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In-line monitoring of cocrystallization process and quantification of Carbamazepine-Nicotinamide cocrystal using Raman spectroscopy and chemometric tools

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Abstract

A cocrystallization process may involve several molecular species, which are generally solid under ambient conditions. Thus, accurate monitoring of different components that might appear during the reaction is necessary, as well as quantification of the final product. This work reports for the first time the synthesis of carbamazepine-nicotinamide cocrystal in aqueous media with a full conversion. The reactions were monitored by Raman spectroscopy coupled with Multivariate Curve Resolution - Alternating Least Squares, and the quantification of the final product among its coformers was performed using Raman spectroscopy and Partial Least Squares regression. The slurry reaction was made in four different conditions: room temperature, 40°C, 60°C and 80°C. The slurry reaction at 80°C enabled a full conversion of initial substrates into the cocrystal form, using water as solvent for a greener method. The employment of MCR-ALS coupled with Raman spectroscopy enabled to observe the main steps of the reactions, such as drug dissolution, nucleation and crystallization of the cocrystal. The PLS models gave mean errors of cross validation around 2.0 (% wt/wt), and errors of validation between 2.5 – 8.2 (% wt/wt) for all components. These were good results since the spectra of cocrystals and the physical mixture of the coformers present some similar peaks.

Keywords: Carbamazepine-nicotinamide cocrystal, In-line monitoring, Partial least squares, Multivariate curve resolution, Cocrystallization.

1. Introduction

Cocrystals are mixed crystals of two or more compounds (which are neither solvates nor simple salts) that when combined forms a unique crystallographic pattern, different from its precursors[1, 2]. Pharmaceutical cocrystals are a class of crystals of which at least one active principle ingredient (API) is present in the crystallographic motif[3, 4]. This class of crystals presents several properties distinct from its coformers, becoming a material of high interest for the pharmaceutical industry[5].

Solubility, thermal and mechanic stability and bioavailability are some of the characteristic that can be altered by the addition of coformer molecules in the crystallographic pattern of the API[6]. The solubility enhancement and high chemical stability are advantageous features for a pharmaceutical compound with polymorphic forms and low solubility[7].

Carbamazepine (CBZ) is a carboxamide highly used in the treatment of epilepsy and trigeminal neuralgia[8]. Although it has been used as medicament for more than 30 years, it presents low solubility, a treatment with high doses becoming necessary[9]. Four crystallographic forms of the drug are well-known, where, Form III (CBZ III) is the most thermodynamically stable at room temperature, which is used in the commercial tablets. Under normal conditions, CBZ III can be hydrated by atmospheric moisture. The hydrated form of carbamazepine (CBZ DH) presents dissolution properties and aqueous solubility lower than its anhydrous form, which is a problem for pharmaceutical industries[10, 11].

The carboxamide group of carbamazepine can originate a homosynthon interaction in the crystallographic motif of this molecule, generating dimers inside the pure crystal[12]. Therefore, it is possible to use homosynthon and heterosynthon interactions

on the carboxamide group with different organic compounds with high water solubility in order to enhance the dissolution features of this API.

Two of the most studied coformers used to crystallize with carbamazepine are saccharin and nicotinamide[13]. Nicotinamide is a vitamin belonging to the B vitamin group; it is widely used in biochemical systems for NAD (Nicotinamide adenine dinucleotide) and NADP (Nicotinamide adenine dinucleotide phosphate) formation, which are used as coenzymes in several biochemical reactions.

In the crystallographic motif of carbamazepine-nicotinamide cocrystal, it is possible to observe the presence of a carbamazepine dimer surrounded by nicotinamide dimers. Carbamazepine organizes in a boat form and the cocrystal presents a monoclinic structure. Carbamazepine-nicotinamide cocrystal present a solubility rate 152 times higher than the pure API (at 23 – 25 °C)[13] .

Among the methods used to crystalize carbamazepine-nicotinamide, the slow evaporation method is the most used and studied in the field. However, the slow evaporation method can only be used when the desired cocrystal is present in an equivalent stoichiometric ratio, i.e. both initial substrates must be at the same amount in the cocrystal form. Furthermore, the slow evaporation method uses hazardous solvents, such as: methanol[14], acetyl acetate[15] and DMSO[10].

The slurry conversion procedure presents several advantages when compared to traditional cocrystallization methods, such as: development of in-situ analysis, easily transferable to industrial scales and environmentally friendly[16]. In 2006, Rodríguez-Hornedo *et. al.* studied the crystallization reaction of carbamazepine-nicotinamide by slurry conversion, using ethanol and water as solvents, at room temperature[17]. With a Raman probe, the authors observed the cocrystal formation by the shifts on one peak of the spectra, relative to the pure carbamazepine spectra.

The reaction monitoring enables better control of the reaction, with more accurate responses (in real time) and decreasing the reprocess[18-21]. However, there are some limitations when univariate analysis is employed in the reaction monitoring. With multivariate analysis it is possible to build models in the presence of interferents, to analyze more than one component present in the reaction mixture and to obtain more robust and accurate responses, which can decrease the errors involved in the process analysis and in the final product[22, 23].

The chemometric tools most used in the process analysis and reaction monitoring are[23]: principal component analysis (PCA), partial least squares (PLS) and multivariate curve resolution (MCR-ALS). PLS enables to correlate experimental data with several different material properties, and thus, quantify some product characteristics, for example API quantification[24], excipients and drugs granulation[25] and monitoring of polymerization reaction[26]. It is a chemometric tool widely used to quantification experiments where the interferent overlay the signal of the analyte.

MCR recovers concentration profiles as a function of time, which allows the monitoring of initial substrates in the final products[27, 28], and different from the PCA, it recovers a spectral information more similar to the real spectra. It is widely used in *in-situ* systems, where it is possible to get data relative to the compounds present in the reaction in real time, and then observe the formation of the final product, either in chemical reactions[29] or in physical processes[30].

Thus, the aims of this work were: to develop a method to synthesize the carbamazepine-nicotinamide cocrystal by slurry reaction using a greener method (with water as solvent); to monitor the reaction at different temperatures using in-line Raman spectroscopy and MCR-ALS; and to develop PLS models to quantify simultaneously the cocrystal and its coformers.

2. Materials and Methods

2.1 Synthesis of CBZ-NCT "standard" cocrystal

The synthesis of the CBZ-NCT "standard" cocrystal was achieved by following the method developed by Nehm et. al.[15]. The synthesis was based on the crystallization reaction, also known as solvothermal synthesis, where equimolar amounts of API and coformer are added in an organic solvent (in this case, ethyl acetate), and then heated until total dissolution of the initial substrates. The cocrystal is obtained by slow evaporation of the solvent.

Carbamazepine was from Jubilant Life Science Limited and nicotinamide from Aarti Drugs Limited. Ethyl acetate was from Quemis, P.A. grade. The "standard" cocrystal was characterized by Raman spectroscopy and powder X-ray diffraction (PXRD), evidencing the high pureness of the cocrystal.

2.2 CBZ-NCT cocrystal quantification among its precursors

A ternary mixture design was used to prepare the samples of the calibration curve. Different amounts of carbamazepine, nicotinamide and cocrystals were weighed, such that the total weight for each point of the calibration curve was around 50 mg. The samples were then homogenized using a vortex mixer and analyzed by Raman spectroscopy. The validation samples were made by preparing five samples with known amounts of each component and prepared following the procedure of the calibration curves. This kind of methodology is largely used to validate PLS quantification models [31-34]. Table 1 shows the mass of each component for each sample.

In order to evaluate the models error, it was used two frequently used measurements for error calculation in multivariate analysis: the values of root means square error of

cross validation (RMSECV) and root mean square error of validation (RMSEV) calculated by Equation 1.

$$RMSE = \sqrt{\frac{1}{n}\sum_{i=1}^{n}(y_i - \hat{y}_i)^2} \quad \text{(Equation 1)}$$

It is based on the difference between the estimated value and the real value of a model, where n is the total samples of calibration for RMSECV, and total samples of validation for RMSEV; $\boldsymbol{y_i}$ is the real value weighted for the analyte in the sample; and $\boldsymbol{\hat{y}_i}$ the estimated value obtained from the calibration regression model.

2.3 Synthesis of cocrystal by slurry conversion and Raman monitoring

For the slurry conversion synthesis, 2 mmol of carbamazepine (472.5 mg) was weighed in a beaker. In a different recipient, 2 mmol of nicotinamide (244.3 mg) and 1.5 mL of ultrapure water were placed. This solution was added to the carbamazepine powder and the reaction was monitored by Raman spectroscopy. The reaction was heated using an IKA heating plate, model C-MAG HS 7, equipped with electronic thermometer and magnetic stirrer.

For all analyses an *i*-Raman BWS 415-785H was used, (B&W Tek, Inc., Newark, DE, USA), with red laser of 785 nm and spectral resolution of 3.5 cm^{-1}. For the in-line monitoring, a B&W Tek Raman probe BAC-100 was used, placed above the reaction system. The spectra were obtained continuously using an integration time of 120 s. The spectral ranges were 500 to 1666 cm^{-1} (Raman Shift), with a laser power of 224 mW. The first spectra obtained was relative to the pure spectra of carbamazepine (time = 0 h). After this, the nicotinamide solution was added into the beaker and the heating started. The reaction was performed in four different temperatures: room temperature (23 °C ± 1 °C), 40 °C (± 2 °C), 60 °C (± 3 °C) and 80 °C (± 3 °C); this last one being the reaction described in the literature as the temperature of polymorphic transition of

dihydrate CBZ into the Form III CBZ[10]. The reactions were monitored for five hours, the first three hours being under heating (heating stage) and the last two hours without heating (cooling stage).

2.4 Raman spectroscopy for quantification of final product

After the synthesis, the dried final product was characterized and quantified. The parameters for the Raman analysis were: integration time of 60 s; laser power from 224 mW to 320 mW; a B&W Tek BAC151 microscope coupled to the Raman system with 20x objective lens and spectral range between 285 and 1708 cm^{-1} (Raman shift). The calibration samples (Table 1) and the products from the different syntheses were manually compacted using a 13 mm die to obtain packed powder, improving the spectra quality. Due to the microheterogeneity of the analyzed samples, ten spectra were obtained from each calibration and validation sample at random points, in order to get a better representativeness of the whole sample, avoiding subsampling problems.

2.5 X-ray powder diffraction (XRD)

To characterize the "standard" cocrystal and the final products of the slurry reactions an X-ray powder diffraction Rigaku Multiflex diffractometer was used with Cu source (0.154 nm), current of 30 mA and voltage of 40 kV. The samples were scanned from 5° to 45° (2θ) at 0.2° min^{-1} and step size of 0.02°.

2.6 Chemometric methods

To quantify the cocrystal of CBZ-NCT, the iPLS routine present in PLS_toolbox 6.2 (Eigenvector Research Inc., WA, USA) was used, available for Matlab® 2011a (Mathworks Inc., MA, USA). The samples were normalized by area, and the average of

the triplicate analysis was used in the iPLS routine. The first derivative was used in the models. Different interval ranges of Raman spectra for iPLS were also used, in order to obtain the lowest validation and cross-validation errors.

For in-line monitoring, the first derivative spectra were used, which were normalized by the area and filtered by PCA. By applying the first derivative, it can be observed slightly changes in the acquired spectra that were not too evident in the unmodified spectra. The PCA filter intends to reduce the instrumental noise without losing spectral information, using the recovered matrix acquired by a product of scores and loadings that only retains principal components related to meaningful data. In this case, 6 principal components were used in order to ensure all significant information obtained during the monitoring. This routine ensures lower residuals in MCR-ALS analysis, and therefore a better value of lack of fit. For the MCR-ALS, the ALS routine was used, available on the MCR GUI 2.0 toolbox[35]. The number of components was determined using the SVD (single value decomposition) function on MATLAB. The initial estimative of the pure spectra was obtained using the PURE routine, also available on the toolbox. The MCR-ALS was performed using the non-negativity constraint only for the concentration profile, since it was used the first derivative for the spectra in order to improve the resolution of MCR-ALS. The pure spectra recovered by MCR-ALS were integrated after the deconvolution.

3. Results and discussion

3.1 Characterization of the "standard" cocrystal

Supplementary Figure 1 presents the X-ray diffractograms of carbamazepine Form III, dihydrate carbamazepine, nicotinamide and synthesized carbamazepine-nicotinamide cocrystal. The crystallographic pattern of CBZ-NCT indicate the

formation of a crystallographic motif distinct from its coformers. The main peaks are: 6.70°, 8.92°, 10.26°, 13.40° and 20.50° 2θ for the cocrystal; 11.34°, 14.80°, 19.06°, 19.54°, 19.92° and 22.24° 2θ for nicotinamide; 6.08°, 8.84°, 12.22°, 15.12°, 18.42°, 18.88°, 19.44°, 24.12° and 24.68° 2θ for the dihydrate and; 10.08°, 13.00°, 15.24°, 24.82° and 27.54° 2θ for CBZ Form III. The diffractograms obtained were in a very good agreement with the literature[10, 36, 37].

Supplementary Figure 2 presents the Raman spectra of carbamazepine-nicotinamide and of its coformers. In the Raman spectrum of CBZ-NCT characteristic peaks appear common to the pure carbamazepine spectrum, with some slight differences that enabled to characterize and distinguish their spectra. In the region between 1550 – 1630 cm^{-1}, relative to C=C stretch and N-H bend, it is possible to observe a more intense peak around 1600 cm^{-1} in the cocrystal spectrum, concerning the N-H secondary amide bend presented in both nicotinamide and carbamazepine molecules. This vibration is accounts for the homosynthon interaction existing between the carbamazepine dimers and nicotinamide amide in the crystallographic motif of the cocrystal. Changes in nicotinamide spectrum were evident, mainly regarding the extinction of the peak around 1675 cm^{-1} relative to C=O stretch of amides, that occurs due to the homosynthon interaction between the nicotinamide and carbamazepine molecules.

Another characteristic region on the cocrystal spectrum was about 1035 cm^{-1}. In the carbamazepine spectrum it is possible to observe two different peaks concerning the 7-membered ring, which contains the carbamate group, in the region of 1040 cm^{-1} and 1030 cm^{-1}. In nicotinamide it is possible to observe a peak around 1040 cm^{-1} originated from the pyridine ring breathing, this being the most intense peak of this molecule. However, in the cocrystal spectrum, besides the carbamazepine peaks, a third peak was observed in an intermediary Raman shift, close to 1035 cm^{-1}, relative to an asymmetric

breathing of the aromatic ring with the heteroatom. This is the most intense peak in the cocrystal spectrum. The spectra obtained was in agreement with the literature[10, 15].

Owing to the results obtained in the characterization, the cocrystal synthesized by the solvothermal method was used as a standard in the calibration curve to quantify the cocrystal among its coformers as well as a comparison material for the products obtained in the slurry reactions.

3.2 Quantification of carbamazepine-nicotinamide cocrystal by Raman spectroscopy and iPLS

For the calibration and validation data sets, the average of 10 spectra acquired in random points of the samples was used. Due to the differences in the Raman scattering between the samples, the spectra were normalized by the area, so that all components of the mixture presented the same relative intensity, i.e., all samples had the same importance for calibration and validation models. The iPLS algorithm was used in order to find different spectral intervals which provided more linear information for each analyte.

Table 2 presents the summarized data for the PLS regression models using Raman spectroscopy. The values of root mean square error of validation (RMSEV) and cross validation (RMSECV), enable to estimate the model efficiency for quantification of the compounds. The lower the RMSECV and RMSEV values, the lower the error to quantify that substance. For the cocrystal quantification, the values of RMSECV and RMSEV were 2.0% and 3.5% (wt/wt), respectively, which are low error values for quantification of crystalline states.

The selected models showed a good linearity and low data dispersion, indicating a small difference between the real and predicted values for each sample. All components

presented a coefficient of determination of cross validation (R^2 CV) and calibration (R^2 cal) higher than 0.99 and a coefficient of determination of validation (R^2 val) higher than 0.93.

It is inferred by the acquired data that it is possible to quantify the carbamazepine-nicotinamide cocrystal among its coformers from a ternary mixture of these components using Raman spectroscopy and iPLS.

3.3 Monitoring of the cocrystal synthesis in aqueous solution

3.3.1 Reaction at room temperature

During the reaction at room temperature we obtained 151 spectra during a 5 hour period. At the end of reaction, these spectra were organized in a matrix with 151 rows (time) and 751 columns (wavelengths). The spectra were pretreated using first derivative, normalization by area and filtered by PCA using 6 principal components. MCR-ALS was performed to obtain the concentration profiles which enable to observe the reaction pathway.

Figure 1a presents some spectra obtained during the room temperature monitoring. The regions that showed more significant changes during the reaction were 500 cm^{-1}, 700 cm^{-1}, 1020 cm^{-1} and 1600 cm^{-1}. All acquired spectra exhibited a spectra profile similar to the carbamazepine spectrum. This occurs because the Raman scattering of carbamazepine is more intense than that of nicotinamide, furthermore, carbamazepine was present in solid-state during the whole reaction, while nicotinamide was in a diluted solution, which also contributes for low Raman scattering.

At the beginning of the reaction, only the spectrum of carbamazepine Form III can be observed, which is the commercial form of this drug. This spectrum can be characterized by two peaks relative to the 7-member ring molecule (characteristic peaks

at 1020 and 1040 cm^{-1}). After 4 minutes of reaction, the acquired spectrum was similar to the initial spectrum of the reaction. However, at 6 minutes the Raman spectrum was related to the dihydrate form of carbamazepine, characterized by the inversion in the intensity of the peaks at 1020 and 1040 cm^{-1}, and the disappearance of the 535 cm^{-1} peak. At the end of the reaction, no significant change was available, and the final spectrum was the same as the dihydrate form of carbamazepine spectrum.

Figures 1b and 1c shows the concentration and spectral profile recovered by the MCR-ALS model, respectively. The concentration profile demonstrated that at room temperature a quick polymorphic transition is observed. The first three spectra acquired for the reaction monitoring (times equal to 0, 2 and 4 minutes) were related to the carbamazepine form III spectrum. After 6 minutes of reaction, all carbamazepine presented in the reaction turned into the dihydrate form of CBZ. Dihydrate CBZ has lower water solubility than CBZ III, inhibiting the solubilization of this drug, even partially in the aqueous media, to interact with nicotinamide molecule. Therefore, at room temperature no evidence of a cocrystal was observed.

It can be observed in the Figure 1c that the recovered spectra present identical spectral profiles of pure carbamazepine Form III and dihydrate form spectra, an expected fact, since it is well known that carbamazepine presents a polymorphic transition for its hydrated forms in the presence of humidity, what is one of the biggest issues regarding carbamazepine handling in pharmaceutical industries[11]. The model generated by MCR-ALS presented good agreement with the experimental data, with a percentage of explained variance of 99.65% ($R^2 = 0.99$) and a low lack of fit, 5.89 %.

3.3.2 Reaction at 40 °C

Figure 2a presents some spectra obtained during the 40 °C reaction monitoring. As in the room temperature reaction, the spectra presented only characteristic peaks relative to the carbamazepine spectra. With 4 minutes of reaction, it is possible to observe a mixed spectrum between Form III and the dihydrate form of carbamazepine, where relative intensity of peaks 1020 cm^{-1} and 1040 cm^{-1} were altered, and the double peak at 530 cm^{-1} starts to decreased. With 6 minutes of reaction, the acquired spectrum is closely similar to the hydrate form spectrum. At the end of the reaction, it is only possible to observe the pure spectrum of CBZ dihydrate.

The concentration and spectral profile recovered by MCR-ALS are presented in Figure 2b and 2c, respectively. The concentration profile shows the polymorphic transition of CBZ III to the dihydrate form of the drug. After 8 minutes of reaction, all CBZ III was converted in CBZ DH making the CBZ-NCT crystallization impossible. By the spectral profile it can be observed that one of the components presented a Raman spectrum identical to the carbamazepine Form III, while the other component presented a spectrum similar to the dihydrate carbamazepine. The recovered data showed a good adjustment related to the experimental data, presenting 99.46 % of explained variance, with a small lack of fit (7.38 %).

3.3.3 Reaction at 60 °C

Figure 3a shows four different spectra acquired during the 60 °C reaction. At the beginning the spectrum obtained is related to the Form III carbamazepine, however, at 80 minutes of reaction it is possible to observe a slight difference between the intensity of the peaks in the 1000 – 1040 cm^{-1} region, this alteration can indicate both a polymorphic transition to dihydrate and cocrystallization. At 150 minutes of reaction, the spectrum is similar to the cocrystal, with the presence of a few selective peaks, such

as the main peak around at 1035 cm^{-1}(instead of 1040 cm^{-1}) and the changes in the relative intensity of the peaks presented at 750 – 800 cm^{-1}. At the end of the reaction, the spectrum was identical to the cocrystal spectra.

The concentration and spectral profiles obtained by MCR-ALS are presented in Figures 3b and 3c. The spectral profile presents two distinct spectra, one of the recovered spectrum is related to the CBZ Form III, and the other spectrum shows as identical to the CBZ-NCT cocrystal spectrum. At the beginning of the reaction, there is only pure carbamazepine. In 60 minutes, no significant changes can be observed. In the previous reactions, the carbamazepine undergoes a polymorphic transition in 6 minutes of reaction, the preservation of spectral profile in over an hour of reaction suggests that at 60 °C the dihydrate form of carbamazepine is not stable, even in aqueous solution.

At close to 80 minutes of reaction, the relative concentration of CBZ Form III started to decrease and the intensity of the cocrystal spectrum increased. After two hours of reaction, the cocrystal is predominant.

The modeled data showed a good agreement with the experimental data, with a percentage of explained variance of 96.62 % and lack of fit of 14.88 %. The reaction at 60 °C enabled a high conversion of the initial reactants to cocrystal.

3.3.4 Reaction at 80 °C

Even with a high conversion at 60 °C, a reaction at 80 °C was conducted, which is the temperature described in the literature[11] as needed for the polymorphic transition of CBZ DH to Form III. As noted in the reactions at 40 °C and at room temperature, the CBZ DH inhibits the cocrystallization reaction with nicotinamide.

Figure 4a presents some spectra obtained during the reaction monitoring. With 170 minutes of reaction, changes can be noticed in the relative intensities of the initial spectrum in four distinct regions: 520 – 550 cm^{-1}, 740 – 800 cm^{-1}, 1000 – 1050 cm^{-1} and

between 1550 – 1650 cm^{-1}. At the out of plane bend of the aromatic ring region (between 500 and 550 cm^{-1}), it was possible to observe an enhancement in the intensity of the peak at 530 cm^{-1}, when compared to the initial spectrum. At 740 – 800 cm^{-1}, there was an emergence of a peak close to 780 cm^{-1}, and a clear alteration in the relative intensities of the peaks in this region. In the aromatic ring C-H bend region (1000 – 1050 cm^{-1}), there was a shift of a peak and appearance of a shoulder, that is an indicative that two or more peaks were overlapped in this area. Between 1550 and 1650 cm^{-1}, the relative intensity among the peaks was different from the expected for the three evaluated forms, DH, Form III and cocrystal.

Right after the heating stage, at 210 minutes of reaction, the acquired spectrum is quite similar to the 170-minute spectrum. However, a decrease can be noticed in the shoulder close to 1045 cm^{-1} and an increase in the peak around 1035 cm^{-1}, characteristic of the cocrystal spectrum. At the end of the reaction, only a spectrum identical to the CBZ-NCT spectrum can be seen.

When the PCA filter was executed for the previous reactions, only two principal components were significant, which were related to the carbamazepine Form III and dihydrate CBZ (for room temperature and 40 °C) or cocrystal (for 60 °C). In the reaction at 80 °C, it was possible to observe three principal components that were significant for the model. Thus, in the 80 °C reaction, the MCR-ALS routine was performed using 3 components. The concentration profile and spectral profile recovered by the model were presented in Figures 4b and 4c. The first recovered spectrum was related to the pure spectrum of Form III, the second one looks like a mixture of cocrystal and the spectrum of amorphous carbamazepine, and the last was the CBZ-NCT spectrum.

Figure 4b displays the concentration profile of the reaction. At the beginning of the reaction, only the Form III spectrum remains. After 150 minutes the Form III is consumed and a mixture of amorphous carbamazepine and cocrystal is observed. This mixture is maintained until the cooling stage. When the solution is cooled, the crystals start to nucleate as a cocrystal form, resulting in almost full conversion of the reactants in the desired product. At the end of the reaction it is possible to observe CBZ III concentration values above zero, this occurs due to the lack of fit of the model and the high similarity of the spectra.

Some studies have reported that the cocrystallization mechanism of carbamazepine combined with nicotinamide in aqueous solution occurs firstly with a polymorphic transition of crystallized carbamazepine (usually in Form III) to the amorphous form. The amorphous form presents a higher dissolution rate and water solubility than other polymorphs[38]. Therefore, the amorphous CBZ solubilize in the nicotinamide solution and due to the homosynthon interaction the coprecipitation occurs. This study also corroborates with the described mechanism evidencing a recovered spectrum which is a mixture of cocrystal and amorphous CBZ, characterized by the shoulder at 1040 cm^{-1} and the relative intensity in the region of 1550 - 1650 cm^{-1}, different from the other CBZ forms.

The recovered data showed a good suitability with the experimental data, obtaining the lowest error values with 99.51 % of explained variance and lack of fit of 6.97%. The reaction at 80 °C enabled a better understanding of the cocrystallization mechanism as well as the almost full conversion of the initial reactants into the cocrystal, this fact being a novelty in aqueous solution.

3.4 Characterization and quantification of the final products

In order to evaluate the final product of each reaction, the materials obtained at the end of the reactions were characterized by powder X-ray diffraction and Raman spectroscopy. Supplementary Figure 3 presents the diffractograms of the final products of each reaction. The crystallographic patterns of the reactions at room temperature and at 40 °C presented a pattern similar to the dihydrate form of carbamazepine, characterized by the main peaks at 8.84° and 12.22° 2θ. The diffractograms of the reactions at 60 °C and 80 °C showed pattern analogous to the cocrystal diffraction pattern, mostly characterized by the first four diffraction peaks at: 6.68°, 8.92°, 10.22°, 13.38° 2θ; and the main peak at 20.46° 2θ. However, in the 60 °C reaction, it is possible to observe a small peak at 12.28° 2θ, indicating a small contamination of CBZ DH in the sample. Moreover, the peaks presented in the 60 °C diffractogram related to the CBZ-NCT are larger than in the 80 °C diffractogram. The broadening of the peaks can be related to the crystal size, thus the crystals obtained at the 60 °C reaction probably are smaller than those at 80 °C.

As both temperatures present the CBZ-NCT formation, and considering that at 60 °C the crystals are smaller, it can infer that 60 °C is the optimum temperature for CBZ-NCT nucleation, once it is found a good conversion (good number of crystal) but with small particle size. While at 80 °C, the nucleation process has occurred and the crystals starts to growth.

Supplementary Figure 4 exhibit the Raman spectra of the final products of each reaction temperature. The acquired spectra at room temperature and 40 °C reactions are similar to the CBZ DH spectrum, characterized by the inversion of the relative intensity of peaks between 1000 and 1040 cm^{-1}, and the single peak at the region of 545 cm^{-1}. The Raman spectra of the products obtained at 60 °C and 80 °C are similar to the cocrystal spectrum. The double peak at 1020 cm^{-1} and the intensity relation of the peaks

between 1550 and 1650 cm^{-1} could characterize the acquired spectra. However, the spectrum of the 60 °C reaction presents a shoulder at 1030 cm^{-1}, and different relation of intensities in the region of 1550 – 1650 cm^{-1}. These results, coupled with the X-ray diffractograms, indicates that the 60 °C reaction presents a small contamination of CBZ DH, while the 80 °C reaction achieved a full conversion of the final products.

In order to evaluate the conversion of each reaction, the samples were analyzed using the iPLS regression models developed in the section *3.2*. Table 3 presents the percentage of each component in the final products of the reactions. The room temperature and 40 °C reactions resulted in a full conversion of Form III to CBZ DH. The reaction at 60 °C presented a high conversion of the initial reactants to cocrystals, although with a small amount of unreacted CBZ DH. The reaction at 80 °C was the only experiment which enabled a full conversion of carbamazepine and nicotinamide into the CBZ-NCT cocrystal.

4. Conclusion

It was possible to perform the cocrystallization reaction of carbamazepine-nicotinamide cocrystal using a slurry reaction method, with an environmentally friendly solvent in two different temperatures (at 60 °C, and 80 °C), with full conversion of the initial substrates at 80 °C.

iPLS regression shows to be robust and reliable to quantify the CBZ-NTC cocrystal among its coformers using Raman spectroscopy, which is very important to verify the quality of the final product. The models gave validation errors lower than 8.2 %, which is an acceptable value for quantification of different crystalline structures of the same molecules, due to the high similarity of the spectra.

The in-line monitoring using Raman spectroscopy and MCR-ALS shows to be a powerful combination to *in-situ* evaluation of crystallization process. The recovered concentration profiles of each reaction showed high correlation with the final results found by Raman and XRD.

The reactions at room temperature and 40 °C showed a quick polymorphic transition from Form III to the dihydrate. Although it was not the main focus of this work, the results showed the great importance of the control of the drug to prevent undesirable changes during the tablet manufacturing. In the reaction at 60 °C, there was a small formation of the dihydrate form, but CBZ-NCT cocrystals were mainly yielded. At 80 °C it was possible to identify the presence of amorphous carbamazepine corroborating the proposed cocrystallization mechanism. The amorphous form interacts with the nicotinamide present in solution and forms the cocrystal. The reaction at 80 °C also allowed a cocrystallization process with several advantages, such as: high final product purity (conversion higher than 98%), easy to control and environmentally friendly.

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Figure 1

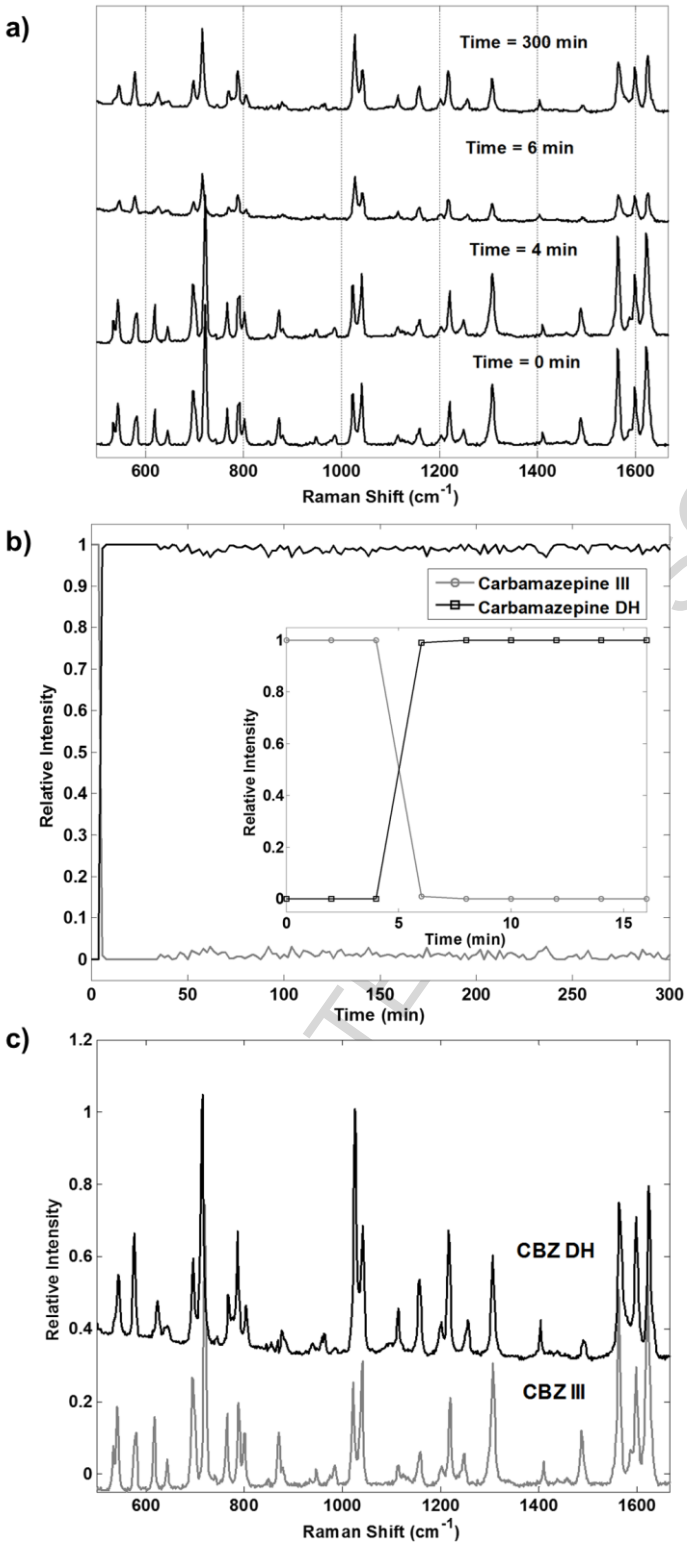


Figure 1. Reaction at room temperature: a) Raman spectra obtained at four different times; b) Concentration profile and; c) Integrated Raman spectral profile.

Figure 2

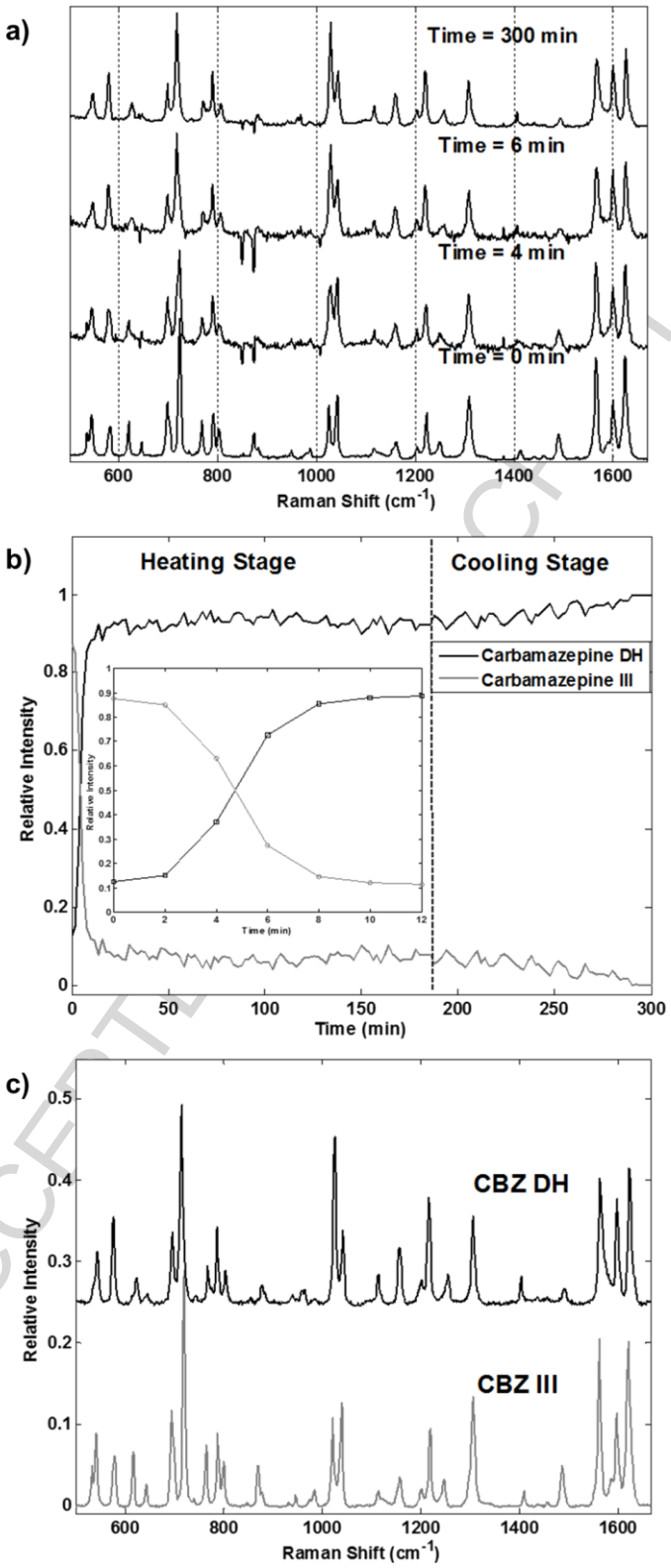


Figure 2. Reaction at 40 °C: a) Raman spectra obtained at four different times; b) Concentration profile and; c) Integrated Raman spectral profile.

Figure 3

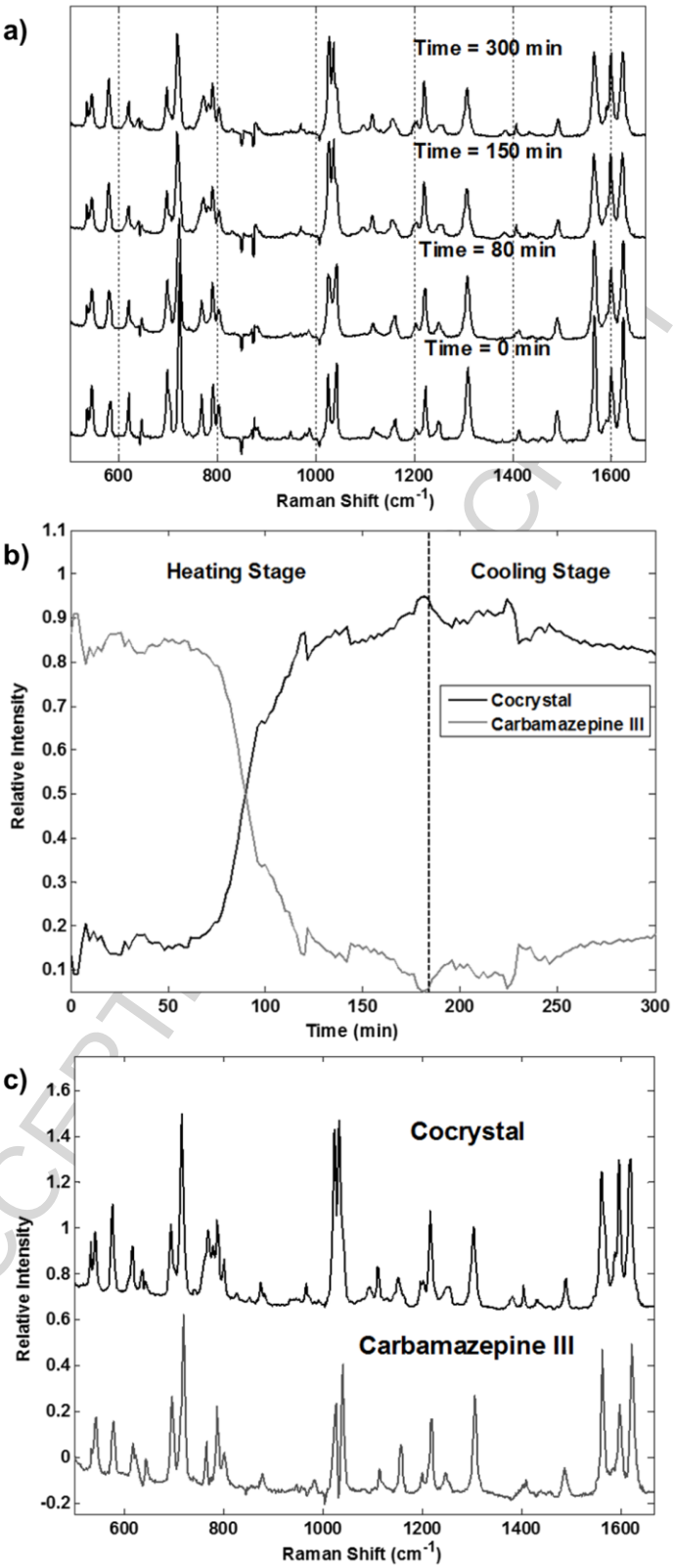


Figure 3. Reaction at 60 °C: a) Raman spectra obtained at four different times; b) Concentration profile and; c) Integrated Raman spectral profile.

Figure 4

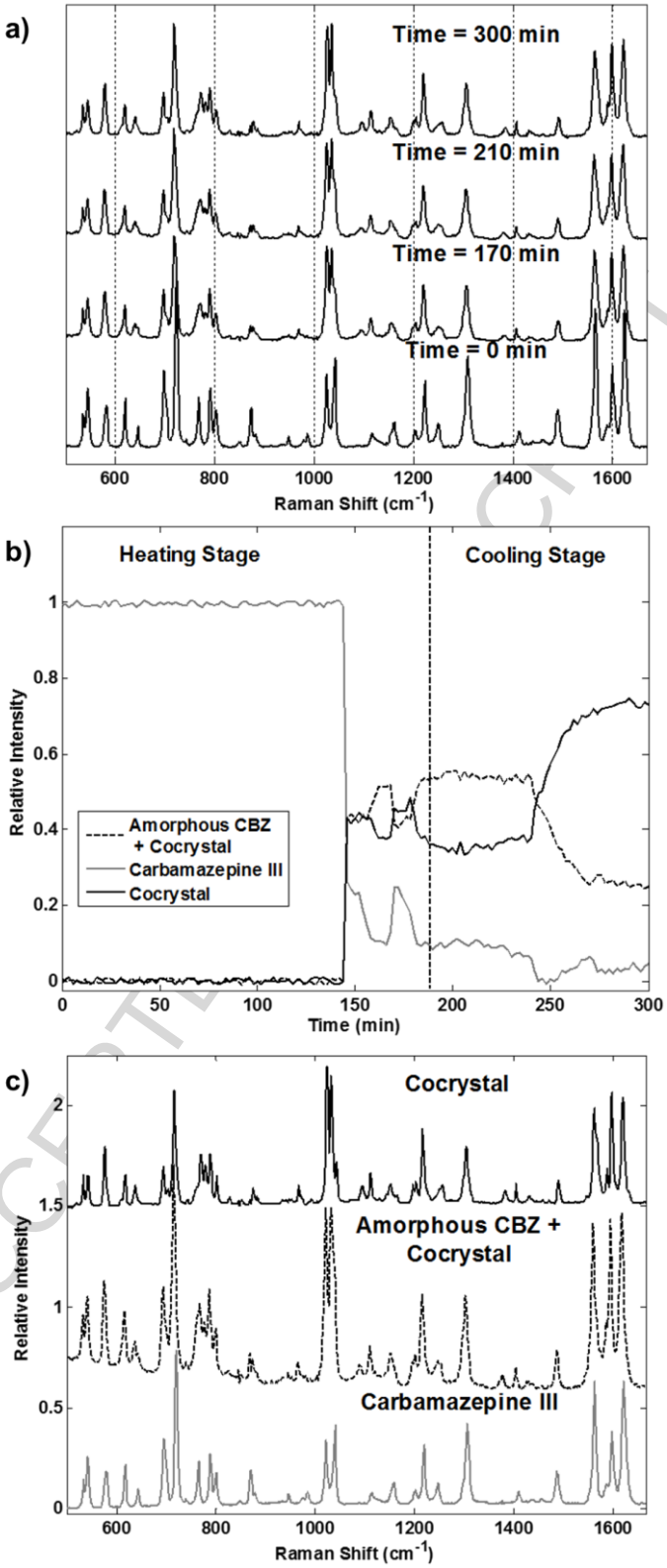


Figure 4. Reaction at 80 °C: a) Raman spectra obtained at four different times; b) Concentration profile and; c) Integrated Raman spectral profile.

Table 1. Ternary mixture design for calibration and validation samples for multivariate calibration using iPLS.

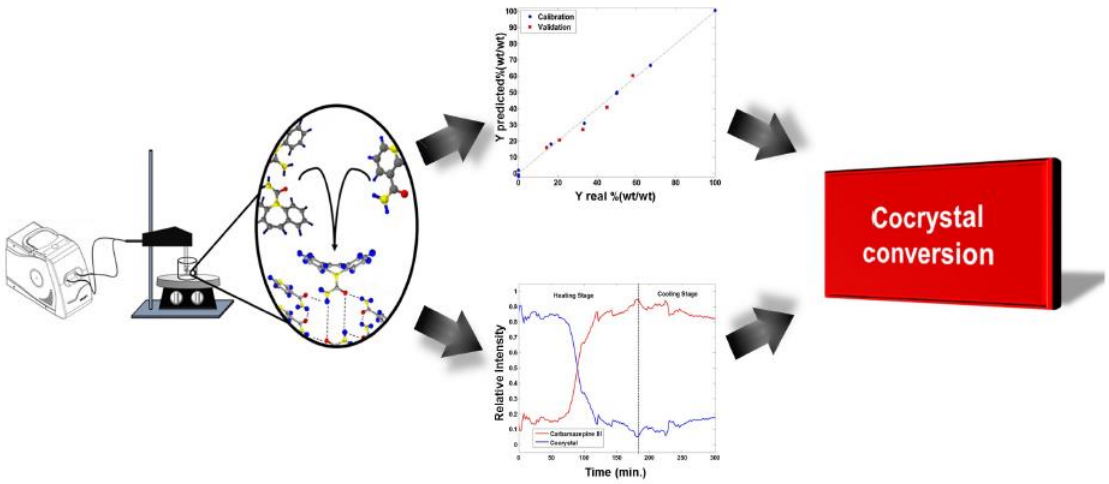
	Percentage (%)		
	CBZ	NCT	CBZ-NCT
Cal1	100.0	0.0	0.0
Cal2	0.0	100.0	0.0
Cal3	0.0	0.0	100.0
Cal4	50.0	50.0	0.0
Cal5	50.0	0.0	50.0
Cal6	0.0	50.0	50.0
Cal7	66.5	16.7	16.8
Cal8	16.6	66.7	16.6
Cal9	16.6	16.6	66.7
Cal10	33.3	33.3	33.3
Val1	75.7	9.2	15.1
Val2	42.0	12.9	45.1
Val3	7.9	33.7	58.4
Val4	38.4	40.4	21.2
Val5	26.5	40.5	33.0

Table 2. Parameters of the iPLS models in the simultaneous quantification of the cocrystal and its coformers.

	Carbamazepine	**Nicotinamide**	**Cocrystal**
RMSEC (%)	1.6	1.1	1.4
RMSECV (%)	2.1	1.4	2.0
RMSEV (%)	5.1	8.2	3.5
R² cal	0.99	0.99	0.99
R² CV	0.99	0.99	0.99
R² val	0.97	0.93	0.96
Pretreatment	Normalization + 1st derivate		
Interval size	40		
Included variables	160 variables		
Spectral ranges	355-426; 777-907; 1100-1161 (cm^{-1})		
Latent variables	3		

Table 3. Characterization of final products of slurry reactions using iPLS.

Temperature	Carbamazepine DH (%)	Nicotinamide (%)	Cocrystal (%)
Room temperature	99.2	0.2	0.6
40°C	98.7	0.3	1.0
60°C	13.4	1.1	85.5
80°C	1.0	0.6	98.4



Graphical abstract

Highlights

- Pure cocrystal bulk was obtained by synthesis in water at 80 °C.
- Raman spectroscopy and MCR-ALS were employed for in-line monitoring.
- The drug dissolution, nucleation and crystallization steps were identified by Raman.
- PLS regression models allowed the quantification of cocrystal among its precursors.
- The quantification models presented RMSEV between 2.5 – 8.2 % for all components.