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Nanonized itraconazole powders for extemporary oral suspensions: Role of formulation components studied by a mixture design

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ABSTRACT

Itraconazole (ITZ) nanocrystal-containing powders were prepared through the combined use of high pressure homogenization (HPH) and spray drying (SD). These powders were intended as base materials for the preparation of extemporary oral suspensions of the drug. The role and the effect of stabilizers on the size of re-dispersed particles were studied using a mixture design and a Scheffé model relating the dried nanosuspension composition to the mean particle diameters. The homogenization process required a surface active agent (Tween 20) to obtain the efficient comminution of itraconazole micronized powder. SD was carried out on ITZ nanosuspensions after addition of a cellulose derivative (Methocel® E5) that allowed the prompt re-dispersion of nanoparticles under "in use" conditions. The powders obtained by drying of homogenized systems showed *in vitro* dissolution profile faster than that of the micronized drug, suggesting a potential ameliorated GI absorption of itraconazole released from the nanosuspensions.

1. Introduction

Among different strategies that can be used for enhancing the dissolution rate of poorly water-soluble drugs and leading to an ameliorated bioavailability, particle size reduction to sub-micron range (typically 100–200 nm) seemed to be the simplest way to face the formulation challenges (Mou et al., 2011; Möschwitzer, 2013; Gao et al., 2012; Plakkot et al., 2011).

In the last ten years, many efforts have been spent in developing pharmaceutical nanosuspensions and now some nanocrystal-based products are available on the market (Rapamune®, Emend®, Tricor® and Megace® ES) and others are currently being evaluated in clinical trials (Baert et al., 2009; Hanafy et al., 2007; Shrewsbury et al., 2009; Tuomela et al., 2014; Kumar and Burgess, 2012). Nanosuspensions are biphasic systems constituted of nanocrystals, with dimensions ranging between 10 and 1000 nm, dispersed in a liquid containing stabilizer agents that lower the free surface energy of the nanoparticles and prevent particle aggregation and/or particle growth.

Several processes can be used for the nanosuspension production; they can be classified into "Bottom-up" such as nanoprecipitation or nanocrystallization (Chan and Kwok, 2011; D'Addio and Prud'homme, 2011; Dandagi et al., 2011) and "Top-down" such as high pressure homogenization (Keck and Müller, 2006) and media milling (Merisko-Liversidge et al., 2003; Merisko-Liversidge and Liversidge, 2011). Each of these technologies possesses advantages and disadvantages; among the major drawbacks, the use of organic solvents and the possible degradation of the active molecule can be cited.

Independently from the strategy adopted for the nanosuspension production, the final product is invariably a system that suffers from physical instability, linked to sedimentation, agglomeration/aggregation of particles and/or Ostwald's ripening phenomena. To avoid such instability issues, the nanosuspension can be converted into a dry powder. This solid material can be conveniently handled and used, either as dosage form or as pre-formulate in the production of granules, tablets, capsules or pellets (Cerea et al., 2015; Dolenc et al., 2009; Pinto and Müller, 1999). Freeze drying (FD) and spray drying (SD) are techniques of first choice to convert solutions and suspensions to powder and their application to this purpose has been extensively investigated (Abdelwahed et al., 2006; Lee, 2003; Vehring, 2008; Yin et al., 2005).

At time of use, the obtained dried nano-particulate systems should be able to give aqueous dispersions characterized by particle size similar to that of the original nanosuspension. To reach this goal, appropriate stabilizers are added to the nanosuspension to lower the free surface energy of the nanoparticles. The high surface free energy of nanoparticles can be readily lowered by stabilizers that decrease the solid–liquid interfacial tension (Rabinow, 2004), while particle aggregation may be efficiently prevented or slowed down through adsorption of

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substances that form electrostatic repulsion or steric barriers (Wu et al., 2011). Surfactants (sodium dodecylsulphate, polysorbates, poloxamers), polymers (cellulose derivatives, povidones) and sugars have been employed, in many cases in mixture, to exploit their synergistic stabilizing effect (Van Eerdenbrugh et al., 2009).

The development of a dried nanosuspension containing itraconazole was the objective of the present study: itraconazole, an antifungal drug for the treatment of local and systemic mycoses, belongs to BCS class II, having high permeability, but a very poor water solubility (less than 1 ng mL^{-1} at pH 7.0) (Peeters et al., 2002). The poor aqueous solubility and high hydrophobicity limit its therapeutic efficiency and consequently it has been object of many studies (Chaubal and Popescu, 2008; Cerdeira et al., 2013; Kumar et al., 2014; Segale et al., 2015) devoted to develop pharmaceutical dosage forms with ameliorated biopharmaceutical characteristics. The reduction of the particle size is one of the strategies pursued for ITZ to improve its solubility and dissolution rate with a view to increasing its bioavailability. Our interest was focused on a dried product containing a high amount of ITZ in form of nanocrystals and able to promptly re-form a nanosuspension in contact with water. Further aims were to model the ITZ particle diameter as a function of system composition, and to investigate the role played by the excipients on the comminution of ITZ particles and on the redispersibility of dried suspension in water.

2. Materials and methods

2.1. Materials

Itraconazole (ITZ) was kindly donated by Chemo Group (E). Hydroxypropylmethylcellulose Benecel® E50 (E50) was provided by Ashland Inc. (KY, USA) and Methocel® E5 (E5) by Colorcon (UK). Sodium carboxymethylcellulose (Blanose® 7 LF — CMC) and hydroxyethylcellulose (Natrosol® 250 HX — HEC) were provided by Hercules (USA). Polyethoxylated sorbitan esters (Tween 20 — TW20 and Tween 60 — TW60), sorbitan monoesters (Span 20 — SP20 and Span 80 — SP80) and sodium lauryl sulfate (SLS) were purchased from Sigma-Aldrich (USA). Poly(ethylene oxide)/poly(propylene oxide) block copolymer (Pluronic® F127 — poloxamer, PLX) and macrogolglycerol ricinoleate (Cremophor® ELP — CRM) were obtained from BASF (D).

2.2. Evaluation of dispersion stability

A set of experiments was performed for selecting the stabilizer or the stabilizer mixture to be used for the production of the nano-powdered formulation.

An amount of 0.125 g of each stabilizer (both surfactant and polymer) was added to a water dispersion (10 mL) containing 1.25 g ITZ and the system was treated by a disperser (UltraTurrax T25 IKA®, D) at 24,000 rpm for 5 min. The suspensions were transferred in a graduated cylinder and visually inspected for homogeneity after 15 min, 1 h and 24 h using an arbitrary 4 points rating scale (see Table 1). An analog arbitrary scale was used at time 24 h for evaluating the consistency of the cake at the bottom of the cylinder (see Table 1).

2.3. High pressure homogenization

In exploratory tests, 3 different systems (100 mL) constituted of water and ITZ alone, ITZ/Tween 20 (10/1 weight ratio) and ITZ/Tween 20/E5 (10/1/1 weight ratio) respectively were processed by a high pressure homogenizer (Microfluidizer M-110L, Microfluidics®, USA). The coarse dispersions were pre-treated by a disperser (UltraTurrax T25 IKA®, 6500 rpm for 1 min) before applying the HPH process. In the high energy process, pressure was set to 1000 bar, temperature at 25 °C and process time at 10 min. After HPH treatment, the suspensions were immediately analyzed for the particle size (Z_{ave}) and surface charge (zeta potential, Z_{pot}) by PCS.

Table 1
Preliminary experiments for component screening: low energy process of drug/stabilizer aqueous dispersion.

System	Homogeneity			Cake aspect
	After 15 min	After 1 h	After 24 h	
ITZ-SLS	+++	++	+	+
ITZ-TW20	++++	++++	+	+
ITZ-TW60	++	++	+	++
ITZ-SP20	−	−	−	+++
ITZ-SP60	−	−	−	+++
ITZ-CRM	+++	++	+	+++
ITZ-E50	−	−	−	+
ITZ-E5	+	−	−	+
ITZ-PLX	+++	++	+	+
ITZ-CMC	−	+	+	+
ITZ-HEC	−	+	+	+

Homogeneity scale. −: absent; +: poor; ++: discrete; +++: good; ++++: very good.
Cake scale. −: absent; +: loose; ++: solid; +++: very solid.

In the body of the experimental work, HPH process was carried out by the following procedure. ITZ coarse powder was dispersed by UltraTurrax (6500 rpm for 1 min) in water containing an appropriate amount of Tween 20. The suspension was transferred to the homogenizer hopper and treated at constant temperature (25 °C) under 1200 bar for the different times (1 cycle lasted about 15 s). The nanosuspension was recovered and immediately analyzed for particle size determination.

Two sets of experiments were carried out: in the first, three suspensions constituted of coarse ITZ in different amounts (corresponding to 3, 10 and 15% *w*/*v*) and TW20 in a fixed ratio with the drug (drug/TW20 = 10/1 *w*/*w*) were submitted to HPH process. At pre-selected time intervals (1, 2, 5, 10, 12, 20, 25, 40 and 60 min), samples (1 mL) were withdrawn and immediately analyzed by PCS for particle size determination. A second set of experiments (see 2.7. Mixture design) was accomplished on systems composed of 10% *w*/*v* coarse ITZ and different percentages of Tween 20 (ranging from 0.7 and 2.42% *w*/*v*): they were homogenized for 25 min and, after withdrawal of 1 mL sample for PCS analysis, the prescribed amount of E5 was added to the suspension, which was dried as below described.

2.4. Spray drying

Nanosuspensions were dried by a Mini Spray Dryer B-290 (Buchi® Labortechnik, CH). The drying conditions were the same for all the formulations (Cerea et al., 2015): inlet temperatures of 160 °C; air flux at 742 L/h, nozzle 0.6 mm diameter and a feeding rate of 4 mL/min. Before drying, the equipment was equilibrated using deionized water. The dried powders were recovered only from the collection chamber.

2.5. Morphology and size of particles

The morphological properties of spray dried powders were investigated using a scanning electron microscope (SEM; Sigma, Carl Zeiss, D). Before scanning, the samples were coated with gold using a plasma evaporator under vacuum. SEM images were acquired at an accelerated voltage of 10 kV using different magnifications.

Particle size of micronized-ITZ was measured on SEM pictures using an image analysis software (Seneco, Motic Image Plus, Ver. 2.0 ML) analyzing 20 particles on two different pictures for each sample.

The average size (Z_{ave}) and size distribution (Polydispersity Index—PI) of nanoparticles were investigated by Photon Correlation Spectroscopy (PCS) with a Zetasizer 3000HS (Malvern instrument, UK) after HPH and after the spray drying process. Liquid formulations were diluted with deionized water to obtain reproducible

analyses. Powder formulations were dispersed (re-dispersed systems), like in the extemporary reconstitution of dispersed dosage forms: an amount of powder corresponding to 0.20 g of ITZ was dispersed in 25 mL water and manually shaken for 30 s. The re-dispersed systems were further diluted with deionized water for the PCS analysis. Two different samples of each formulation batch were assessed: for each aliquot the results of three runs were collected and their average value was calculated. Zeta potential (Z_{pot}) was determined by Zetasizer 3000HS at 25.0 ± 1 °C. The re-dispersed systems were diluted with 1 mM NaCl and the samples showed pH values in the range 6.0–6.5. The Zeta potential values were calculated using the Smoluchowski equation.

2.6. Differential scanning calorimetry

DSC analyses were performed with a Pyris 1 (Perkin Elmer, USA). The samples were prepared introducing an aliquot of the sample equivalent to 5 mg of ITZ in an aluminium pan that was then sealed. Thermal scans were recorded at a heating rate of 5 °C min^{-1} under dry nitrogen purge (20 mL min^{-1}) from 25 to 200 °C.

2.7. Mixture design

The size of particles constituting the dried powders obtained by HPH and SD combined processes was modelled as a function of the percentage composition of the systems. A mixture design was used for planning the systems to be tested: the proportions of ITZ, TW20 and E5 in the dried product were considered as factors and the log 10 of the size values of re-suspended powder particles represented the response variable.

The experimental points were identified based on restrictions suggested by the previous screening experiments: systems containing percentages of ITZ higher than 66.0% and lower than 86.0%, TW20 and E5 in the range 6.0–16.0% and 6.0–28.0% respectively were considered. Based on these constraints, a sub-region of the simplex (the complete domain of ITZ/TW20/E5 mixture) was identified as the experimental domain (Fig. 1). The mixture design was constructed selecting the most informative experimental points as dictated by Cornell (2002): they were the extreme vertices of the polygonal experimental domain, the middle points of its edges and the centroid (Table 2).

Table 2
Setting of mixture design.

Exp. point*	Original coordinates			"Pseudo" components		
	ITZ	TW20	E5	ITZ	TW20	E5
NS 1	0.660	0.060	0.280	0.000	0.000	1.000
NS 2	0.860	0.060	0.080	0.897	0.000	0.103
NS 3	0.860	0.083	0.057	0.897	0.103	0.000
NS 4	0.783	0.160	0.057	0.552	0.448	0.000
NS 5	0.660	0.160	0.180	0.000	0.448	0.552
NS 6	0.760	0.060	0.180	0.448	0.000	0.552
NS 7	0.822	0.122	0.057	0.724	0.276	0.000
NS 8	0.722	0.160	0.119	0.276	0.448	0.276
NS 9	0.660	0.110	0.230	0.000	0.224	0.776
NS 10	0.765	0.105	0.131	0.469	0.200	0.331

* The middle point on the edge 2–3 has been deliberately omitted because it gave no significant information.

Because of bounds placed on the factors, the region of interest is confined to a limited region of the whole system domain and seemed natural to redefine its coordinates in term of "pseudo" components (combination of the original component coordinates). This transformation made the comprehension of the design and the fitting of models easier and increased the precision of coefficient estimates. In Table 2, the design setting in the original variables and in "pseudo" components of dried suspensions is reported; the same was also illustrated in Fig. 2. Such a design allowed to calculate up to a complete third degree model and provided information for detecting the curvature of the response surface and for validating the model itself.

A special cubic Scheffé model was fitted to the logarithm of experimental data in "pseudo" coordinates (denoted with a prime):

$$\log Z_{ave} = b_1 ITZ' + b_2 TW20' + b_3 E5' + b_{12} ITZ' \cdot TW20' + b_{13} ITZ' \cdot E5' + b_{23} TW20' \cdot E5' + b_{123} ITZ' \cdot TW20' \cdot E5'.$$

Multivariate regression analysis was performed by a cross-validated procedure implemented via R-software (version 3.1.0).

The total effect of each system component was evaluated as proposed by Piepel (Cornell, 2002; Piepel, 1982). In brief, the centroid of our experimental domain was taken as reference mixture and the line

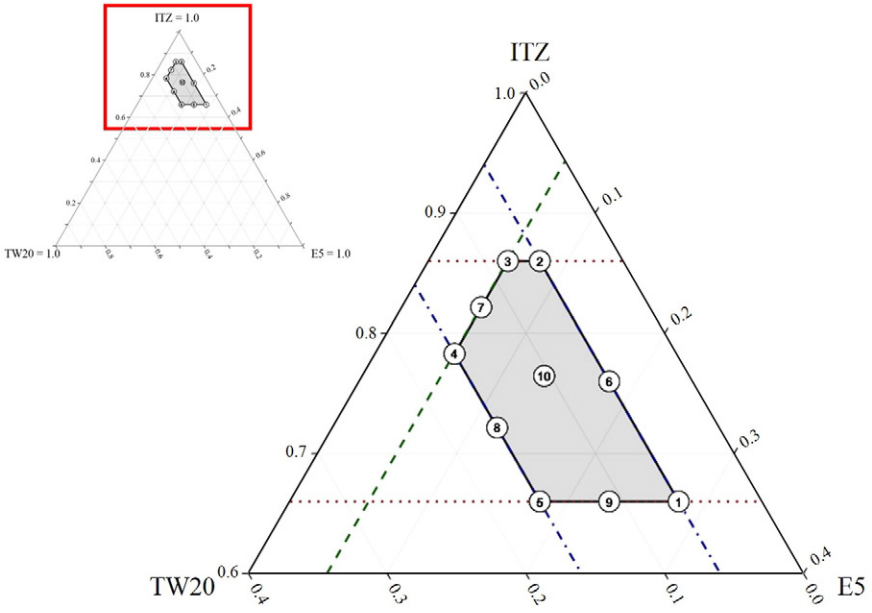

Fig. 1. Region of interest with experimental domain (grey zone) and experimental points of mixture design: in detail the whole simplex of ITZ-TW20-HPMC mixture with a box surrounding the region of interest.

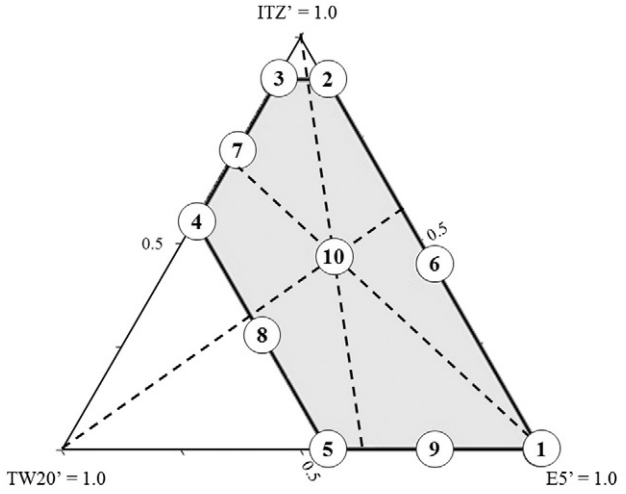

Fig. 2. Experimental domain of mixture design: components are re-defined in "pseudo" components; dashed lines represent the "Piepel's directions" of each component.

connecting the reference to the vertex of each "pseudo" component *i* was defined as "Piepel's direction" (Fig. 2). This is the direction along which the proportion of component *i* increases (or decreases), those of remaining 2 components decrease (or increase), but the ratio among the remaining components stay the same. Coordinates of mixtures along the Piepel's direction of component *i* were calculated and substituted into the fitted model to obtain their predicted response values. Plotting them against incremental values of the selected component (within the experimental region) gave the curves showing the effect of each component.

2.8. Dissolution tests

The dissolution tests were carried out on some of the dried nanosuspensions and on a physical mixture (PM 5) constituted of coarse ITZ (66.0%), TW20 (16.0%) and E5 (18.0%). A dissolution approach published in the literature for nanoparticulate formulations (Chaubal and Popescu, 2008; Crisp et al., 2007) was applied. Dissolution testing was conducted by first adding the dried nano-powder or the physical mixture to water and mixing for up to 5 min at 37 °C to allow thorough wetting of the particles; then the suspension was transferred to the dissolution medium.

An USP dissolution apparatus 2 (AT7 Smart, Sotax, CH; 100 rpm, 37.0 ± 0.5 °C, n = 6) was employed. The dissolution medium was Simulated Gastric Fluid without enzymes (SGF) (USP 36) (900 mL); the use of surfactants in the dissolution medium was avoided to primarily assay the effects of the dimensional characteristics of particles on the dissolution process. An amount of material corresponding to 4 mg of ITZ (ITZ solubility in SGF is 5.0 μg mL^{-1}) was introduced into the vessel. Samples were withdrawn at fixed time points, filtered and spectrophotometrically assayed for the drug content (UV 220 nm, Lambda 35, Perkin Elmer, I).

For comparing the drug release profiles, the similarity factor *f2* was used considering that *f2* values greater than 50 (50–100) assess similarity or equivalence of the two curves (FDA, 1997).

3. Results and discussion

3.1. Preliminary studies

The first set of preliminary experiments was carried out by a low energy homogenization process with the aim of selecting suitable excipients for an efficient homogenization of suspensions. Among the various surfactants and polymers, Tween 20 seemed to be the most performing one, since it gave a suspension, which remained homogeneous for at least 1 h (Table 1).

All of the polymers, with the exception of the poloxamer (PLX), led to suspensions less stable and homogeneous than those obtained with the surfactants, but they gave a loose settling of the suspension, reducing the risk of an irreversible agglomeration of drug particles.

Since in our case, the nanosuspensions were intended to be spray dried immediately after the size reduction treatment, the long-term stability of the systems was not investigated. These results suggested the selection of Tween 20 and of a cellulose derivative as components of the formulation under development. Among polymers, our choice was E5 because this compound has a lower MW than E50. As a result, when it comes in contact with water it exhibits a faster dissolution and gives a colloidal solution characterized by a lower viscosity (Dow, 2002). These characteristics could be particularly important for the production of a powder that should re-disperse promptly in water at time of dispensing giving rise to an extemporary suspension.

The preliminary HPH experiments, carried out on aqueous dispersions containing pure drug, drug/TW20 or ITZ/TW20/E5, showed that the surfactant is essential for an efficient comminution of the drug. In fact, after HPH, the particle size of pure ITZ suspension was significantly higher than that of ITZ/TW20 system (Z_{ave} > 2000 nm and 677.4 ± 53.1 nm respectively). It has also been observed that the polymer does not have a relevant effect on the extent of size reduction of particles: the particle size for ITZ/TW20/E5 system accounted for 714.3 ± 42.8 nm, not significantly different from that of ITZ/TW20 suspension ($p > 0.01$). The comparison of measured Z_{pot} values for these systems indicated that neither the surfactant, nor the cellulose derivative modified remarkably the surface charge characteristics of particles: $Z_{pot} = -32$ mV for pure ITZ system; $Z_{pot} = -30$ mV for ITZ/TW20 system and $Z_{pot} = -27$ mV for ITZ/TW20/E5 system. Since E5 did not meaningfully modify the performances and the outcome of HPH process, the cellulose polymer was added to the nanosuspension subsequently to the homogenization treatment and just before the spray drying process.

In the HPH experiments, it was also observed that suspensions containing the drug in concentration higher than 25% (*w*/*v*) led to the clogging of the homogenizer and did not allow the profitable homogenization treatment of dispersions.

3.2. Homogenization and spray drying of nanosuspensions

The degree of comminution obtainable by HPH is related to the operative conditions applied in the process and, most of all, to the homogenization time and pressure and to the processing temperature (Pardeike and Müller, 2010). In the present work, the homogenization pressure was set at 1200 bar, so that the energy applied per time unit was equal for all the formulations: the temperature was maintained at 25 °C throughout the process. Systems constituted of ITZ and Tween 20 at constant weight ratio 10:1, differing for the percentage of solid dispersed in water (3, 10 and 15%), were submitted to transit into the homogenizer chamber for different times (cycles). Size and size distribution of particles after selected processing times were measured. Starting from a ITZ powder constituted of particles 8.9 μm in mean diameter, suspensions with particles in the nanometric range were obtained. Increasing the number of homogenization cycles, the particle size diminished and simultaneously a continuous narrowing of the width of the particle size distribution was observed (Fig. 3a–b).

The profile of size reduction of three ITZ suspensions (Fig. 3a) followed a trend similar to that observed by other researchers (Pardeike and Müller, 2010). The most conspicuous decrease was within about 20 min (about 75 cycles) and after this time the change in particle size was less evident: as previously reported by Plakkot et al. (2011), the comminution in the latter stages of process occurred by abrasion rather than fragmentation of particles. The Z_{ave} curve for the 10% ITZ system almost overlapped that of the 3% drug suspension,

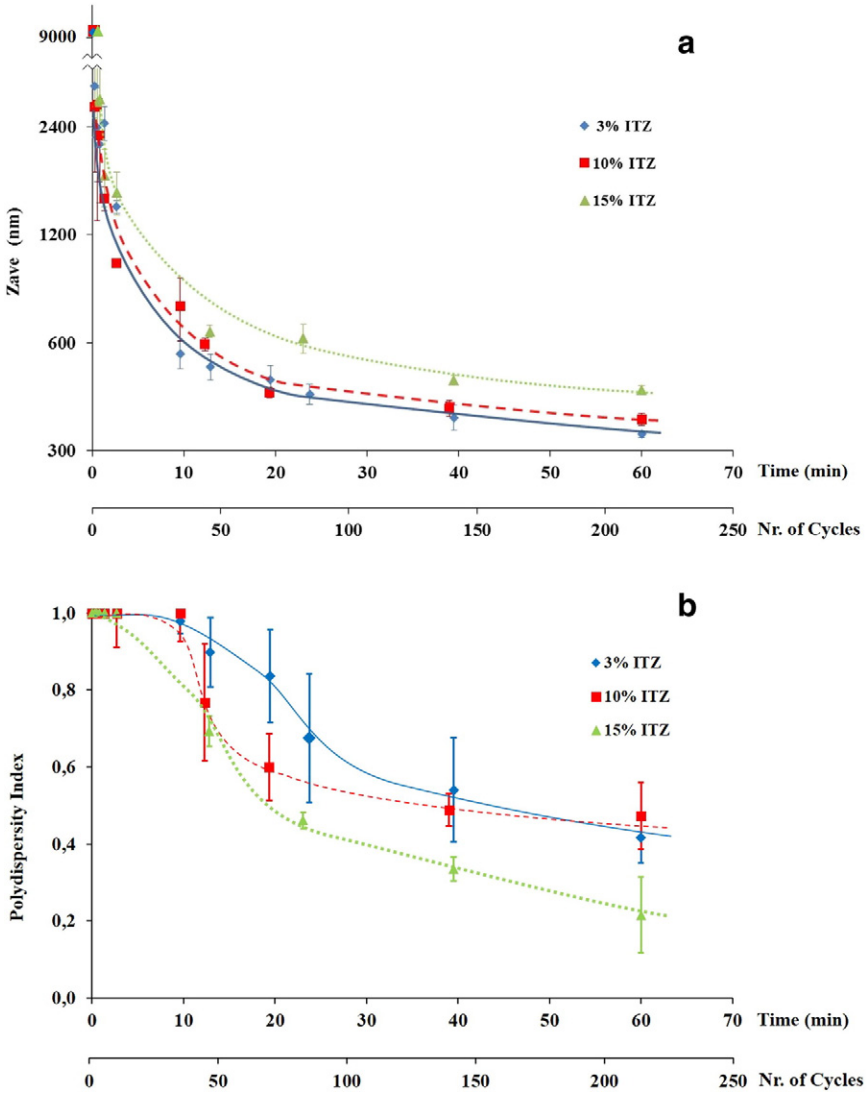

Fig. 3. Size reduction of ITZ particles by HPH process: particle size (Z_{ave}) (a) and Polydispersity Index (PI) (b) as a function of time and number of HPH cycles. ITZ/TW20 weight ratio was kept constant (10/1).

while 15% ITZ system showed significantly higher mean diameter values, suggesting that the high concentration of particles reduced the efficacy of their collisions during the treatment. Even though the number of the investigated systems was limited, it can be asserted that amounts of solid ≤10% did not affect the particle size reduction of the product. Increasing the number of homogenization cycles, the polydispersity index (PI) diminished (Fig. 3b), providing nanonized powders with a higher degree of homogeneity and thus less prone to the potential effects of Ostwald's ripening. The diminution of PI (increase of homogeneity) on increasing times (cycles) was particularly evident for the 15% ITZ suspension.

These findings oriented the choices for the HPH treatments in the successive mixture design: the processing conditions were fixed at 1200 bar for 25 min (90 homogenization cycles) at 25 °C employing suspensions containing 10% ITZ in order to obtain products constituted of particles of nanometric dimensions and stable enough for the drying process.

Liquid vehicle removal was carried out by spray drying with the aim of obtaining powders that, when re-dispersed in water, were able to form again suspensions containing particles of original nanometric size. A further desired feature of the powders is their complete de-agglomeration in water when shaken for few seconds, i.e. in conditions miming those used by a patient at the administration of the medicinal. The presence of the employed cellulose derivative E5 would delay the crystal growth by reducing particle interactions and consequently would diminish the potential for agglomeration (Plakkot et al., 2011). The hydrophilic polymer E5 was added to ITZ/TW20 suspensions before spray drying. Drying conditions used for all nanosuspensions were the same to avoid any influence of the process on the aggregation of nanocrystals, as experienced by other authors (Chaubal and Popescu, 2008).

3.3. Mixture design study

The influence of the cellulose derivative and the possible effects of the surfactant on the size of re-dispersed ITZ powders were investigated and a regression model describing the relationship among the size of particles and the amount of powder components was searched. The study was accomplished by a mixture design approach (Table 2), which involved the preparation of ten suspensions constituted of different proportions of ITZ, TW20 and E5. ITZ and TW20, in the proportions of design, were dispersed in water, homogenized and analyzed for particle size determination: for all nanosuspensions (NS) (Table 3), mean particle diameters were lower than 500 nm and PI values accounted for a discrete size distribution homogeneity (<0.52). After the addition of E5, the suspensions were spray dried.

Table 3
Dimensional characteristics of nanosuspensions (NS) and re-dispersed dried nanopowders (r-NS): S.D. values in brackets, the values are means of three samples.

Exp. point	After HPH (NS)		After redispersion (r-NS)	
	Z_{ave} (nm)	P.I.	Z_{ave} (nm)	P.I.
1	313.0 (4.6)	0.38 (0.05)	424.8 (14.2)	0.32 (0.09)
2	338.6 (1.4)	0.41 (0.05)	703.7 (38.6)	1.00 (0.00)
3	371.6 (12.1)	0.52 (0.05)	1169.8 (105.3)	1.00 (0.00)
4	441.0 (13.1)	0.45 (0.03)	712.8 (58.5)	0.98 (0.04)
5	439.5 (32.5)	0.37 (0.04)	492.5 (35.1)	0.62 (0.12)
6	443.0 (12.7)	0.30 (0.03)	399.1 (15.5)	0.44 (0.08)
7	455.9 (9.6)	0.40 (0.08)	1187.7 (49.4)	1.00 (0.00)
8	379.9 (9.1)	0.36 (0.04)	440.3 (14.5)	0.42 (0.11)
9	371.4 (13.5)	0.36 (0.03)	432.6 (20.5)	0.34 (0.05)
10	368.1 (11.6)	0.33 (0.05)	440.7 (7.2)	0.45 (0.08)

Spray dried powders were analyzed by DSC in order to point out possible modification of the physical status of the drug. DSC traces for the pure drug, a physical mixture of the three components and the corresponding NS 5 powder are reported in Fig. 4. For the physical mixture, a thermal profile very different from that of the pure drug was observed: as expected, the presence of E5 and Tween 20 determined the shift of the onset and peak temperatures of the drug. The comparison between formulation NS 5 and the physical mixture revealed a thermal behaviour very similar, that suggests a practically identical structure for the two types of systems and the assignment of the peak around 160 °C to the melting of ITZ nanocrystals. The slight reduction of DSC parameters for the formulation NS 5 can be attributed to the more efficient heat transfer during the DSC analysis for the nanonized-ITZ-containing powder in comparison to that for physical mixture PM 5 that contains ITZ particles of higher dimension.

The powders were also microscopically observed and the inspection gave evidence of their structure. From the SEM pictures of NS 5 powder (Fig. 5), it can be appreciated that SD powders were agglomerates of variable dimensions, constituted of big, spherical and collapsed particles (whose diameter ranged from 5 to 25 μm) with adhered and embedded small particles (<2 μm in diameter) on their surface. We suppose that the largest units were E5 particles formed during the drying process, while the smallest ones nanocrystals of ITZ covered/agglomerated by the excipients.

The powders re-dispersed quickly in water by shaking (250 mg in 25 mL of water, r-NS systems) and gave suspensions that did not show sedimentation for 5 h. Cellulose derivative E5 units, when in contact with water, re-hydrated and dissolved in few seconds leading to the complete separation of agglomerates present in the powder. PCS analysis (Table 3) showed increased particle size values for most of r-NS systems due to the adhesion of E5 on the surface of ITZ nanocrystals. For powders containing low percentages of E5 (r-NS 3 and r-NS 7), the size values were particularly higher than those before drying: this result indicated strong interactions among nanocrystals during SD process, that led to their stable aggregation. This finding could probably be due to the low proportion of E5 in the system.

In the study of the relationship among the particle dimension and the components of mixtures, we used the logarithm of Z_{ave} instead of Z_{ave} values: this transformation obtained a set of data for which the distribution of the error terms is close to a normal distribution. Logarithmic particle size values of the different batches of powders were fitted to the Scheffé special cubic model and a cross-validated regression algorithm was applied to the data of Table 4. The method gave a model whose coefficient estimates and statistical parameters are reported in Table 5. This model adequately described the correlation between the particle size and the proportion of components, as proved by resulting statistical parameters.

The contour plot depicted in Fig. 6 illustrates the complexity of the relationship between response and formulation variables. An estimate of the effects of mixture components was calculated substituting the "pseudo" coordinates of blends along each "Piepel's direction" into the fitted model. A set of predicted response values were obtained and the response trace constructed. The plot of Fig. 7 graphically illustrates the effects of each mixture component in "pseudo" coordinates.

A significant effect of ITZ on the log mean diameter of particles can be appreciated, log Z_{ave} diminishing for systems constituted by proportions ≤0.713 of ITZ (0.239 in "pseudo" coordinates) and increasing for higher proportions of the drug. A more remarkable effect is shown by E5, the log mean diameter of particles diminishing for increasing proportions of the stabilizing agent, with a minimum value for the suspension containing a proportion of 0.210 E5 (0.671 in "pseudo" coordinates) and low increment for higher content of this component. Tween 20 has limited effect on the particle size, as testified by the low variation of response over all TW20 proportion range along the direction this component effect is measured. These results indicate that E5 plays a very important role in determining the size of particles of redispersed powder; a proportion of the stabilizer around 21% represents the optimal level for powders that will give suspensions with particles

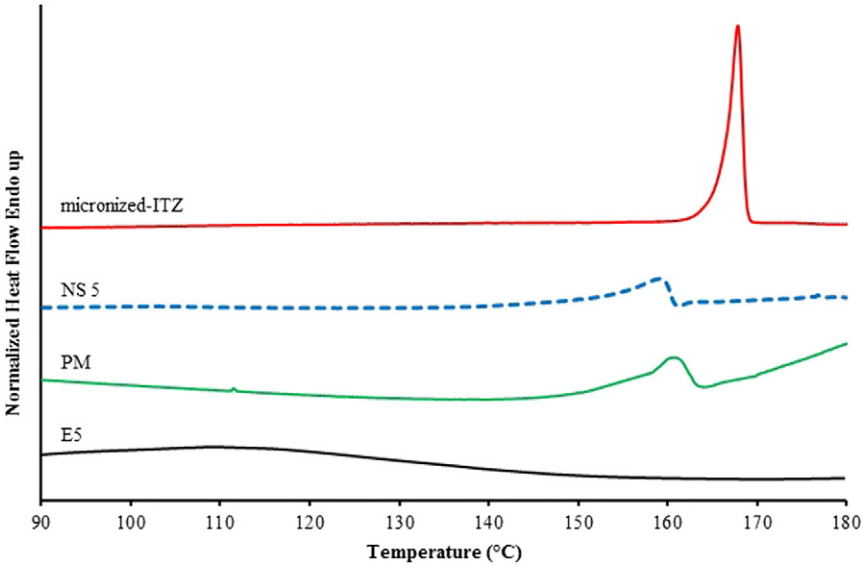

Fig. 4. DSC profile of E5, ITZ-TW20-E5 physical mixture PM 5, NS 5 powder and micronized-ITZ powder.

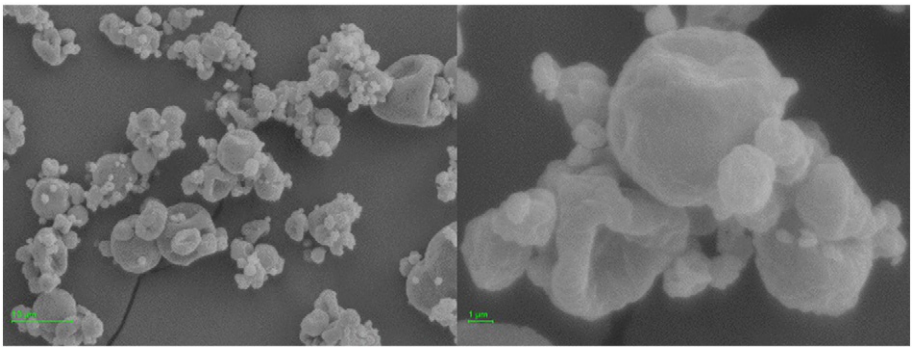

Fig. 5. SEM pictures of NS 5 system at two different magnifications.

of minimum size. Moreover, the surfactant seems to have a very limited effect on the re-suspended particle size, but, as above reported, it is essential for the comminution of ITZ during HPH.

The good predictive ability of the calculated model is demonstrated by the high value of R^2_{CV} (=0.9073). To reinforce this, the model was used to predict the particle size value of a mixture inside the experimental space that could represent a valuable formulation for the extemporary ITZ suspension. The mixture used as validation point was selected taking into account the effects of polymeric stabilizing agent and drug on the particle size: the proportion of surfactant was calculated as complement of the system. The validation mixture, constituted of 68.7% ITZ, 13.3% Tween 20 and 18.0% E5 (corresponding to 0.448, 0.179 and 0.373 in "pseudo" coordinates, respectively), was homogenized and dried, as previously described, and diameter and size distribution of particles were determined by PCS after re-suspension of the powder in water. The experimental size value (434.1 ± 25.5 nm) of this system was very close to the predicted one (415.0 nm), confirming the high reliability of the model for the prediction of the particle size of our ITZ blends.

Dissolution behaviour of dried nanoparticles was tested in SGF for simulating the gastric environment. As an example, in Fig. 8, the dissolution profile of re-dispersed ITZ powder r-NS 5 is reported and compared with the corresponding physical mixture prepared with the micronized drug (PM 5). Differently from other authors (Chaubal and Popescu, 2008), the release profiles did not reveal an initial lag time which could have been referred to a disintegration step of aggregates: the pre-dispersion treatment of powders and the good disaggregating properties of E5 accounts for this finding. As expected, the dissolution of nanonized ITZ powder was markedly more rapid than that of physical mixture PM 5, being the surface area of drug particles substantially increased by HPH process. For the nanonized formulation, after 15 min the solubilized ITZ accounts for 83.0%, while, for the micronized ITZ system PM 5, the percentage of drug in the medium was only 46.0%. Less than 1 h is required for the complete dissolution of the drug from the system containing nanoparticles (492.5 nm in diameter), allowing to expect an increased bioavailability of itraconazole when administered *in vivo*.

Table 4
Matrix of data for model calculation.

r-NS exp. point	N. of batches	"Pseudo" components			Z_{ave} (nm)*	Log Z_{ave}	P.I.
		ITZ	TW20	E5			
					412.8	2.616	0.29
1	3	0.000	0.000	1.000	439.0	2.642	0.30
					422.7	2.626	0.36
					731.1	2.864	1.00
2	3	0.897	0.000	0.103	658.9	2.819	1.00
					721.3	2.858	1.00
					1105.3	3.028	1.00
3	3	0.897	0.103	0.000	1248.1	3.096	1.00
					1091.5	3.037	1.00
					728.3	2.862	1.00
4	3	0.552	0.448	0.000	675.2	2.829	0.96
					735.0	2.865	1.00
					447.8	2.651	0.46
					547.8	2.738	0.63
5	5	0.000	0.448	0.552	509.2	2.707	0.72
					505.5	2.704	0.65
					492.0	2.691	0.69
					385.4	2.586	0.39
6	3	0.448	0.000	0.552	409.2	2.612	0.56
					402.7	2.605	0.38
					1213.3	3.084	1.00
					1990.0	3.299	1.00
7	5	0.724	0.276	0.000	1206.9	3.081	1.00
					1143.1	3.058	1.00
					1404.0	3.147	1.00
					445.4	2.649	0.37
8	3	0.276	0.448	0.276	448.0	2.651	0.46
					427.6	2.631	0.43
					454.2	2.657	0.32
9	3	0.000	0.224	0.776	433.8	2.637	0.34
					409.7	2.612	0.37
					432.4	2.636	0.44
					443.7	2.647	0.47
					437.5	2.641	0.48
10	7	0.469	0.200	0.331	449.1	2.652	0.42
					528.5	2.723	0.90
					476.5	2.678	0.53
					432.5	2.636	0.52

* Each value is the mean of two replicates.

4. Conclusions

High pressure homogenization and spray drying combination allowed the production of ITZ-containing powders that can be used as a base of extemporaneous suspensions. These powders contained high proportions of drug in form of agglomerates of nanocrystals coated and/or embedded by a mixture of excipients. The agglomerates easily re-disperse upon contact with water in conditions simulating those of actual use in administrating suspensions. The increased drug dissolution rate allows expecting an enhanced bioavailability of ITZ when administered in form of nanosuspension. Based on these findings, it has been hypothesized that the nanonized-ITZ powders could be employed as intermediate for the production of other oral drug dosage forms and further works are in progress in order to demonstrate this hypothesis.

Table 5
Coefficient estimates of the Scheffé special cubic model and related statistical parameters.

Coefficient	Estimate	St. Error	t value	p
b_1	3.119	0.035	88.378	$<2.00 \cdot 10^{-16}$
b_2	2.712	0.064	42.341	$<2.00 \cdot 10^{-16}$
b_3	2.640	0.038	70.401	$<2.00 \cdot 10^{-16}$
b_{13}	−1.331	0.177	−7.506	$1.03 \cdot 10^{-08}$

$R^2 = 0.9992$; Adjusted $R^2 = 0.9992$; Res. St. Error = 0.0809 (34 df); $F_{4,34} = 1.12 \cdot 10^4$; $p < 2.20 \cdot 10^{-16}$; $R^2_{CV} = 0.9073$.

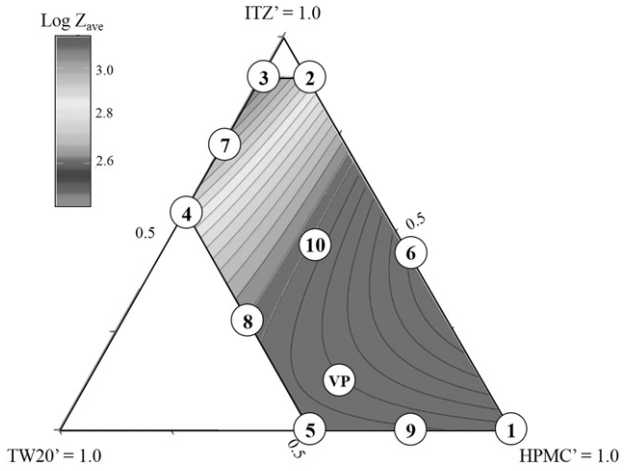

Fig. 6. Contour plot of log particle size as a function of component proportions in nanopowders.

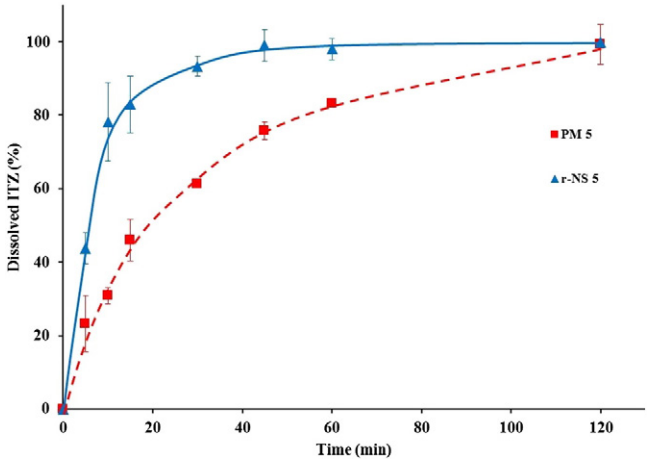

Fig. 8. Dissolution profiles of nanonized ITZ powder (r-NS 5) and of corresponding physical mixture (PM 5).

The mathematical model adequately describes the relationship between the size of particles and the composition of suspensions and clarifies the role played by the employed stabilizing mixture. The surfactant seems primarily involved as wetting agent in the comminution process and the cellulose polymer as de-agglomerating/separating agent of spray-dried formulation. The resulting model can be used as a valuable tool for obtaining systems with the desired dimensional characteristics.

List of main abbreviations

CMC	Blanose® 7 LF (sodium carboxymethylcellulose)
CRM	Cremophor® ELP (polyoxyl 35 hydrogenated castor oil)
E5	Methocel® E5 (hydroxypropylmethylcellulose; 5 mPa·s)
E50	Benecel® E50 (hydroxypropylmethylcellulose; 50 mPa·s)
F	F test value
FD	freeze drying
HEC	Natrosol® 250 HX (hydroxyethylcellulose)
HPH	high pressure homogenization
ITZ	itraconazole
NS	nanosuspension
PCS	Photon Correlation Spectroscopy
PI	polydispersity index
PLX	Pluronic® F127 (poly(ethylene oxide)/poly(propylene oxide) block copolymer)

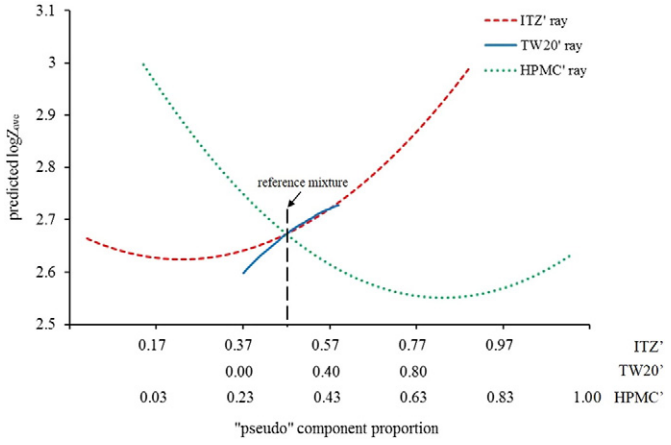

Fig. 7. Effect of formulation components on the particle size of re-dispersed dried nanosuspensions (r-NS).

PM	physical mixture
r-NS	reconstituted nanosuspension
R^2_{CV}	cross-validated correlation coefficient
SD	spray drying
SGF	simulated gastric fluid
SLS	sodium lauryl sulphate
SP20	Span 20 (sorbitane monolaurate)
SP60	Span 60 (sorbitane monostearate)
TW20	Tween 20 (polyoxyethylene (20) sorbitan monolaurate)
TW60	Tween 60 (polyoxyethylene (20) sorbitan monostearate)
Z_{ave}	average size of particles
Z_{pot}	zeta potential

Declaration of interest

The authors report no declarations of interest.

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