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Milling and comilling Praziquantel at cryogenic and room temperatures: Assessment of the process-induced effects on drug properties

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ABSTRACT

This study is a comprehensive evaluation of praziquantel (PZQ) behavior upon grinding considering the influence of milling temperature (cryogenic vs room temperature), frequency and time and presence of polymers (milled raw PZQ vs comilled PZQ/povidone and PZQ/crospovidone at 50:50 w/w) on two experimental responses (residual crystallinity and PZQ recovery). To this aim a full factorial design was set up and the responses of the experimental design were statistically assessed.

The powder temperature, measured in different milling conditions, was found to increase with increasing milling frequency and time, up to a maximum recorded value of 46.9 °C (after 90 min at R.T.), for all the three powder systems. When PZQ was ground in RT environment, the recovery was 100%, independently from frequency and time of milling. Its residual crystallinity remained pronounced (>70%) upon milling, even if treated at the most severe conditions. Conversely, when the drug was milled in presence of the polymers, it showed a higher tendency to degradation and amorphysation, independently from the choice of the polymer. The use of cryogenic conditions, operating at temperatures lower than PZQ glass transition, permitted to dramatically reduce PZQ residual crystallinity when the drug was ground by itself. In the case of binary mixtures, the switch to a cryogenic environment did not affect significantly the experimental responses, but permitted to obtain a more predictable trend of both drug recovery and residual crystallinity when varying time and frequency of milling.

1. Introduction

Among the several available therapeutic entities, Praziquantel (PZQ) is the first-line drug used for the shistosomiasis treatments. PZQ has been widely used for 40 years and the disease control entirely depends on this single drug [1]. Its main advantages are the low cost, the therapeutic regimen as it is required a one day treatment and the low toxicity [2]. On the other hand, the high dose needed (40 mg/kg bodyweight) to reach the ED_{50} (due to its low aqueous solubility and the high first pass effect) and its poor palatability represent the main drawbacks [3]. Moreover, the non-appropriateness of the available dosage forms (600 mg PZQ tablets) makes dose adjustment and swallowing a real problem in pediatric patients. In fact, young children have been reported not to be able to swallow those large tablets. Several strategies have been undertaken to improve its solubility in view of a possible PZQ dose reduction [3–7]. More recently PZQ has been processed by comilling with several pharmaceutical excipients with the aim to alter the drug solid state versus an amorphous state [8].

Milling is a common mechanical process that is used regularly within the pharmaceutical industry for the reduction of particle size [9]. However, high levels of mechanical energy involved may result in the crystal lattice disruption of materials [10], causing polymorphic transformations, crystal defect generation, amorphization and other additional physical and chemical changes in a crystalline drug [9,11,12]. It is well documented that amorphization is mainly due to accumulation of crystal defects during grinding which ultimately results in a loss of long-range order which can

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be gradual or spontaneous. [13]. Once reached a critical limit of defects, the amorphous phase has greater thermodynamical stability than the disordered crystal and thus the transformation may occur [11]. Furthermore, amorphization increases typically with increasing milling time [14,15]. Amorphous phases typically show better dissolution properties; however due to the increased molecular mobility, are often susceptible to chemical degradation [11] and to conversion into the crystalline form [16].

Cryomilling consists in milling materials at cryogenic temperatures using liquid nitrogen as cryogenic media [17]. This process represents an effective method of reducing the effect of temperature-induced changes as well as the risk of recrystallisation of the amorphous material [9]. Cryogenic temperatures display further advantages: the material becomes brittle to be milled, reducing the specific energy required for milling; the materials are protected from thermal damage and undesirable chemical reactions between phases and particles aggregation is reduced [12]. It is reported that grinding a compound far below its Tg is more efficient to prepare the amorphous state of drugs than grinding it at room temperature [10,18], although cryo-milled drugs exhibited reduced physical stability [9]. In the last years cryogenic grinding has been applied to investigate APIs having either high glass forming ability as piroxicam [19] and indomethacin [11,20] or an easy tendency of polymorphic transformations as carbamazepine [21] and ranitidine [22]. In the case of cryo-comilling, results evidenced that the piroxicam-PVP system was less stable than that of indomethacin-PVP [20]; in addition, for both systems the polymer amount should be more than 50% with respect to the drugs to obtain their amorphous state. Moreover, mefenamic acid was found to transform in the polymorphic form when pure API was cryogenically milled, while formed a solid dispersion when cryo-milled with PVP [23].

Generally, it might be stated that the majority of studies reported in the literature have been focused on the use of milling/cryomilling to obtain an amorphous drug phase [10,11] and on the evaluation of its physical stability [9,24]. However the effect of the API chemical degradation along with the disruption of the starting crystal phase has been poorly investigated. Recently, we obtained a coground having a 50% w/w PZQ content and showing a solubility 4.6-fold higher than raw drug. Crospovidone followed by povidone were the polymers that increased the PZQ solubility to a higher extent [8], appearing physically stable over six ageing months. The comilling process at room temperature induced a relatively high extent of amorphous phase (residual crystallinity was about 12%). Nevertheless, the results evidenced a partial degradation of the API showing an effective reduced drug recovery (ranging about 90%).

Therefore this study aimed to develop a comprehensive evaluation of PZQ behavior upon the milling process considering the influence of milling temperature (cryogenic vs room temperature), frequency, time and presence of polymers (milled raw PZQ vs comilled PZQ-CROSPVP and PZQ-PVP at 1:1 wt ratio) on two experimental responses (residual crystallinity and PZQ recovery). To reach this aim, a full factorial design of experiments (DoE) was set up and the statistical assessment of the DoE responses was performed.

2. Materials and methods

2.1. Materials

Praziquantel (PZQ) Ph. Eur. grade ((11bRS)-2-(Cyclohexylcarbonyl)-1,2,3,6,7,11b-hexahydro-4-H-pyrazino[2,1-*a*]isoquinolin-4-one) was kindly donated by Fatro S.p.A. (Bologna, Italy). PZQ impurity A (2-Benzoyl-1,2,3,6,7,11b-hexahydro-4-H-pyrazino[2,1-*a*]isoquinolin-4-one) and impurity B (2-Cyclohexanecarbonyl-2,3,6,7-tetrahydro-pyrazino[2,1-*a*]isoquinolin-4-one) were Ph. Eur. grade and purchased from Endotherm Gmbh (Saarbruecken, Germany).

Povidone (Kollidon K30, Fluka Chemie- Buchs, Germany) (PVP) and micronized crospovidone (Kollidon-CL-M, BASF-Ludwigshafen, Germany) (CROSPVP) were used in the co-milling experiments. HiPersolv Chromanorm Methanol (Ph. Eur. for HPLC Gradient Grade) was from VWR Chemicals BHD PROLABO® Milano, Italy.

2.2. Preparation of binary mixtures

Before grinding (at room temperature or cryo-milling), PZQ and each polymer (PVP, CROSPVP) were manually gently mixed in an agate mortar in a 1:1 drug-to-polymer weight ratio for the standardized time of 3 min (batch size ranging about 1 g).

2.3. Milling experiments

The grinding process was performed in a vibrational mill-Retsch MM400 (Retsch GmbH, Haan, Germany) which was equipped with 2 screw-type zirconium oxide jars, each with a capacity of 35 ml. A ceramic material like zirconium oxide was selected due to its high density (5.9 g/cm^3), allowing for a high energy input. On the basis of previous experiences [8], the amount of powder to be introduced in the milling jar was determined to be 1.072 g per jar, and three zirconium oxide 15 mm spheres were used as milling media. PZQ was ground by itself and in combination with the polymers (PVP or CROSPVP) one by one (50% w/w). No cooling was provided to the grinding jar during room temperature (RT) milling. For cryogrinding (CC) experiments, prior to milling, the jars containing the samples were immersed in liquid nitrogen for 1 min; re-cooling of the milling jars with liquid nitrogen for 1 min was performed every 15 min of milling. Vibrational frequency and milling duration were varied according to the experimental plan reported below (paragraph 2.5).

Post milling, the samples were collected and stored in glass vials in a desiccator at room temperature for further characterization and processing.

2.4. Sample temperature measurement

Temperatures of the samples were recorded using a 35XP-A Amprobe K-type thermocouple (Amprobe, Test Tools Europe, Glottertal, Germany). After opening each jar, the K-type bead thermocouple was immediately placed in touch to the powder. To understand the general trend of temperature during milling and comilling, the temperature was recorded every 15 min from time 0–90 min at the frequency of 15, 20 and 25 Hz during cryogrinding (CC) and room temperature (RT) milling. Average measurements values were made from three replicates.

2.5. Experimental design

For planning the milling trials, a factorial design was employed by means of JMP software (SAS Institute Inc). As reported in Table 1, three process variables (milling time, frequency and temperature) were considered at 2 levels, whilst a formulation variable was evaluated at 3 levels. In particular, the following levels (lower and upper, respectively) for each process variable were considered: 30 min and 90 min for time, 15 Hz and 25 Hz for frequency, room temperature (RT) and cryogenic conditions (CC) for the qualitative variable milling temperature. As for the formulation variable, to allow comparison between the performance of the drug ground by itself and in combination with the polymers one by one, the