by applying the liquid assisted grinding (LAG) mechanochemical method. Samples with (1:1) and (1:2) molar ratios of thiamine hydrochloride to succinic acid resulted after two hours grounding with few drops of acetonitrile were investigated by X-ray diffraction, thermal analysis and infrared spectroscopy. Both XRD and FTIR analysis show new diffraction lines and absorption bands, respectively, denoting that there is not simply a mechanical mixture of components. The FTIR results indicate that a new salt of thiamine hydrochloride with succinic acid was obtained owing to proton transfer.

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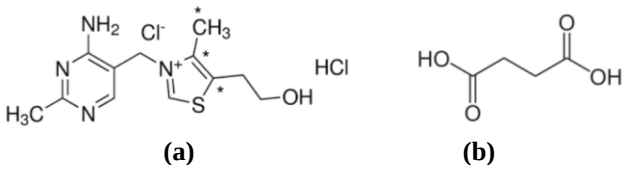
1. Introduction

Special attention is paid in the last years to increasing the safety and quality of pharmaceutical products. In the field of pharmaceutics, identification of new solid forms like solvates, hydrates, co-crystals or salts of organic molecules plays a basic role in the design of new drugs [1]. By obtaining new solid forms of the active pharmaceutical ingredients (API), most of the times is achieved an improvement of thermodynamic parameters and physico-chemical properties, such as solubility, dissolution rate, stability and mechanical properties, of the final solid form [2].

New pharmaceutical solid forms with improved bioavailability are expected to be obtained by co-crystallization [3]. The solid-state assembly of API co-crystals can be achieved using a second agent as coformer. Whether the interaction between API and coformer takes place via proton transfer, it results a salt, but whether this interaction is via hydrogen bonds, the result consists in the formation of a co-crystal [4].

The mechanochemical method consisting in milling of an active pharmaceutical compound and a conformer with solvent droplets is a suitable and frequently used method for preparing new solid forms of APIs. It is an economical method that requires a small amount of solvent and, for this reason, it makes the chemical processes more environmentally friendly [5]. Moreover, they often make posible the synthesis of co-crystals which are not traditionally obtained [6]. The mechanochemical synthesis method is based on chemical reactions that are induced by the direct absorption of mechanical energy. Mechanochemical reactions are generally conducted using automated ball mills, which can be shaker or vibration mills, and planetary mills. Otherwise, the mechanical milling is increasingly applied in synthesis of advanced materials with possible applications in very different fields, from bio-nano-systems to devices for optoelectronics [7-10].

The vitamin B1 (thiamine hydrochloride - $C_{12}H_{18}Cl_2N_4OS$ / 336.06 g/mol) is one of the 13 essential vitamins for the human body [11]. Its molecule contains a pyrimidine ring linked to primary amine and thiazole ring linked to HCl (Scheme 1a). The small molecule of succinic acid (butanedioic acid – $C_4H_6O_4$ / 118.01 g/mol) is often used as excipient in pharmaceuticals [12], and appears attractive also as conformer due to the presence of carbonyl and hydroxyl groups (Scheme 1b) offering both hydrogen bond acceptor and donor sites. Moreover, a positive effect of vitamin B1 deposition on Si solar cells [13] as well as on the properties of N- and S-doped carbon dots [14] and other optoelectronic devices [15, 16] were reported, and all these make more challenging the investigation of new forms of vitamin B1. Therefore, the main purpose of this study was to obtain thiamine co-crystals using as coformer succinic acid, following a mechanochemical route.



(a) **(b)**

Scheme 1. Chemical structure of thiamine hydrochloride (a), and succinic acid (b)

The mechanochemical reactions supported by solvents used as lubricants for improving the mixing of reactants and reaction kinetics are actually accomplished by liquid-

assisted grinding (LAG). They may be described by a specific η parameter, defined as the ratio of the quantity of liquid (in µl) to the combined weights of solid reactants (in mg) [17]. This parameter allows the comparison and the differentiation between certain grinding techniques as follows: for neat grinding (η=0), for liquid-assisted grinding (0< η ≤1), and for the formation of slurries and homogenously solutions its values is η > 1.

The aim of this study was to obtain new solid forms, most desirable co-crystals, of thiamine hydrochloride with succinic acid in two molar ratios 1:1 and 1:2, following the liquid-assisted grinding technique with few drops of acetonitrile. The preliminary analysis of the samples indicates the achievement of a new solid form which is not a co-crystal but a salt.

2. Experimental

Thiamine hydrochloride (337.3 g/mol molar mass), and succinic acid (118.09 g/mol molar mass) were provided by Alfa Aesar and Sigma-Aldrich and were used without any further purification. Acetonitrile solvent at HPLC purity ≥99.5% (Sigma-Aldrich) was used as lubricant.

The investigated samples were obtained following the liquid-assisted grinding technique. The sample-jar with the reagents in powder form together with few drops of solvent was rapidly oscillated for grinding of the reagents. Two mixtures of thiamine hydrochloride (THC) and succinic acid (SU) in molar ratios 1:1 and 1:2 were prepared. After adding few drops of acetonitrile, the powder mixtures were ground for two hours in a Retsch MM200 ball-mill, with two stainless steel balls inside of sample-jar. Thereafter the samples were dried at room temperature.

- The sample with THC / SU in 1:1 molar ratio, noted THCSU_1-1, was prepared from 100 mg THC (0.296 mmol), 35.08 mg SU (0.296 mmol), and 60 µL acetonitrile ($\eta = 0.44\ \mu L \cdot mg^{-1}$).

- The sample with THC / SU in 1:2 molar ratio, noted THCSU_1-2, was prepared from 100 mg THC (0.296 mMol), and 70.17 mg SU (0.593 mMol), 60 µL acetonitrile ($\eta = 0.47\ \mu L \cdot mg^{-1}$).

Both 0.44 and 0.47 values of η parameter for THCSU_1-1 and THCSU_1-2, respectively, are in agreement with $0 < \eta \leq 1$ condition for mechanochemical reactions within the liquid-assisted grinding method.

The THC, SU and THCSU samples were analysed by X-ray powder diffraction (XRD), Fourier transform infrared spectroscopy (FTIR), and differential thermal analysis (DTA / TGA).

The structure was analysed by X-ray diffraction (XRD) with a Shimadzu XRD-6000 diffractometer with a Ni-filter. The powder XRD measurements were performed with Cu radiation Kα (λ = 1.5418 Å, operating conditions 40 kV and 30 mA) at room temperature, in 2θ range 3–40°.

The infrared spectra were recorded in 4000-400 cm^{-1} spectral range with a JASCO 6200 FTIR spectrometer, using KBr pellets. About 1.2 mg of each sample was mixed with 150 mg KBr of spectroscopic grade purity, and pressed into 13 mm diameter disks under a pressure of 6 tons. The spectra recorded by acquisition of 256 scans, with 4 cm^{-1} resolution, were processed with Spectra Analysis software.

Differential thermal and thermogravimetric analysis (DTA/TGA) was performed with a simultaneous analyser Shimadzu DTG-60H. All investigated samples were heated in the temperature range of 24–600°C, with a heating rate of 10 °C/min, under dry nitrogen purge (70 mL/min), using an alumina sample cell (5.8 × 2.5 mm^2).

3. Results and discussion

3.1. X-ray powder diffraction

The structure of the samples obtained by ball milling (THCSU_1-1 and THCSU_1-2), as well as of the starting materials thiamine hydrochloride (THC) and succinic acid (SU) was first analysed using X-ray powder diffraction. In Figure 1 are shown the PXRD patterns of starting materials thiamine hydrochloride and of succinic acid, compared with THCSU_1-1 and THCSU_1-2 investigated samples. Highly similar diffraction patterns, which are not the superposed diffraction features of thiamine hydrochloride and succinic acid, are recorded for both samples prepared by mechanochemical method.

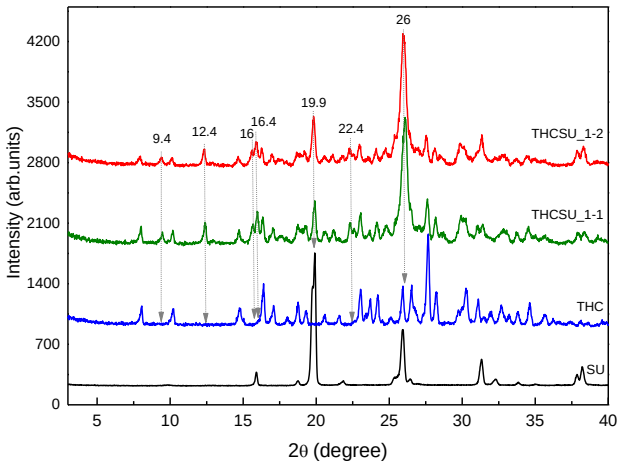


Fig. 1. XRD patterns of starting materials THC and SU, compared with the prepared THSU samples (color online)

The most intense diffractions lines of THC and SU are recorded at 27.5º and 19.9º, respectively, and no one of these are recorded both in THC and SU. At the diffraction angle of 26º a lower intensity line is recorded for both THC and SU. Beside diffraction lines of starting materials, the THCSU_1-1 and THCSU_1-2 XRD patterns contain new diffraction lines which prove that after two hours grinding of thiamine hydrochloride and the succinic acid together with few drops of acetonitrile a new solid form has been formed. On the other hand, in the diffraction patterns obtained for THCSU_1-1 and THCSU_1-2

samples the peak at 2θ of 26.5º present in THC disappears. An increase of the intensity of 26º peak would be expected even for a simple mix of THC and SU, on the account of both THC and SU contributions to this diffraction line, but such a simple mechanical mix is not the case of THCSU_1-1 and THCSU_1-2 samples, as can be observed by further inspection of this 26º line and the most intense diffraction lines recorded at 19.9º and 27.5º for SU and THC, respectively. Both for THCSU_1-1 and THCSU_1-2 the ratio between 26º and 19.9º line intensities is close to 2.6, while the relative intensity of the most intense diffraction line of THC recorded at 27.5º for THCSU_1-1 and THCSU_1-2 is not close to 0.5 as expected for a merely physical mixture of THC:SU in 1:1 and 1:2 compositions. At the same time, new peaks are recorded at 2θ of 9.4, 12.4, 16, 16.4, 19.9 and 22.4º (Fig.1). These diffraction results evidently support the development of a new structure of thiamine hydrochloride with succinic acid, but without any indication on the formation of a co-crystal or salt. This assessment was attempted using the infrared spectroscopy.

3.2. Fourier transform infrared spectroscopy (FTIR)

The infrared spectra are very sensitive to the position of the proton, and this may provide information on co-crystal or salt formation [18]. The changes occuring during mechanochemically processing of THCSU, involved by interactions between thiamine hydrochloride and succinic acid, may be evidenced by analysis of FTIR spectra recorded from starting materials and the new prepared THCSU samples (Fig. 2). An easier inspection of the spectral features may be obtained at expanded scale in the range of high and low wavenumbers (Fig. 3).

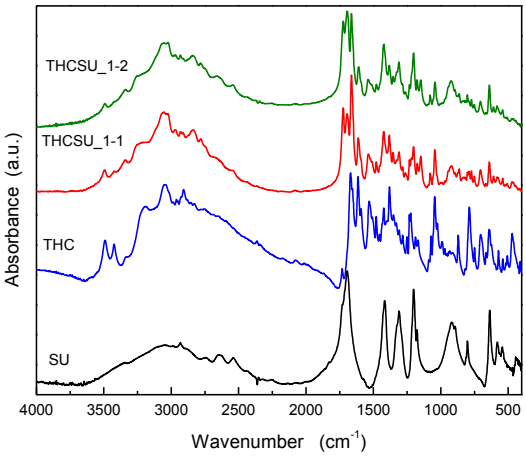


Fig. 2. Infrared spectra of vitamin B1 (THC) and succinic acid (SU) and of the samples resulted from their liquid assisted grinding (color online)

In the high wavenumber region 4000-2000 cm^{-1} of FTIR spectrum recorded from succinic acid (Fig. 3a), the weak 3348 cm^{-1} shoulder evidences O-H stretching vibration in free water, while the absorption bands at 2931, 2651 and 2537 cm^{-1} correspond to C-H stretching vibrations. In the low wavenumbers region 2000-400 cm^{-1} (Fig. 3b), at 1730 and 1692 cm^{-1} are observed the -C=O stretching vibrations, and at 1418 cm^{-1} the in-plane C-OH bending vibration and/or COO^- symmetric stretching vibrations [19-21]. An older study on analytical profile of thiamine hydrochloride [22] shown that the infrared spectrum of thiamine hydrochloride evidences characteristics bands at 3495 and 3420 cm^{-1} from -NH_2, 3255 cm^{-1} from -OH, 3000 cm^{-1} from aromatic -CH, 2930 cm^{-1} from aliphatic -CH, 1650 cm^{-1} from C=N, and 1600 cm^{-1} from C=C vibrations, mentioning also that other infrared bands for thiamine hydrochloride were also reported at 3290, 2940, 2700, 1670, 1610, 1520, 1390, 1250, 1220, 1040, 890, 830, 770, 750 and 700 cm^{-1}.

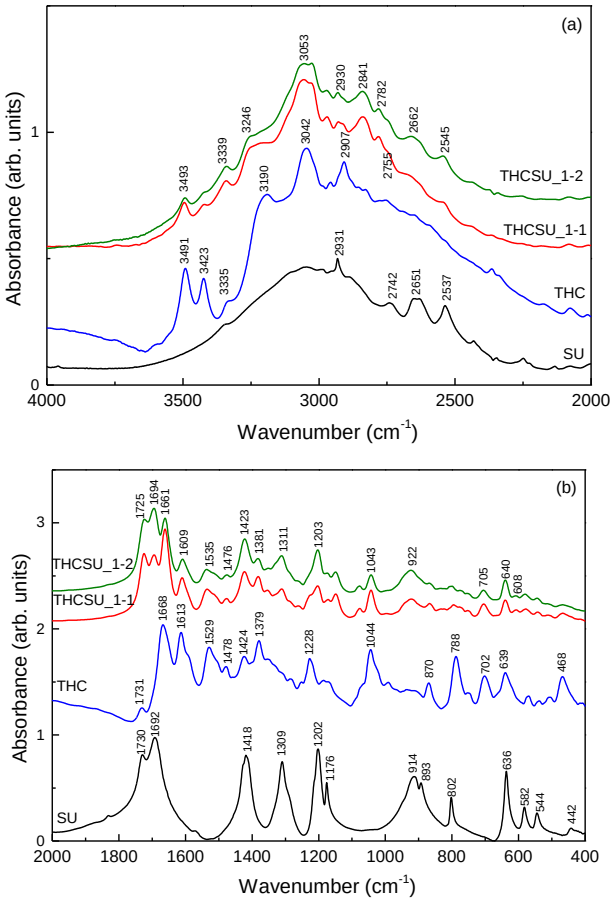


Fig. 3. FTIR spectra of SU, THC, THCSU samples, in 4000-2000 cm^{-1} (a) and 2000-400 cm^{-1} (b) spectral range (color online)

The FTIR spectrum of thiamine hydrochloride used in the present study (Figs. 2 and 3) contains a weak band at 3335 cm^{-1} from O-H groups of water molecules, and characteristic bands at 3491, 3424, 3190 cm^{-1}, ascribed to the stretching frequencies of primary amino group NH_2 [22, 23]. The bands at 3045, 2957, and 2907 cm^{-1} are assigned to C-H aromatic and aliphatic stretching vibrations [22]. Between 1700-1500 cm^{-1} (Fig. 3b) two bands of medium intensity are observed at 1668 cm^{-1}

assigned to the C=N stretching vibrations [22], and at 1613 cm^{-1} due to primary amine deformation vibration [24]. The antisymmetric and symmetric stretching vibrations of COO^- are identified at 1529 and 1379 cm^{-1} [22]. The low intensity bands at 1478 and 1424 cm^{-1} can be assigned to stretching vibrations of the C=C bond in the thiazole ring and pyrimidine ring [23]. The C-S stretching vibration was observed at 1044 cm^{-1} and C-Cl vibrations at 788 cm^{-1} and 639 cm^{-1} [23].

By comparing the absorption bands of the starting compounds with those obtained after liquid assisted grinding process, it was observed that the vibration of primary amine appears diminished in intensity and at a weakly modified frequency in the new solid forms, namely from 3346 cm^{-1} in THC, to 3342 cm^{-1} in THCSU samples. This fact denotes protonation of primary amine from THC molecule [24-26].

The 3045 cm^{-1} band from THC spectrum, which corresponds to the stretching vibration of the C-H group, is shifted towards higher wavenumbers in the spectra of the new solid forms, at 3055 cm^{-1}. In the spectral range 1625-1516 cm^{-1} of the new solid forms, the primary amine identified in thiamine spectrum at 1613 cm^{-1}, has an absorption band at lower wavenumber, at 1609 cm^{-1}, and the 1730 cm^{-1} band of C=O bonds in succinic acid is also down shifted to 1725 cm^{-1}. The changes in the infrared absorption bands of thiamine and succinic acid observed in the spectra recorded from LAG prepared THCSU samples can be attributed to proton transfer and ionic interaction of the amino group from thiamine hydrochloride and carboxyl groups of succinic acid. These changes denote the formation of a THCSU salt [27].

The 3042 cm^{-1} absorption band from THC spectrum, which corresponds to the stretching vibration of the C-N group is shifted towards higher values in the spectra of the new solid forms. In the spectrum of THCSU samples a new vibration band appears at 2841 cm^{-1}, which is not present in the spectra of starting materials and is attributed to protonated secondary amine [23]. In the spectral range 1625-1516 cm^{-1} of THCSU samples, the band corresponding to the stretching vibration of primary amine identified for thiamine at 1613 cm^{-1}, is shifted to lower frequency, at 1609 cm^{-1} wavenumber, which proves the formation of the salts between thiamine with succinic acid [22]. In the low-frequency region of the FTIR spectra recorded from the new THCSU forms, a new band appears at 608 cm^{-1} suggesting that new bonds are formed due to the intermolecular interaction between succinic acid and thiamine.

3.3. Differential thermal analysis and thermogravimetry (DTA/TGA)

Both thiamine hydrochloride and succinic acid are crystalline solids. The melting temperature of thiamine hydrochloride is reported between 196 ºC and 254ºC, and that of decomposition at 248°C [28, 29]. The succinic acid has the melting temperature around 185°C and it boils around 235°C [28].

The DTA/TGA curves for the thiamine hydrochloride (Fig. 4a) show that this compound is stable up to 101ºC, and dehydrates in 101-210º temperature range, with water weight loss of about 4.55%. Between 210-243ºC the thiamine hydrochloride melts, as denoted in DTA curve by the endothermic event, and starts to decompose. The thermal degradation may be accompanied by emissions of sulfur and nitrogen atoms, heterocyclic structures, nitric oxide, sulfur oxide and chlorine [30, 31] is continuously recorded up to 600ºC, with a mass loss of 74.5 % between 210 and 600ºC.

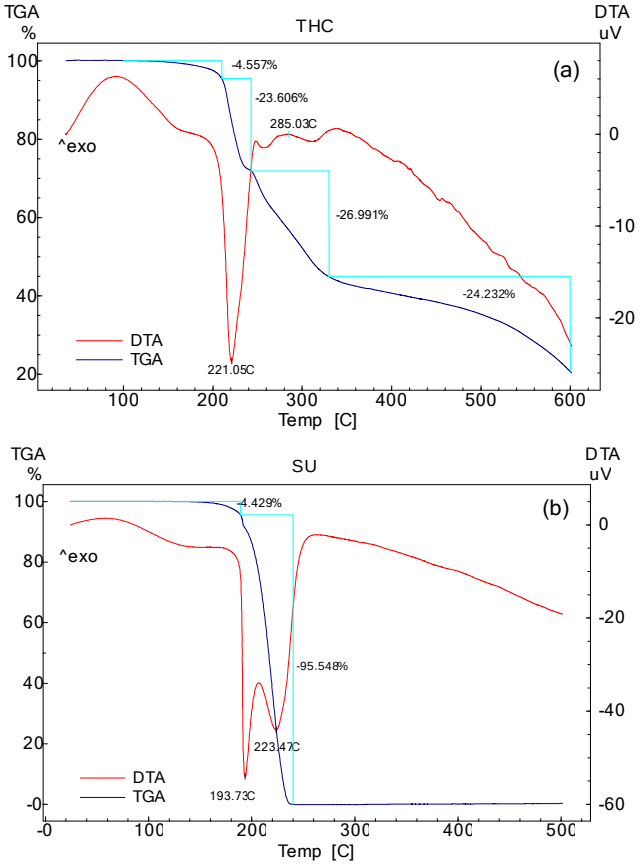


Fig. 4 DTA /TGA curves of thiamine hydrochloride (a) and succinic acid (b) (color online)

The analysis of DTA/TGA curve recorded from succinic acid (Fig. 4b) points out its mass stability up to 150°C, and a mass loss of about 4.43% between 150-190°C, due to dehydration. Two endothermic events are recorded between 190 and 240°C. The endothermic peak at 194°C corresponds to melting and the second one at 223°C corresponds to boiling and evaporation of succinic acid, with a single mass loss step of 95.55%. The succinic acid melts and evaporates without decomposition [32].

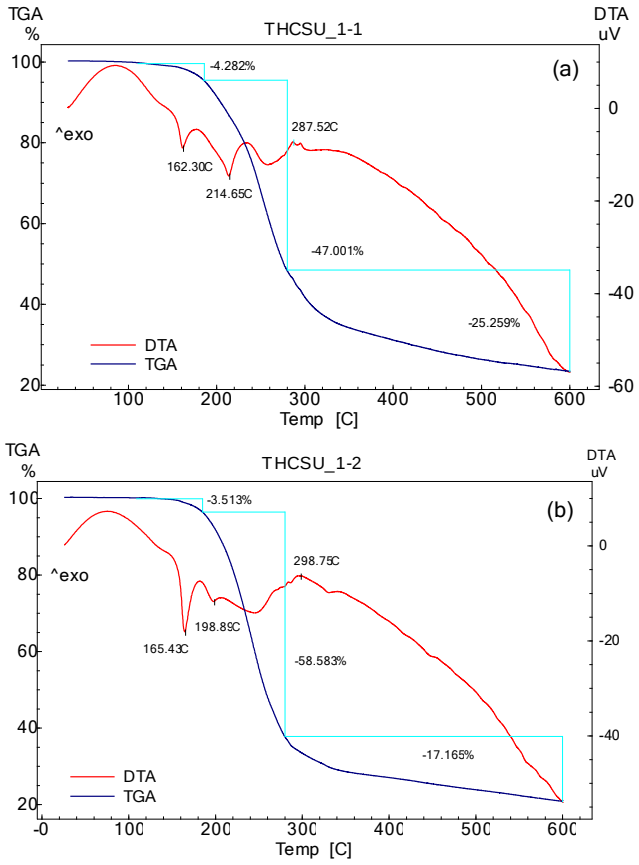


Fig. 5. DTA /TGA curves of THCSU samples (color online)

The DTA/TGA curves for THCSU_1-1 and THCSU_1-2 (Fig. 5) show that in these solid forms the dehydration starts at 109ºC, and continues by further heating, up to 185ºC. A mass loss of 4.28% for THCSU_1-1 and about 3,51% for THCSU_1-2 is observed, corresponding to water loss. Between 185-280ºC, the compounds undergo melting and decomposition, as evidenced in the DTA thermograms by the endothermic signals with minima at 162.3 and 214.7ºC for THCSU_1-1 and at 165.4 and 198.9ºC for THCSU_1-2. These events are accompanied by a significant mass loss about 47% for THCSU_1-1 and 58,58% for THCSU_1-2. In 280-600ºC temperature range, mass losses of about 22.26% and 17.16% for THCSU_1-1 and THCSU_1-2, respectively, denoting the advanced volatilization of the decomposed samples up to 80 % of their initial mass. The same 80 % mass loss was observed for thiamine hydrochloride by heating to 600ºC.

The clear difference observed in DTA runs of mechanochemically synthesized THCSU samples and their THC and SU components is the occurrence of the thermal event recorded around 165ºC (Fig. 5) that infers the formation of new THCSU_1-1 and THCSU_1-2 solid forms.

Further remarks are related to dehydration and decomposition processes. Considering that dehydration starts at 101°C for THC and at 150°C for SU, the dehydration of the new THCSU solid forms would be expected to start at a higher temperature than that of THC, but this is only slightly enhanced to 109ºC. Concerning the thermal decomposition rate, this is higher in the new THCSU salt than in the involved vitamin B1 (THC).

4. Conclusions

This study proves the synthesis of a novel salt of thiamine hydrochloride with succinic acid by liquid-assisted grinding method (LAG), under mechanochemical reactions (with η parameter obeyed to $0 < \eta \leq 1$ condition). The XRD results indicate the development of the new solid form, which yields additional, characteristic diffraction lines. Also FTIR analysis points out the appearance of new vibrations and shows that the new solid form is a salt formed during the LAG process, after proton transfer and ionic interaction of amino group from thiamine hydrochloride and carboxyl groups of succinic acid. New thermal events pointed out by DTA/TGA measurements are due to the formation of a solid form between thiamine hydrochloride and succinic acid.

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