An explorative analysis of process and formulation variables affecting comilling in a vibrational mill: The case of praziquantel

Beatrice Perissutti[a,*], Nadia Passerini[b], Ramona Trastullo[b], Jennifer Keiser[c], Debora Zanolla[a], Guglielmo Zingone[a], Dario Voinovich[a], Beatrice Albertini[b]

[a] Department of Chemical and Pharmaceutical Sciences, University of Trieste, P.le Europa 1, 34127 Trieste, Italy
[b] Department of Pharmacy and BioTechnology, University of Bologna, Via S. Donato 19/2, 40127 Bologna, Italy
[c] Helminth Drug Development Unit, Department of Medical Parasitology and Infection Biology, Swiss Tropical and Public Health Institute, Socinstr. 57, CH-4051 Basel, Switzerland

ARTICLE INFO

Accepted 22 May 2017

Keywords:
Praziquantel
Experimental design for screening
Vibrational mill
Solubility
Drug recovery
Residual crystallinity

ABSTRACT

Praziquantel, a BCS II class anthelmintic drug used for the treatment of schistosome infections, was coground in a vibrational mill with different polymers (linear and crosslinked povidone, copovidone and sodium starch glycolate). An explorative analysis of formulation variables (drug-polymer wt ratio and polymer type) and process parameters (type of grinding media, grinding time and frequency) was carried out with the help of an experimental screening design. The influence of the above mentioned factors on three PZQ characteristics (residual crystallinity, water solubility enhancement and drug recovery) was studied. The variation of carrier amount proved to be by far the most important variable affecting all the experimental responses. A lower impact and, in some cases, rather null effect, had the variation of the process variables. All coground systems were characterized by a high amorphous degree and a solubility significantly higher than the API. A very promising product was obtained by processing at 20 Hz for 4 h, using 3 spheres of 15 mm as grinding media, i.e. a coground having a 50% API content, showing a 4.6-fold greater solubility at 20 °C than pure praziquantel. This product maintained the same antischistosomal activity of pure API and was both physically and chemically stable for at least 6 months.

1. Introduction

Praziquantel (PZQ) is the only available drug used to treat people infected with parasite flatworms that cause the neglected tropical disease schistosomiasis (bilharzia), one of the most prevalent parasitic diseases (Nogi et al., 2009). It is included in the WHO model list of essential drugs for adults (World Health Organization, 2015a) and for children (World Health Organization, 2015a), and is at the core of preventing chemotherapy programs. This drug, discovered in the seventies, is both unexpensive and of low toxicity (Cioli et al., 2014), however its utilization is limited by many important drawbacks. The main disadvantages, especially for pediatric patients, are related to its exclusive marketed formulation, i.e. oral tablet, which have a bitter and disgusting taste (Meyer et al., 2009) and a high dose needed (40 mg/kg bodyweight). Young children have been reported not to be able to swallow those large 600 mg tablets (Talaat and Miller, 1998). The high dose is required for PZQ to be effective *per os* administration due to the low aqueous solubility (PZQ belongs to BCS class II) (Lindenberg et al., 2004) and the high first pass effect (Huang et al., 2010).

A few studies have focused on this challenging enhancement of PZQ solubility, carrying out both classical approaches (fast dispersible granules, solid dispersions with polymers and cyclodextrin complexation) and modern technologies (spray congealing and melt granulation, solid lipid nanoparticles) (Becket et al., 1999; Yang et al., 2009; Chaud et al., 2013; Passerini et al., 2006; Trastullo et al., 2015).

In this paper, we describe how vibrational milling can be used to enhance the solubility of praziquantel by co-milling with selected pharmaceutical excipients. It has been proven that cogrinding with suitable polymers might alter the solid state of a drug through its deconstruction versus an amorphous state (Carli et al., 1987; Shakhtshneider and Boldyrev, 1999; Colombo et al., 2009). Several previous studies demonstrated that this procedure was helpful for enhancing solubility and dissolution rate of many BCS class II or IV drugs (Sugimoto et al., 1998; Shin et al., 1998; Mura et al., 2002; Voinovich et al., 2009; Hasa et al., 2011). On the other hand

* Corresponding author.
E-mail address: bperissutti@units.it (Beatrice Perissutti).

experiments performed in our lab evidenced that cogrinding failed for several drugs, as gliblencamide comilled with crospovidone and β-CD and croscarmellose sodium, leading to a poorly soluble composite substance/complex (depending on the drug to polymer weight ratio), as benznidazole comilled with superdisintegrants being subjected to massive chemical degradation and mechanochromism depending on the mechanical input (time and frequency of milling). Therefore this quite novel technological approach has great potentiality in the solubility enhancement field but further investigation is necessary for a better comprehension of process and formulation conditions to obtain the desired product characteristics. To carry out an explorative analysis of these aspects (process and formulation factors) of comilling via neat grinding praziquantel and 4 different pharmaceutical carriers, a screening design was employed. Even though there are a few studies applying the design of experiments in the context of mechanochemical activation (Hasa et al., 2011a, 2011b), this is the first research aimed to screen simultaneously the effect of formulation and vibrational mill conditions at each assigned level on the three properties. In addition, this is the first attempt of cogrinding PZQ, therefore, a proper design of experiments would permit to check the effect of the process and formulation factors on the physical and chemical characteristics of the antihelminthic drug.

Screening designs are typically used in the early stages of experimentation, they are among the most common experimental designs and generally they require fewer experimental trials than other designs (Voinovich et al., 1999). Screening studies are in fact carried out in attempt to provide the experimenter the most relevant information (identification of important factors) with the minimum effort (Cela et al., 2009). In such research, many factors, both continuous and discrete, have been considered and examined at different levels, to identify which ones has the greatest effect on the responses. As process variables, milling time, type of grinding media and milling frequency were studied, whereas as formulation factors the type of pharmaceutical polymer used as a process adjuvant and its percentage in the coground systems were examined.

As experimental responses of the screening design, the solubility enhancement (with respect to simple physical mixtures), the residual crystallinity (by quantifying the melting enthalpy by Differential Scanning Calorimetry) and the percentage of PZQ recovered (as determined by HPLC) were chosen. The designed experimental plan of the screening design consisted of 8 duplicated runs, which is only a fraction of the possible combinations of factor levels.

At the end of the screening one promising coground system was selected for further analyses (through powder X-ray diffraction (PXRD), Attenuated Total Reflectance- Fourier Transform–IR (ATR-FTIR) spectroscopy, Scanning Electron Microscopy (SEM)) in comparison to pure materials and corresponding physical mixture. The physical and chemical characterizations were also repeated over a period of 6 months to perform a short-term stability of the PZQ in the binary coground. Finally, an in vitro assay on adult *S. mansoni* flatworms was performed on the same sample to verify that PZQ maintained its unaltered antischistosomal activity after cogrinding in presence of the excipient. These further analyses were aimed to know additional solid state characteristics, physical stability and maintaining of antischistosomal activity in a sample complying at least one experimental response of the screening design.

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), Copovidone – 1-vinylpyrrolidone-co-vinyl acetate (Sigma Aldrich Chemie- USA) (PVPVA), micronized Crospovidone (Kollidon-CL-M, BASF-Ludwigshafen, Germany) (CROSPVP), Sodium Starch Glycolate (Explotab-Milano, Italy) (SSG), Sodium croscaramellose (Ac-Di-Sol, BH Schilling- Milano, Italy), Soluplus – polyvinylcaprolattame-vinylacetate-polyethilenglycol-graftcopolymer (BASF-Ludwigshafen, Germany) and Syloid 74 FP (Grace Davison-Worms, Germany) were used as received. HiPersolv Chromanorm Methanol (Ph. Eur. for HPLC Gradient Grade) was from VWR Chemicals BHD PROLABO® Milano, Italy.

2.2. Preparation of the binary mixtures and coground systems

The binary coground systems were prepared by grinding the PZQ with each polymer in a vibrational mill-Retsch MM400, whose description and operating conditions will be reported in the following paragraph.

The considered active ingredient-to-polymer weight ratios were the following: 1:1 and 1:2. These wt ratios were chosen in accordance to previous mechanochemical experiences (Hasa et al., 2011a, 2013a; Shin et al., 1998). In particular in this case percentages of carrier superior to 66% by wt were excluded to avoid excessive dilution of the API, which is highly dosed. To prepare the samples to be coground, the accurately weighed components were mixed in an agate mortar for a standardized time of 3 min and then introduced in the jars.

In preliminary tests, various grinding trials were carried out with different hydrophilic commonly used excipients. Among those tested, Soluplus was immediately discarded due to its very low glass transition temperature and the coground with PZQ showed a rubbery consistence and massive adhesion to the grinding media. Sodium croscaramellose was also discarded because the coground systems showed a very poor attitude to enhance praziquantel solubility. Four polymers were thus selected: PVP, PVPVA, CROSPVP and STG.

For comparison purposes, binary physical mixtures were prepared in the same agate mortar by manually mixing PZQ and each polymer (PVP, PVPVA, CROSPVP and SSG) in the same weight ratios as the coground systems for the standardized time of 3 min. In addition, only for DSC evaluation, 1:4 by wt PZQ-polymer physical mixtures were prepared.

2.3. Comilling experiments

The grinding process was performed in a vibrational mill-Retsch MM400 (Retsch GmbH, Haan, Germany) which was equipped by two screw-type zirconium oxide jars, each one with a capacity of 35 ml. In this mill, the grinding is carried out by vibrational shocks involving the grinding media and the jar, taking place along the horizontal axis. A ceramic material like zirconium oxide was chosen due to its high density (5.9 g/cm^3), allowing for a high energy input.

In order to ensure the 1:30 wt ratio of powder:grinding media, chosen on the basis of previous experiences, the amount of powder to be introduced in the milling jar was determined in 1.072 g or 0.843 g per jar, when using three 15 mm spheres or one 20 mm sphere, respectively. Also during preliminary trials, the following intervals of milling frequencies and milling times were selected: 20–30 Hz and 1–4 h.

The temperatures of the powder at the end of the grinding experiments were recorded using a 35XP-A Amprobe K-type thermocouple (Amprobe,Test Tools Europe, Glottertal, Germany).

2.4. Experimental design

A screening design was performed to find the most significant factors among formulation and process variables in cogrinding experiments of binary mixtures polymer-PZQ in the oscillating mill Retsch MM400. The factors included: X_1 drug-polymer ratio (w/w) (quantitative/continuous variable), X_2 type of grinding media (qualitative/discrete variable), X_3 grinding time (quantitative/continuous variable), X_4 frequency (quantitative/continuous variable), X_5 polymer type (qualitative/discrete variable). Each of them was analyzed at different levels.

Based on the preliminary trials and research objectives, and to ensure that the experimental design space included feasible conditions, the following level (lower and upper, respectively) for each quantitative variable were considered: 1:1 and 1:2 for X_1, 1 h and 4 h for X_3, 20 Hz and 30 Hz for X_4.

Regarding qualitative variables, the type of grinding media (X_2) could be chosen between a combination of 3 balls with a 15 mm diameter and 1 sphere with a 20 mm diameter, whereas the type of polymer (X_5) was selected among PVP, CROSPVP, PVPVA or SSG.

Therefore, the variables were considered at different levels: X_1, X_2, X_3, X_4 at 2 levels while X_5 at 4 levels (as reported in Table 1).

For generating the multilevel experimental plan required and collapsing the number of levels and factors, we designed an experimental plan with the help of the NEMRODW software (Mathieu et al., 2015). The total number of runs performed was 16, comprised of 8 experiments performed in duplicate, as reported in Table 2.

The effect of the variation of these variables on 3 experimental responses was then studied and data processing was carried out using NEMRODW software. The properties of interest were: (Y_1) percent residual crystallinity (the heat of PZQ fusion (J/g)-ΔHfus determined by DSC- in the coground in comparison to that of original PZQ; (Y_2) percent amount of drug recovered (determined by HPLC analysis); (Y_3) drug solubility enhancement in the coground systems (at 20 °C in water, also determined by HPLC). This latter value was calculated as the difference between the PZQ solubility in the coground and in the corresponding physical mixture, prepared with the same drug-to-polymer ratio.

Table 1
The process and formulation factors, tested levels, and experimental responses.

Experimental variables	levels			
	1	2	3	4
X_1 drug-to-polymer wt ratio	1:1	1:2		
X_2 Grinding media	1 large sphere	3 small spheres		
X_3 Milling time (h)	1	4		
X_4 Frequency (Hz)	20	30		
X_5 Type of Polymer	CROSPVP	PVPVA	SSG	PVP
Y_1 Residual PZQ crystallinity				
Y_2 Recovered PZQ				
Y_3 drug solubility enhancement				

2.5. Differential Scanning Calorimetry (DSC)

For this analysis a differential scanning calorimeter DSC Mettler TA 4000 (Greifensee, Switzerland) connected to a cell Mettler DSC20 calorimeter, equipped with the STARe software version 9.30, was used. All samples, each containing about 2 mg of accurately weighed PZQ and placed in aluminum perforated crucibles with a capacity of 40 μl, were heated at a scanning rate of 10 °C/min in a temperature range between 25° and 160 °C, under air atmosphere. Coground systems, physical mixtures and pure raw materials were analyzed. The calibration of the instrument was performed with indium, zinc and lead for the temperature, and with indium for the measurement of the enthalpy.

To test for linearity of the calorimetric response of the PZQ-polymers physical mixtures for each polymer, different drug-to-polymer wt ratios (1:0, 1:1, 1:2 and 1:4) were prepared in an agate mortar and analyzed. The heat of fusion of the drug, calculated as the area of the endothermic peak, was then plotted versus percentage of PZQ in the mixtures, and linear regression was calculated. Once the linearity of the calorimetric response for the binary systems ($R^2 \geq 0.998$ in all cases) was verified, the drug enthalpy of fusion in each coground was determined, and the percent of residual crystallinity (Y_1) calculated with respect to the enthalpy of fusion of pure drug.

2.6. Determination of drug content

Reverse phase HPLC method was used for the quantification of PZQ, using a method adapted from literature (Sun and Bu, 2012) and validated according to a slight modification in column length. The HPLC system used consisted of two mobile phase delivery pumps (LC-10 ADVP, Shimadzu, Japan), a UV–vis detector (SPD-10Avp, Shimadzu, Japan), an autosampler (SIL-20A, Shimadzu, Japan), an interface (SCL-10Avp, Simadzu, Japan) for the acquisition of data through a software Ez-Star. The mobile phase comprised of methanol and water (65:35 V/V), the flow rate was 1 ml/min and absorbance readings were conducted at fixed wavelength of 220 nm. A Kinetex 5 μm C18 column (150 × 4.60 mm, Phenomenex, Bologna, Italy) was used. The retention time of PZQ was about 5.5 min and the run time was set at 12 min. Quantitation was carried out by integration of the peak areas using the external standardization method. Under these conditions, the linear calibration curve of PZQ was obtained in the range of 0.3–10 mg/L ($r^2 = 0.99996$). As reference solution, the standard of the day was prepared each time before starting the analysis. The standard solution was prepared by dissolving about 10 mg, exactly weighed, of PZQ in 20 ml of methanol HPLC-grade. The solution was stirred for several minutes and then 1:10 and 1:20 dilutions with the mobile phase were prepared to obtain a drug concentration of approximately 2.5 mg/L. According to the PZQ monograph in the Eur. Ph. (Ed. 8.0), specified impurities, named impurity A and impurity B, have to be detected. The linear calibration curve of each impurity was obtained in the range of 0.05–1 mg/L ($r^2 = 0.9993$ and 0.9994, for impurity A and B, respectively). The retention time of the impurities were at 3.45 min and 11.2 min. Both impurities were absent in the reference solution.

The determination of the PZQ content and eventually of the related impurities into the coground was determined by dissolving, depending on the drug-polymer ratio, about 20 mg or 30 mg, exactly weighed, of sample in 20 ml of methanol HPLC-grade. The obtained solution was then diluted 1:200 with the mobile phase, corresponding to about 2.5 mg/L of PZQ, in order to ensure the linearity of the analytical response. In the sample solution of each coground system, the retention time of the major peak was at about 5.4–5.5 min. Each sample solution was analyzed in triplicate

Table 2
Experimental plan and observed response values.

Exp	X_1 (ratio by wt)	X_2	X_3 (h)	X_4 (Hz)	X_5	Y_1 (%)	Y_2 (%)	Y_3 (mg/l)
1	1:1	3s*	4	20	CROSPVP	9.61	87.22	436.77
2	1:1	3s	4	20	CROSPVP	11.99	89.93	448.26
3	1:1	1ls*	4	30	PVPVA	21.36	94.94	282.08
4	1:1	1ls	4	30	PVPVA	37.39	94.12	220.01
5	1:1	3s	1	30	SSG	69.37	100.00	285.59
6	1:1	3s	1	30	SSG	73.45	100.00	282.05
7	1:1	1ls	1	20	PVP	42.54	94.60	229.49
8	1:1	1ls	1	20	PVP	25.66	96.51	290.68
9	1:2	1ls	1	30	CROSPVP	0	88.63	210.71
10	1:2	1ls	1	30	CROSPVP	0	91.65	200.71
11	1:2	3s	1	20	PVPVA	0	90.47	113.35
12	1:2	3s	1	20	PVPVA	1.49	92.09	93.56
13	1:2	1ls	4	20	SSG	0	90.32	127.48
14	1:2	1ls	4	20	SSG	0	91.24	135.51
15	1:2	3s	4	30	PVP	0	94.13	110.11
16	1:2	3s	4	30	PVP	10.85	94.84	140.29

X_1 = drug/polymer wt ratio Y_1 = Residual PZQ crystallinity (%).
X_2 = type of milling media Y_2 = Recovered PZQ (%).
X_3 = milling time (h) Y_3 = Coground solubility enhancement (mg/l).
X_4 = milling frequency (Hz).
X_5 = kind of polymer.
3s* = 3 spheres (having a 15 mm diameter).
1ls* = 1 large sphere (having a 20 mm diameter).

and the mean of the sum of the peak responses of praziquantel was then calculated. The results were expressed as the percentage of PZQ recovery with respect to the sum of all peaks (PZQ and related impurities and/or other detectable related products). As negative control (blank solution), each polymer was ground in the same condition of the corresponding coground system and dissolved similarly to the sample solution and injected in HPLC.

The percentage of PZQ recovery detected in the coground systems was considered as the experimental response Y_2.

2.7. Determination of drug solubility

Solubility measurements of PZQ were carried by adding an excess amount of drug or coground system, or physical mixture to 10 ml of deionized water. The suspensions were agitated in the dark, at 20 °C for 48 h, to ensure that equilibrium was attained, and then filtered through a membrane (pore size 0.2 μm). Finally, 1 ml of each solution, previously diluted 1:200 with the mobile phase, was assayed by HPLC analysis, following the previously described method. Forty-eight hours was found to be enough for equilibrium, whilst an agitation time of 72 h or superior was found to cause a pronounced chemical degradation of the drug. This noticed behaviour is also in accordance to results previously published by Moutasim et al. (2004). Experiments were performed in duplicate for all samples.

"Coground solubility enhancement" (Y_3) was calculated as the difference between the solubility of the drug in the coground system and the solubility of the drug in the corresponding physical mixture (prepared with the same polymer in the same weight drug-polymer ratio) and expressed as mg/l.

2.8. Additional characterizations of a selected coground system

2.8.1. X-ray powder diffraction studies (PXRD)

PXRD patterns were recorded using a D500 (Siemens, Munich, Germany) diffractometer with Cu-Kα radiation (1.5418 Å), monochromatised by a secondary flat graphite crystal. All the analyses were performed in duplicate using a current of 20 mA and the voltage was set at 40 kV. The powder samples were scanned in the range from 3 to 30° of 2θ angle, steps were of 0.05° of 2θ, and the counting time was of 5 s/step. The samples subjected to the analysis were the following: the selected coground mixture, the corresponding physical mixture and raw materials.

2.8.2. ATR-FTIR spectroscopy

ATR-FTIR spectra in the solid state (the sample as undiluted) were recorded in a spectrophotometer PerkinElmer Spectrum 100 FT-IR (Beaconsfield, England), with the equipped software PE version 6.3.4. copyright 2008, attached to a Universal ATR sampling accessory. The range analyzed was from 650 cm-1 to 4000 cm-1, with scan number equal to 4. The coground, pure PZQ and polymer were analyzed.

2.8.3. Scanning Electron Microscopy (SEM)

Samples were metallized with S150A Sputter Coater (Edwards High Vacuum, Crawley, West Sussex, UK) and then observed under a scanning electron microscope Leica Stereoscan 430i (Leica Cambridge Ltd., Cambridge, UK). Pure PZQ and the coground system were analyzed.

2.8.4. Activity against Adult Schistosomes

Studies were carried out in accordance with Swiss national and cantonal regulations on animal welfare (permission no. 2070) at the Swiss Tropical and Public Health Institute (Basel, Switzerland) as described earlier (Meister et al., 2014). Female mice (NMRI strain; weight ~20–22 g) were purchased from Charles River, Germany, kept under environmentally-controlled conditions (temperature ~25 °C; humidity ~70%; 12 h light and 12 h dark cycle) with free access to water and rodent diet and acclimatized for one week before infection. *Cercariae* of *Schistosoma mansoni* were obtained from infected intermediate host snails (*Biomphalaria glabrata*).

Adult Schistosomes obtained via dissection from infected mice were incubated in the presence of the test compounds at different concentrations (0.33–0.021 μg/ml) for up to 72 h. Phenotypes were monitored at several time points based on motility, viability and morphological alterations under an inverse microscope (Carl Zeiss, Germany, magnification 80×). Parasite viability values of treated

and untreated worms obtained from microscopic evaluation were averaged (means ± standard deviation) using Microsoft Excel software. IC_{50} values were calculated using CompuSyn software.

2.8.5. Aging Studies

In order to check possible modifications of the chemical integrity and solid state (e.g. recrystallization) within time, drug content analysis by HPLC, PXRD, DSC, solubility analyses of a selected coground system were repeated over 6 months. During storage time, the solid samples were kept at room temperature in a dessiccator.

3. Results and discussion

3.1. Screening design

In this paper, the process of mechanochemical activation in vibrational mill with selected pharmaceutical excipients was employed to enhance water solubility of the BCS class II drug, PZQ. To study the effect of the process and formulation factors on the physical and chemical characteristics of the antihelminthic drug a screening design was employed. As experimental responses of this explorative analysis, the percentage of drug residual crystallinity, the percentage of PZQ recovered after the milling process and the enhancement of drug solubility were selected.

With regards to the choice of these dependent variables, the improved solubility is the aim of many mechanochemical experiences reported in literature (Colombo et al., 2009; Shakhtshneider and Boldyrev, 1999): the novelty is here to consider it as a proper dependent variable of a screening design. It is also worth of notice that the experimental response Y_3 named "Coground solubility enhancement" was calculated as the difference between the solubility of the drug in the comilled system and the solubility of the drug in the corresponding physical mixture. This choice was made to screen the carrier subjected to the process in given conditions rather than the influence of the carrier itself.

Residual crystallinity of the sample was also selected as experimental response to check possible structural changes in the product, evidence of the mechanical "activation" of the sample, possibly influencing drug solubility and physical stability. This parameter (Y_1) was classically calculated (by DSC analysis) as the ratio of the enthalpy of fusion of the drug in the coground to that of the starting drug (96.15 ± 0.75 J/g, mean ± S.D; $n = 3$).

Finally, the drug recovery, the possible drug degradation and/or formation of some impurities due to the mechochemical treatment, was checked. This parameter has been rather neglected in previous mechanochemical experiences apart from few examples (e.g. Kaminska et al., 2013; Belenguer et al., 2016; Adrjanowicz et al., 2011). In our opinion this is an interesting parameter to study in the case of PZQ, which tendency to degrade in certain conditions has been reported in literature (Moutasim et al., 2004; Hashem et al., 2017; Cizmic et al., 2016). Therefore, this explorative analysis would be particularly useful to check whether the cogrinding process can be suitably applied in the PZQ case.

Sixteen cogrinding experiments were performed varying process and formulation variables, as reported in Table 2. As visible from this table, in general, the reproducibility of the 3 experimental responses did not contradict the trends, but a given condition did not always provide an equivalent result, as already noticed in our previous experiences and as reported in literature (Belenguer et al., 2016).

The 'weight' of each factor level was estimated by means of the least squares method. For each factor the weight of each level was related to the upper level weight, which became the 'reference state' among each factor. It should be noticed that the use of a screening design to compare different factor levels does not give any information about the effect of the factors themselves. The only considerations that can be drawn regard the weight of each level, and hence a comparison between the levels of the factors considered one at a time (Lewis et al., 1999; Cela et al., 2009).

For a better interpretation of the influence of the different variable levels on the product properties analyzed (Y_1, Y_2 and Y_3) a graph-mode representation was used (Fig. 1). The highest level of each factor was taken as the level unit measure for each single factor. Therefore, all the highest factor levels (red bars in Fig. 1) were drawn with the same length. This graphical procedure enabled visualization and allowed for comparison of effects on Y_1, Y_2 and Y_3, simultaneously. In this manner, the factor levels that influence the final result in a remarkable way were pointed out.

As the cogrinding product was intended for a praziquantel oral formulation, the solubility enhancement and the percentage of drug recovery were to be maximized in order to achieve the

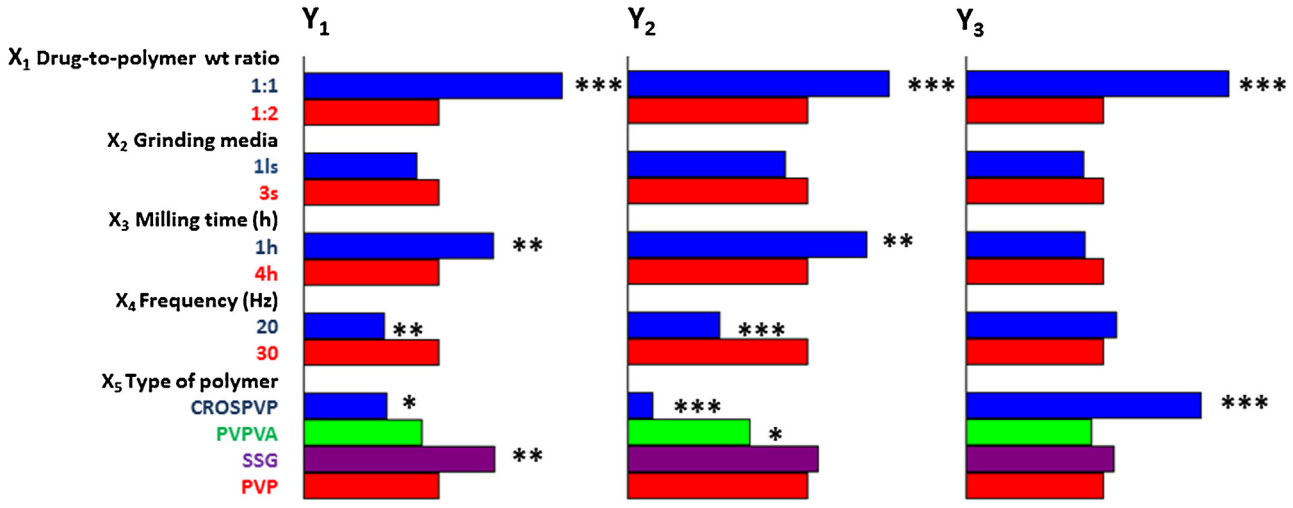

Fig. 1. Total effects of the variation of the 5 factors, each considered at different levels, on the three considered experimental responses. (For interpretation of the references to colour in the text, the reader is referred to the web version of this article.)
(Significance codes (α): $^{***}p = 0.001$, $^{**}p = 0.01$, $^{*}p = 0.05$).

desired product; thus, from the graphical analysis the levels giving the highest value of Y_2 and Y_3 could be easily identified. In addition, Y_1 trend, also visible from the graph, gave us the information about the milling-induced disorder as a function of the variation of the variables. Hence, the following observations could be driven.

The analysis of the percentage of drug recovery (Y2), as seen from Fig. 1, stated that the variable "formulation" was significant ($p = 0.133\%$); in particular the formulation 1:1 resulted in a reduced percentage of impurities with respect to the formulation having a higher polymer content. Due to the fact that none of the carriers in simple physical mixture with PZQ (independently from the composition) gave origin to degradation products (data not shown for brevity), a chemical interaction between PZQ and the carriers at the solid state can be excluded. Thus the drug degradation occurs during grinding in presence of the polymer and it is favored by the polymer itself. This result could be surprising at a first glance, since it is expected that the higher the polymer content the lower the drug degradation, with a mechanism of drug protection during the process operated by the excipient. Conversely, the presence of a larger amount of polymer determines in this case a higher drug degradation. It is likely that a larger amount of polymers permits a higher transmission of energy to the drug, thus a higher mechanochemical degradation.

An alternative explanation could be the presence of water: the higher the content of carrier, the higher the water content, since all polymers are hygroscopic (e.g. PVP contains originally 15% in weight). The water could in turn contribute to degradation (as seen in the "Determination of Drug Solubility" paragraph) and could act as a plasticizer to reduce glass transition of the coground and hence improve propensity to degradation.

Since the influence of the variable formulation on Y_2 is parallel to that observed on Y1 (% residual crystallinity) it could be postulated that a reduced polymer content leaded to a less degraded and less destructured sample at the same time. Thus the degradation was likely to be caused by an enhanced chemical reactivity of the amorphous material rather than to any negative effect caused by high impact milling process itself (as previously postuled by Adrjanowicz et al., 2011 in the case of furosemide).

The other formulation variable, type of polymer, has also important effect on Y_2. CROSPVP led to the highest amount of degraded API, and its effect was significantly different ($p = 0.02\%$) from the other three polymers. As a matter of fact, the influence of the polymers on drug recovery had the same trend as residual crystallinity (e.g. CROSPVP had produced the lowest drug recovery and residual crystallinity). Thus, once again, it can be hypothesized an enhanced chemical reactivity of the amorphous material rather than any detrimental consequence of the milling itself.

As a confirmation of this hypothesis, the process variables had only a minor effect on Y_2. "Type of grinding media" was an irrelevant variable. It must be underlined that in this case only the geometry of the milling media was varied, whereas the milling media-to-powder wt ratio and the empty volume inside the jars were kept constant. Thus, the energy input can be considered substantially constant in both cases. This was not the case of the variables "time" and "frequency", whose increase determines obviously an increase in the mechanical energy input given to the powders. However, contradictory results were obtained from the two variables: increasing milling time a minor effect of reducing Y_2 ($p = 0.859\%$) was noticed, while increasing frequency the drug recovery unexpectedly increased ($p = 0.081\%$). This once again highlights that the degradation of the drug did not directly correlate to the intensity of mechanical stress.

As for the experimental response Y_3, solubility enhancement, as seen from Table 2, all the coground systems are more soluble than corresponding physical mixtures, in fact all values are largely positive, attesting the superiority of the preparation technique with respect to simple physical mixtures. Once again, process variables (geometry of grinding media, milling time and frequency) did not influence significantly the solubility ($p = 5.7\%$, 7.7% and 17.3%, respectively); instead, variables regarding formulation (drug to polymer ratio and type of polymer) had a great importance. The 1:1 drug to polymer wt ratio determined a significant improvement of solubility performance ($p < 0.01\%$). Moreover CROSPVP was by far the best carrier for enhancing PZQ solubility ($p < 0.01\%$), while the other three carriers had similar impact on solubility enhancement. Both these trend are in accordance to literature data. Crospovidone is frequently resulted to be the most efficient solubilizing agent among several carriers (Moneghini et al., 1998). As for the influence of amount of polymer on PZQ solubility, this behavior was in accordance to Costa et al. (2016) also reporting that the solubility does not directly correlate with the fraction of polymer present. This was possibly due to the polymer effect on drug wettability and to the different amount of crystalline phase in the samples.

Finally, the variable Y_1 was studied, to characterize the samples and knowing the milling induced destructuration. As visible from Table 2, the amorphous content was very high in at least half of the samples. Even though the high amorphous content has been frequently associated to sample color changes (Sheth et al., 2005), no relevant phenomena of mechanochromism were noticed in our samples. The variable "drug-to-polymer wt ratio" is the most significant one ($p < 0.01\%$): 1:1 w/w formulation showed a remarkably increased percentage of residual crystallinity. This was in accordance to previous studies conducted in a planetary mill with different actives, such as vinpocetine (Hasa et al., 2011a), vincamine (Hasa et al., 2013a) and silimarine (Voinovich et al., 2009): in systems containing a greater polymer content, the energy transfer to the powder mixture was more effective, thus allowing for a more destructured system. The qualitative variable "type of polymer" was less important and, specifically, the use of SSG as carrier resulted in the highest residual PZQ crystallinity than other polymers ($p = 0.870\%$), whereas crospovidone was the best amorphysizing carrier ($p = 1.38\%$). The variable "type of grinding media" was once again irrelevant, whereas a positive variation of "time" and "frequency" were inversely correlated ($p = 0.158\%$ and $p = 0.167\%$) to the residual crystallinity.

After this screening analysis of the influence of process and formulation variables of the three experimental responses the following general considerations could be driven.

All coground samples had significantly higher solubility than the corresponding physical mixtures (as shown in Table 2, Y_3 responses were always positive). With respect to pure PZQ (140.30 mg/L) a 4.6 – 1.5 fold improvement was achieved. The drug crystallinity was significantly reduced, reaching the complete amorphization in many coground systems. Comilling promoted the formation of a certain percentage of PZQ related products, as the PZQ recovery was lower than the 100% theoretical amount in the majority of the comilled samples.

Parallel trends were observed for responses Y_1 and Y_2 (both in the case of process variables and formulation variables). It seemed that a variation of the variables determined at the same time solid de-structuration and reduced drug recovery; a greater degree of amorphous in the system (which is characterized by a greater molecular mobility) corresponded to a greater tendency to degradation during comilling. Conversely, it is interesting to note that solubility did not increase analogously to crystal lattice de-structuration. In fact, the trend of experimental responses Y_1 and Y_3 was not parallel. While the reduction of the crystallinity was influenced both by formulation (drug to polymer ratio) and by process variables, the solubility was affected only by the type of polymers and their percentage. For example, to reach a higher solubility (experimental response Y_3) a 1:1 drug-to-polymer ratio

formulation was preferred, albeit giving rise to a higher drug melting enthalpy than the 1:2 drug-to-polymer ratio.

The type of grinding media used did not affect any of the responses considered. This showed that the geometry of the grinding media was not so important (nor is the different number or ball diameter). Since the variation of this factor level did not affect the empty volume in the chamber nor the grinding media-to-powder weight ratio, which have been both maintained constant, this also drawn attention to the significance of studying these aspects in a future experience.

To the purpose of comparing polymers, CROSPVP proved to be the best for experimental response Y_3, and the worst for the experimental response Y_2, reaching also the lowest value of Y_1. This indicated that CROSPVP had an "outsider " behavior with respect to the other carriers. The solid state features obtainable via a mechanochemical process with a cross-linked polymer were, also in this case, remarkably different from those mediated by the linear counterparts (Hasa et al., 2013a). As in many experiences reported in literature (Hasa et al., 2011a; Voinovich et al., 2009; Shin et al., 1998), CROSPVP confirmed in this research its ability to act as a solubility enhancer, due to its amphiphilic nature as well as its chemically and physically crosslinked structure. Nevertheless, in comparison to previous literature, this experimental evidence highlighted its influence on drug recovery after comilling.

Conversely, SSG acts as sort of protection in respect of the chemical degradation of the API, also delaying or preventing the physical destructuration of the drug.

At the end of this explorative analysis, in consideration of the aims of the screening design, e.g. a coground product intended for an oral formulation, the product having the best performance in terms of drug solubility enhancement was selected: the coground system 2 composed of PZQ: CROSPVP 50:50 by wt processed at 20 Hz for 4 h, using 3 spheres (having 15 mm diameter) as grinding media. This mechanochemically treated system had a solubility of 642.54 mg/L compared to 194.28 mg/L of the untreated physical mixture and compared to 140.30 mg/L of pure PZQ, thus representing a 3.3 and a 4.6 fold improvement, respectively. As for the other experimental responses, this sample had a reduced drug recovery (ranging about 90%) and a high amorphous content (residual crystallinity 11.99%). To know whether this sample is anyhow a promising sample for further development, it was subjected to additional physical characterizations to check the physical stability (due to the high amorphous content), to compare thermoanalythical results with other techniques and to verify the maintaining of the antischistosomal activity (in consideration of the incomplete drug recovery, the presence of the polymer and the physical changes).

3.2. Additional characterization of a selected coground sample and stability studies

First of all, the DSC analysis of the coground 2 was compared to corresponding physical mixture and raw materials. In the temperature range from 25 to 160 °C, the DSC trace of pure PZQ (Fig. 2) exhibited only an endothermal event at 141.98 ± 0.03 °C (mean ± S.D., n = 3) attributable to the melting of the racemic compound in accordance to El-Arini et al. (1998a), Liu et al. (2004). In the physical mixture this event was anticipated by the dehydration of the hygroscopic polymer. The drug melting temperature and enthalpy corresponded to those of original API. Conversely, the coground exhibited typical DSC trace of a highly destructured system, with the almost complete disappearance of drug melting endotherm, which was accompanied by a scarce enthalpy of fusion (Table 2). In addition, the endotherm underwent at temperatures lower than the original racemic compound in agreement with a very scarce residual crystallinity and with the prevalent presence of nanocrystals of PZQ. In fact, the lower the nanocrystal size, the lower its melting temperature and enthalpy (Hasa et al., 2013b).

Both PXRD and ATR-FTIR analyses confirmed these data. In the diffraction pattern of the comilled system 2, a high disorder could be observed. Besides, the diffractogram of starting PZQ (Fig. 3) showed the intense, narrow and sharp peaks, typical of the racemic compound (e.g. at 4.01, a doublet at 8.00–8.19, 14.74, 15.35, 16.39, 18.48, 19.29, 20.06, and a doublet at 22.36–22.57 of 2 theta) in accordance to literature (El-Arini et al., 1998a; Espinosa-Lara et al.,

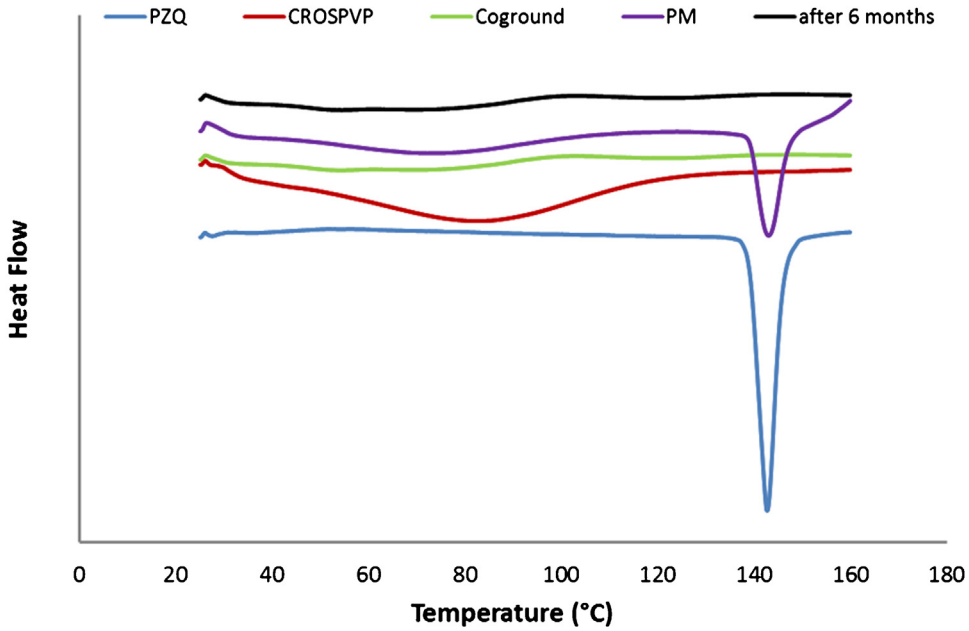

Fig. 2. DSC traces of pure PZQ, pure CROSPVP, coground 2, 1:1 wt PM, and coground 2 analyzed 6 months after preparation (bottom-to-top reading order).

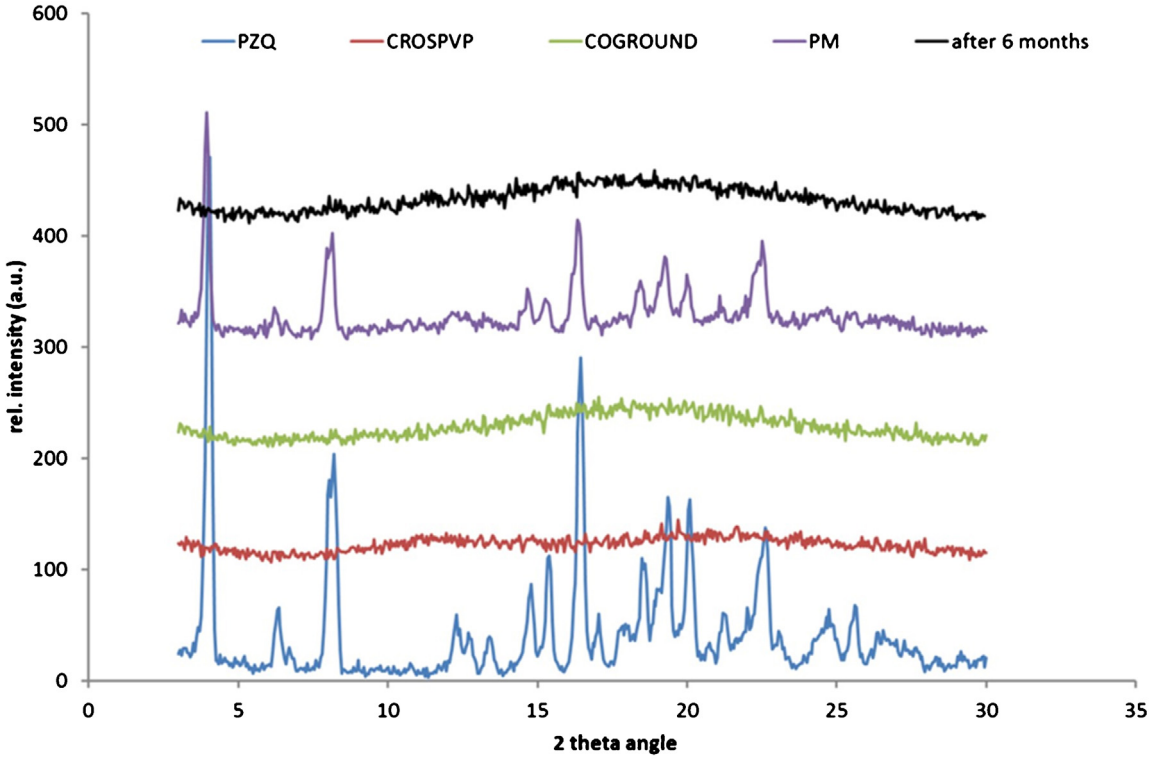

Fig. 3. PXRD pattern of pure PZQ, pure CROSPVP, 1:1 wt PM, coground 2, and coground 2 analyzed 6 months after preparation (bottom-to-top reading order).

2013; Passerini et al., 2006; De La Torre et al., 1999). In the coground pattern only a halo-pattern and a conspicuous scattering are visible. For example, the very intense peak originally present at about 4° of 2 theta, completely disappeared. The lack of new signals different from those of original API indicated that the cogrinding with CROSPVP did not lead to the formation of a new polymorphic form. It is important to note that the reduction of peak intensity of praziquantel in the coground could be attributed only to the dilution effect of the polymer, which was verified by analyzing the simple physical mixtures having the same composition. This physical mixture pattern appeared to be the sum of the pattern of the individual components, with the drug typical signals reduced in intensity by the dilution in the amorphous polymer.

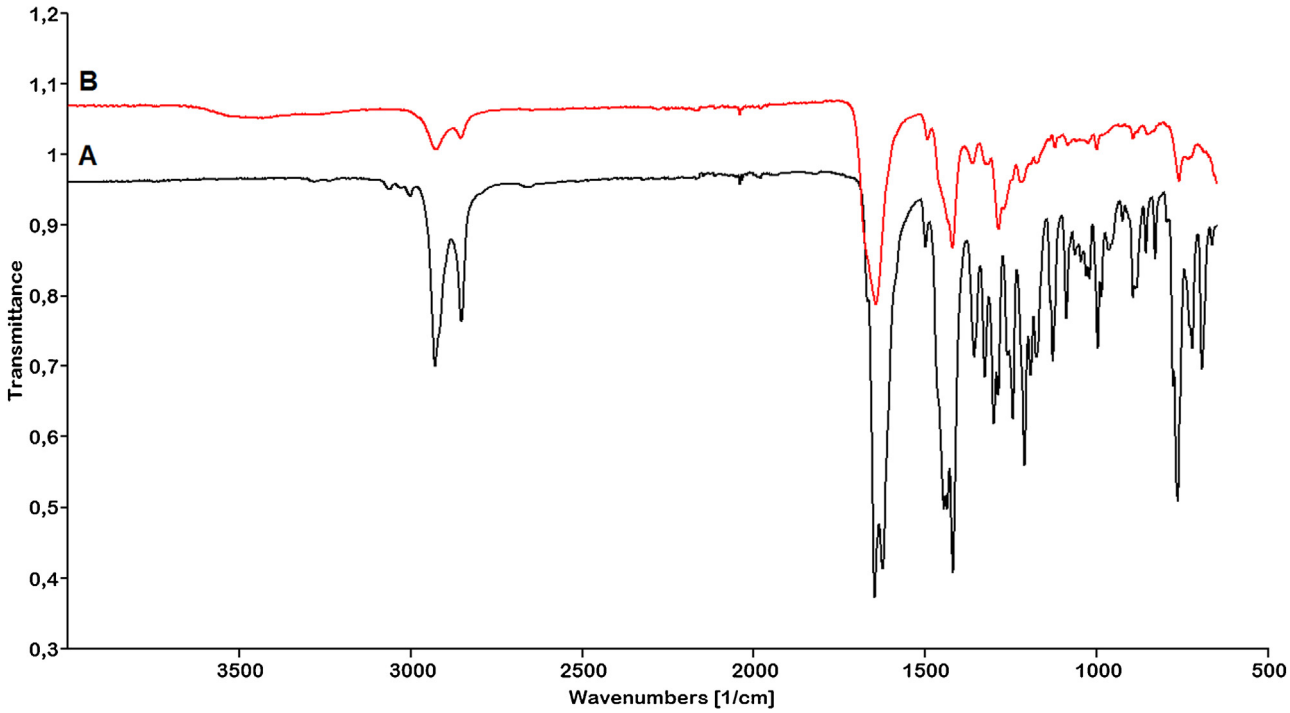

Fig. 4. ATR-FTIR spectra of pure PZQ (a), and coground 2 (b).

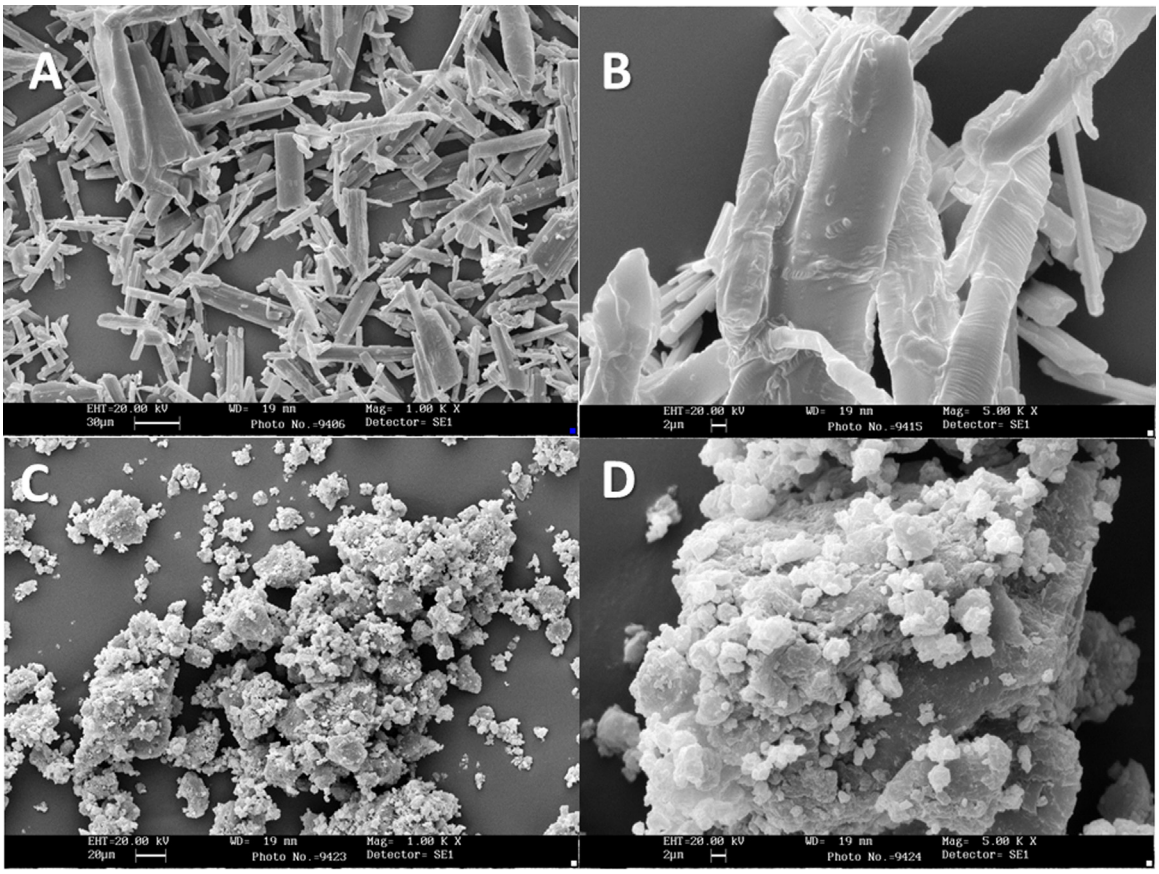

Fig. 5. SEM images of pure praziquantel (A and B) and sample 2 (C and D) [1.00 K × and 5.00 K × magnification, respectively].

The ATR-FTIR spectrum of coground 2 (Fig. 4) showed broader and less intense signals with respect to original API's, which was compatible with the dilution of the polymer and the high amorphous content of system. Particularly evident the differences with the starting drug in correspondence of the stretching of C=O groups (1550 and 1629 cm^{-1}), which are involved in molecular packing in the crystal form (Espinosa-Lara et al., 2013): in the coground spectrum the two original sharp peaks were replaced with a very broad band.

Fig. 5A and B presents the scanning electron micrographs (SEM) of original elongated PZQ crystals, similar to matchwood, analogous to those reported in previous published studies (Passerini et al., 2006; Liu et al., 2004). Original acicular PZQ particles with smooth surface could not be longer detected in SEM images of the coground 2 system (Fig. 5C and D), in accordance to the almost complete loss of crystallinity detected from DSC and PXRD data. Moreover, the coground was characterized by the massive presence of small particles of cubic shape laid on aggregates of greater size, showing a clear difference from the typical popcorn-like shape of commercial CROSPVP polymer, reported in literature (Bühler, 2005).

The in vitro antischistosomal activity of starting PZQ and coground 2 against adult *S. mansoni* was then determined. The IC_{50} values of PZQ-loaded coground (Table 3) was similar (even slightly lower) to that observed with the pure PZQ, proving that the in vitro activity remained unaffected after cogrinding in presence of CROSPVP, even though the physical changes and the incomplete drug recovery.

Finally, for the period of 6 months, chemical integrity, solubility and melting enthalpy of the drug in the coground 2 was checked once a month by repeating HPLC, solubility and DSC-PXRD assays, respectively. The percentage of PZQ recovered remained constant (around 90%) in the considered period and not quantifiable signs of recrystallization phenomena of PZQ were testified (see previous Figs. 2 and 3 reporting the characterizations performed after 6 months). This probably means that CROSPVP acted as a stabilizer towards the finely dispersed state of the drug, and towards the amorphous state of the active component, in accordance with its stabilizing capacity already tested in several coground systems (Shin et al., 1998; Voinovich et al., 2009). This was reflected on solubility performance of the drug in the coground, remaining constant in the considered period.

Table 3
In vitro IC50s of starting PZQ and coground against adult *S. mansoni* at 72 h postincubation.

Sample	IC50 (72 h) adult *S. mansoni* µg/ml[a]	r=
PZQ	0.165	0.92
Coground 2	0.102	0.97

[a] Extrapolated values determined by Com-puSyn.software.

4. Conclusions

The mechanochemical activation of PZQ in the vibrational mill with different polymers increased the solubility of the antischistosomal drug. First of all, the experimental screening design led to the recognition of the variables with the greatest impact on

solubility enhancement, on residual crystallinity and on the percentage of PZQ recovery. The excipient selection and the variation of carrier amount proved to be particularly important variables affecting all the experimental responses. A lower impact and, in some cases, rather null effect, had the variation of the process variables. All coground systems were characterized by a high amorphous degree and a significantly higher solubility than the API, thereby attesting the remarkable potential of the preparative technique. Among different coground systems, the best performance in terms of drug solubility, was achieved by the coground system composed of PZQ: CROSPVP 1:1 by wt processed at 20 Hz for 4 h, using 3 large spheres as grinding media. This mechanochemically treated system exhibited a solubility of 642.54 mg/L compared to 194.28 mg/L of the untreated physical mixture and to 140.30 mg/L of pure PZQ, i.e. a 3.3-fold and a 4.6-fold improvement, respectively. Even though this system revealed an incomplete drug recovery (89.3%), it was able to maintain the in vitro bioactivity of the PZQ against *S. mansoni* and appeared to be chemically and physically stable over 6 ageing months.

In conclusion, this study illustrates that this manufacturing process has a potential for the production of a highly soluble and active coground system, having a drug content of 50% w/w. Such results are very promising in view of a possible PZQ dose reduction and developing of smaller tablets, suitable for children. Ongoing studies are directed towards improving this technological approach to reduce the extent of PZQ degradation and to identify main degradation products formed upon milling.

Acknowledgements

The authors thank "Università degli Studi di Trieste – Finanziamento di Ateneo per progetti di Ricerca Scientifica – FRA 2015" for funding, Prof. Francesco Princivalle and Dr. Davide Lenaz for kind hospitality in the Dept. of Earth Sciences-University of Trieste, and Cristina Vidonis for the preparation of the samples.

References

Adrjanowicz, K., Kaminski, K., Grzybowska, K., Hawelek, L., Paluch, M., Gruszka, I., Zakowiecki, D., Sawicki, W., Lepek, P., Kamysz, W., Guzik, L., 2011. Effect of cryogrinding on chemical stability of the sparingly water-soluble drug furosemide. Pharma. Res. 28 (12), 3220–3236.

Bühler, V., 2005. Polyvinylpyrrolidone Excipients for Pharmaceuticals. Springer-Verlag Wachenheim/Weinstraße, Germany.

Becket, G., Schep, L.J., Tan, M.Y., 1999. Improvement of the in vitro dissolution of praziquantel by complexation with α-,β- and γ-cyclodextrins. Int. J. Pharm. 179, 65–71.

Belenguer, A.M., Lampronti, G.I., Cruz-Cabeza, A.J., Hunter, C.A., Sanders, J.K.M., 2016. Solvation and surface effects on polymorph stabilities at the nanoscale. Chem. Sci. 7, 6617–6627.

Carli, F., Colombo, I., Torricelli, C., 1987. Drug/cyclodextrin systems by mechano-chemical activation. Chimicaoggi 5, 61–64.

Cela, R., Claeys-Bruno, M., Phan-Tan-Luu, R., 2009. Screening strategies. In: Brown, S. D., Tauler, R., Walczak, B. (Eds.), Comprehensive Chemometrics, vol. 1. Elsevier B. V., pp. 251–300.

Chaud, M.V., Lima, A.C., Vila, M.M.D.C., Paganelli, M.O., Paula, F.C., Pedreiro, L.N., Gremiao, M.P.D., 2013. Development and evaluation of praziquantel solid dispersions in sodium starch glycolate. Trop. J. Pharm. Res. 12, 163–168.

Cioli, D., Pica-Mattoccia, L., Basso, A., Guidi, A., 2014. Schistosomiasis control: praziquantel forever? Mol. Biochem. Parasit. 195, 23–29.

Cizmic, M., Ljubas, D., Curkovic, L., Skoric, I., Babic, S., 2016. Kinetics and degradation pathways of photolytic and photocatalytic oxidation of the anthelmintic drug praziquantel. J. Hazard. Mater. 323 (Part A), 500–512.

Colombo, I., Grassi, G., Grassi, M., 2009. Drug mechanochemical activation. J. Pharm. Sci. 98 (11), 3961–3986.

Costa, E.D., Priotti, J., Orlandi, S., Leonardi, D., Lamas, M.C., Nunes, T.G., Diogo, H.P., Salomon, C.J., Ferreira, M.J., 2016. Unexpected solvent impact in the crystallinity of praziquantel/poly (vinylpyrrolidone) formulations. A solubility, DSC and solid-state NMR study. Int. J. Pharm. 511, 983–993.

De La Torre, P., Torrado, S., Torrado, S., 1999. Preparation, dissolution and characterization of praziquantel solid dispersions. Chem. Pharm. Bull. 47 (11), 1629–1633.

El-Arini, S.K., Giron, D., Leuenberger, H., 1998a. Solubility properties of racemic praziquantel and its enantiomers. Pharm. Dev. Technol. 3, 557–564.

Espinosa-Lara, J.C., Guzman-Villanueva, D., Arenas-García, J.I., Herrera-Ruiz, D., Rivera-Islas, J., Román-Bravo, P., Morales-Rojas, H., Hopfl, H., 2013. Cocrystals of active pharmaceutical ingredients praziquantel in combination with oxalic, malonic, succinic, maleic, fumaric, glutaric, adipic, and pimelic acids. J. Cryst. Growth Des. 13, 169–185.

Hasa, D., Voinovich, D., Perissutti, B., Bonifacio, A., Grassi, M., Franceschinis, E., Dall'Acqua, S., Speh, M., Plavec, J., Invernizzi, S., 2011a. Multidisciplinary approach on characterizing a mechanochemically activated composite of vinpocetine and crospovidone. J. Pharm Sci. 100 (3), 915–932.

Hasa, D., Voinovich, D., Perissutti, B., Grassi, M., Bonifacio, A., Sergo, W., Cepek, C., Chierotti, M.R., Gobetto, R., Dall'Acqua, S., Invernizzi, S., 2011b. Enhanced oral bioavailability of vinpocetine through mechanochemical salt formation: physico-chemical characterization and in vivo studies. Pharma. Res. 28 (8), 1870–1883.

Hasa, D., Perissutti, B., Grassi, M., Chierotti, M.R., Gobetto, R., Ferrario, V., Lenaz, D., Voinovich, D., 2013a. Mechanochemical activation of vincamine mediated by linear polymers: assessment of some "critical" steps. Eur. J. Pharm. Sci. 50, 56–68.

Hasa, D., Voinovich, D., Perissutti, B., Grassi, G., Fiorentino, S., Farra, R., Abrami, M., Colombo, I., Grassi, M., 2013b. Reduction of melting temperature and enthalpy of drug crystals: theoretical aspects. Eur. J. Pharm. Sci. 50, 17–28.

Hashem, H., Ibrahim, A.E., Elhenawee, M.A., 2017. A rapid stability indicating LC-method for determination of praziquantel in presence of its pharmacopoeial impurities. Arab. J. Chem. 10 (1), S35–S41. doi:http://dx.doi.org/10.1016/j.arabjc.2012.07.005.

Huang, J., Bathena, S.P., Alnouti, Y., 2010. Metabolite profiling of praziquantel and its analogs during the analysis of in vitro metabolic stability using information-dependent acquisition on a hybrid triple quadrupole linear ion trap mass spectrometer. Drug Metab. Pharmacokinet. 25, 487–499.

Kaminska, E., Adrjanowicz, K., Kaminski, K., Wlodarczyk, P., Hawelek, L., Kolodziejczyk, K., Tarnacka, M., Zakowiecki, D., Kaczmarczyk-Sedlak, I., Pilch, J., Paluch, M., 2013. A new way of stabilization of furosemide upon cryogenic grinding by using acylated saccharides matrices. The role of hydrogen bonds in decomposition mechanism. Mol. Pharm. 10 (5), 1824–1835.

Lewis, A.G., Mathieu, D., Phan-Tan-Luu, R., 1999. Pharmaceutical Experimental Design. Dekker, New York.

Lindenberg, M., Kopp, S., Dressman, J.B., 2004. Classification of orally administered drugs on the World Health Organization Model list of essential medicines according to the biopharmaceutics classification system. Eur. J. Pharm. Biopharm. 58, 265–278.

Liu, Y., Wang, X., Wang, J.K., Ching, C.B., 2004. Structural characterization and enantioseparation of the chiral compound praziquantel. J. Pharm Sci. 93 (12), 3039–3046.

Mathieu, D., Nony, J., Phan-Tan-Luu, R., 2015. Nemrodw: New Efficient Methodology for Research Using Optimal Design (NEMRODW) Software (version 2015). LPRAI, Marseille, France.

Meister, I., Ingram-Sieber, K., Cowan, N., Todd, M., Robertson, M.N., Meli, M., Patra, M., Gasser, G., Keiser, J., 2014. Activity of praziquantel enantiomers and main metabolites against schistosoma mansoni. Antimicrob. Agents Chemother. 58 (9), 5466.

Meyer, T., Sekljic, H., Fuchs, S., Bothe, H., Schollmeyer, D., Miculka, C., 2009. Taste, a new incentive to switch to R-Praziquantel in Schistosomiasis treatment. PLoS Negl. Trop. Dis. 3, e357.

Moneghini, M., Carcano, A., Zingone, G., Perissutti, B., 1998. Studies in dissolution enhancement of atenolol. Part 1. Int. J. Pharm. 175, 177–183.

Moutasim, I.S., Elfatih, I.A.K., Kamal, E.E.I., Babiker, M.A., Ahmed, E.M.S., Ahmed, E.M. E.H., 2004. Photothermal stability of praziquantel. Saudi Pharm. J. 12, 157–162.

Mura, P., Cirri, M., Faucci, M.T., Gine's-Dorado, J.M., Bettinetti, G.P., 2002. Investigation of the effects of grinding and co-grinding on physicochemical properties of glisentide. J. Pharm. Biomed. Anal. 30, 227–237.

Nogi, T., Zhang, J., Chan, J.D., Marchant, J.S., 2009. A novel biological activity of praziquantel requiring voltage-operated Ca2+ channel b subunits: subversion of flatworm regenerative polarity. PLoS Negl. Trop Dis. 3 (6), 1–13 (e464).

Passerini, N., Albertini, B., Perissutti, B., Rodriguez, L., 2006. Evaluation of melt granulation and ultrasonic spray congealing as techniques to enhance the dissolution of praziquantel. Int. J. Pharm. 318, 92–102.

Shakhtshneider, T.P., Boldyrev, V.V., 1999. Mechanochemical synthesis and mechanical activation of drugs. In: Boldyreva, E., Boldyrev, V. (Eds.), Reactivity of Molecular Solids. Wiley, pp. 271–311.

Sheth, A.R., Lubach, J.W., Munson, E.J., Muller, F.X., Grant, D.J.W., 2005. Mechanochromism of piroxicam accompanied by intermolecular proton transfer probed by spectroscopic methods and solid-phase changes. J. Am. Chem. Soc. 127, 6641–6651.

Shin, S.C., Oh, I.J., Lee, Y.B., Choi, H.K., Cho, C.W., 1998. Enhanced dissolution of furosemide by coprecipitating or cogrinding with crospovidone. Int. J. Pharm. 175, 17–24.

Sugimoto, M., Okagaki, T., Narisawa, S., Koida, Y., Nakajima, K., 1998. Improvement of dissolution characteristics and bioavailability of poorly water soluble drugs by novel cogrinding method using water-soluble polymer. Int. J. Pharm. 160, 11–19.

Sun, Y., Bu, S.J., 2012. Simple, cheap and effective high-performance liquid chromatographic method for determination of praziquantel in bovine muscle. J. Chromatogr. B 899, 160–162.

Talaat, M., Dewolfe Miller, F., 1998. A mass chemotherapy trial of praziquantel on Schistosoma haematobium endemicity in Upper Egypt. Am. J. Trop. Med. Hyg. 59 (4), 546–550.

Trastullo, R., Dolci, L.S., Passerini, N., Albertini, B., 2015. Development of flexible and dispersible oral formulations containing praziquantel for potential schistosomiasis treatment of pre-school age children. Int. J. Pharm. 495 (1), 536–550.
Voinovich, D., Campisi, B., Moneghini, M., Vincenzi, C., Phan-Tan-Luu, R., 1999. Screening of high shear mixer melt granulation process variables using an asymmetrical factorial design. Int. J. Pharm. 190, 73–81.
Voinovich, D., Perissutti, B., Magarotto, L., Ceschia, D., Guiotto, P., Bilia, A.R., 2009. Solid state mechanochemical simultaneous activation of the constituents of the silybum marianum phytocomplex with crosslinked polymers. J. Pharm. Sci. 98, 215–228.
WHO, 2015a. Model List of Essential Medicines, 19 ed. Available at: http://www.who.int/selection_medicines/committees/expert/20/EML_2015_FINAL_amended_JUN2015.pdf?ua=1http://www.who.int/selection_medicines/committees/expert/20/EML_2015_FINAL_amended_JUN2015.pdf?ua=1 via the internet (April 2015–Revised June 2015).
WHO, 2015b. Model List of Essential Medicines for Children, 19 ed. Available at: http://www.who.int/selection_medicines/committees/expert/20/EMLc_2015_FINAL_amended_JUN2015.pdf?ua=1http://www.who.int/selection_medicines/committees/expert/20/EMLc_2015_FINAL_amended_JUN2015.pdf?ua=1 via the internet (April 2015–Revised June 2015).
Yang, L., Geng, Y., Li, H., Zhang, Y., You, J., Chang, Y., 2009. Enhancement the oral bioavailability of praziquantel by incorporation into solid lipid nanoparticles. Pharmazie 64, 86–89.