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A combined approach using differential scanning calorimetry with polarized light thermomicroscopy in the investigation of ketoprofen and nicotinamide cocrystal

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ABSTRACT

A screening of ketoprofen cocrystal with nicotinamide as a coformer was performed using the Kofler contact method and mechanochemistry. The crystallization of KET (with cocrystal formation) can occur during the heating process. However, although it is thermodynamically favorable, it was found that the kinetic reactions were slow, since the KET crystallization took about 30 days. The compounds obtained were analysed by differential scanning calorimetry (DSC), polarized light thermomicroscopy (PLTM), Fourier transform infrared spectroscopy (FTIR), and X-ray powder diffraction (XRPD). Cocrystal formation between ketoprofen and nicotinamide has great interest because the nicotinamide has FDA/GRAS status. At the adopted conditions, the achievement of cocrystal between ketoprofen and nicotinamide was not possible by mechanochemical methods However, experiments conducted by the Kofler method confirmed that ketoprofen interacted, under determined conditions, with nicotinamide giving rise to a new cocrystal. Furthermore, biological tests revealed that the KET + NA (1:1) eutectic system obtained by grinding had a greater anti-inflammatory action when compared to pure ketoprofen.

1. Introduction

Even after the marketing stage, an active pharmaceutical ingredient (API) is still the subject of research aimed at improving its physical and chemical properties, especially its solubility in water, since it operates as a solvent in the main biological systems [1].

There are several ways to improve the physicochemical properties of an API, such as salt formation, polymorphic forms and solvates or hydrates [2]. Recently, cocrystal screening has become an important and innovative approach to improve the physicochemical properties of an API by using appropriate molecules as coformers [2,3].

Ketoprofen (KET) or (*RS*)-2-(3-Benzoylphenyl)propionic acid (see Fig. 1a) is a non-steroidal anti-inflammatory and pertains to Class II (low solubility, high permeability) of the biopharmaceutics classification system (BCS) [4,5]. which is derived from phenylpropionic acid and characterized pharmacologically by its anti-inflammatory, antipyretic and analgesic action. For those reasons it is widely used in the medical and veterinary fields. Its mechanism of action is through the inhibition of prostaglandin and the synthesis of leukotriene [6].

The formation of supramolecular heterosynthons (Fig. 1b) is highly favored [7]. Based on this information, molecules that presented the functional groups shown in Fig. 1b were tracked and the choice of nicotinamide (NA) (Fig. 1c) as coformer was based on the fact that the FDA regards this substance as safe [8].

Another essential factor considered for the choice of the coformers was the pKa difference (ΔpKa) between the API and the other molecules. An acid-aromatic nitrogen hydrogen bond can be formed if the ΔpKa is less than 3.75 [9]. KET and NA fully satisfy this condition, since their pKa values are 4.53 [10] and 3.35 [11], respectively.

Kofler has published about 250 papers which describe the contact method to determine the phase diagrams of the binary mixtures of organic compounds. This method can provide a qualitative

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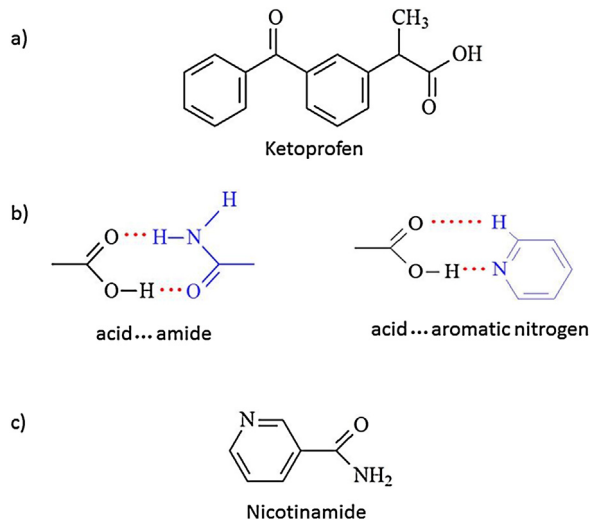

Fig. 1. (a) Ketoprofen, API. (b) Supramolecular heterosynthons. (c) Aromatic acid amide cocrystal formers.

and rapid indication about the formation of cocrystals and because of that it was used as an initial approach [12,13]. Subsequently, a grinding experiment was used to get possible cocrystals, that were analysed by DSC, FTIR and XRPD.

2. Experimental procedures

2.1. Materials

(*RS*)-Ketoprofen 98% and nicotinamide 99% were purchased from Sigma-Aldrich Chem. Co. (St. Louis, MO, USA) and used without purification.

2.2. Grinding

A total mass of 50 mg (KET + NA) was grinded in a Retsch MM400 mill for 30 min at a frequency of 15 Hz, in a 10 mL stainless steel jar with two stainless balls (7 mm diameter) inside.

2.3. Polarized light thermal microscopy (PLTM)

PLTM studies were performed in a Linkam hot stage system, model DSC600. For optical observation, it was used a Leica DMRB microscope and a Sony CCD-IRIS/RGB video camera. The images formed (200× magnification) were analysed through the Real Time Video Measurement System software by Linkam. The experiments of Kofler's contact method were accomplished according to Berry et al. [13].

2.4. Differential scanning calorimetry (DSC)

The DSC curves were obtained through the calorimeter Pyris1, PerkinElmer, with power compensation. A 30 μL aluminum crucible hermetically sealed was used as a sample holder. A similar empty crucible was used as reference. All experiments were performed in nitrogen atmosphere with a flow rate of 20 mL min^{-1}, with an approximate mass of sample equal to 2 mg. The enthalpy calibration was made with indium [14] and the temperature calibration was performed with the onset temperature of indium and standard benzoic acid [14,15]. A 5 °C min^{-1} heating rate was adopted for all calibration processes.

2.5. Fourier transform infrared spectroscopy (FTIR)

Spectra of the solids were recorded at room temperature utilizing the attenuated total reflectance (ATR) technique and using a Bruker Vertex 70 spectrometer with a scanning range between 400 and 4000 cm^{-1} (resolution 4 cm^{-1}) and a diamond crystal as support.

2.6. X-ray powder diffraction (XRPD)

X-ray powder diffractograms were obtained on a Siemens DMAX 2000 X-ray diffractometer using Cu Kα radiation (λ = 1.5406 Å) and settings of 20 kV and 2 mA. The samples were placed on a glass support and exposed to the radiation ($3° \leq 2\theta \leq 50°$).

3. Biological activity

3.1. Isolation of peripheral blood mononuclear cells

Blood from six healthy blood donors was collected with Vacutainer tubes containing heparin as an anticoagulant. The peripheral blood mononuclear cells (PBMC) were isolated by density gradient centrifugation on Histopaque® -1077 (Sigma-Aldrich, St Louis, MO, USA). Cell viability, as determined by 0.2% trypan blue, was >80% in all the experiments. The PBMC were counted using Turkís solution and the monocytes were counted using 0.02% neutral red. Both population cells were suspended to a concentration of 1×10^6 cells/ml in complete medium.

3.2. Cytotoxicity assay

The cytotoxic activity of the KET + NA (1:1) was determined using the colorimetric microculture MTT assay (MTT = 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2H-tetrazolium bromide) [16]. The PBMCs were seeded in 100 μL aliquots in RPMI-1640, supplemented with 20% heat-inactivated fetal bovine serum (SBF), into 96-well microculture plates. The eutectic compound KET + NA (1:1) was added in the PBMC culture and the plate was incubated for 96 h at 37 °C in a humid atmosphere with 5% CO_2. Dimethylsulfoxide (DMSO) was subsequently added to dissolve the formazan crystals. Optical densities were measured using an ELISA microreader (EL800, BIO-TEK Instruments, INC) at a wavelength of 490 nm. The quantity of viable cells was expressed by comparison between the treated cell cultures and the untreated control cultures. The cytotoxicity represented the concentrations that caused 50% inhibition.

3.3. Monocyte cell culture

Monocytes were seeded in 100 μL aliquots in RPMI-1640, supplemented with 20% SBF, into 96-well culture plates. To assess the influence of the KET + NA (1:1) on the inflammatory milieu, the monocytes were submitted to four treatments: 1) simultaneous stimulation with lipopolissacaride (LPS) – 10 μg ml^{-1} and KET + NA (1:1) (initial concentration); 2) LPS; 3) KET + NA (1:1); and 4) non-stimulated cell culture. Cells were incubated at 37 °C and 5% CO_2. After 24 h, the cell-free supernatants were collected to determine the cytokines and the cells were submitted to determination of the hydrogen peroxide release.

3.4. Production of hydrogen peroxide (H_2O_2)

A phenol red solution [dextrose 1% (Sigma-Aldrich Chem. Co. (St. Louis, MO, USA), phenol red 1% (Sigma-Aldrich Chem. Co. (St. Louis, MO, USA), horseradish peroxidase type II – 5 uni (Sigma-Aldrich