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Evaluation of melt granulation and ultrasonic spray congealing as techniques to enhance the dissolution of praziquantel

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Abstract

Praziquantel (PZQ), an anthelminthic drug widely used in developing countries, is classified in Class II in the Biopharmaceutics Classification Systems; this means that PZQ has very low water solubility and high permeability, thus the dissolution is the absorption rate-limiting factor. The aim of this work was to evaluate the suitability of melt granulation and ultrasonic spray congealing as techniques for enhancing the dissolution rate of PZQ. Granules in high shear mixer were prepared by melt granulation, using polyethylene glycol 4000 or poloxamer 188 as meltable binders and α-lactose monohydrate as a filler. Quite regularly shaped granules having main size fraction in the range 200–500 μm were obtained using both formulations; however, only poloxamer 188 granules demonstrated a significant ($P = 0.05$) increase of the PZQ dissolution rate compared to pure drug. To evaluate the potential of ultrasonic spray congealing, Gelucire 50/13 microparticles having different drug to carrier ratios (5, 10, 20 and 30%, w/w) were then prepared. The results showed that all the microparticles had a significant higher dissolution rate ($P = 0.05$) respect to pure PZQ. The increase of the PZQ content considerably decreased the dissolution rate of the drug: 5 and 10% PZQ loaded systems evidenced dissolution significantly enhanced compared to 20 and 30% PZQ microparticles. The microparticle's characterisation, performed by Differential Scanning Calorimetry, Hot Stage Microscopy, X-ray powder diffraction and FT-Infrared analysis, evidenced the absence of both modifications of the solid state of PZQ and of significant interactions between the drug and the carrier. In conclusion, melt granulation and ultrasonic spray congealing could be proposed as solvent free, rapid and low expensive manufacturing methods to increase the in vitro dissolution rate of PZQ.

Keywords: Praziquantel; Dissolution; Melt granulation; Spray congealing

1. Introduction

Praziquantel (PZQ) is a broad spectrum anthelminthic drug: it is widely used in developing countries as the drug of choice in the treatment of schistosomiasis and it is also effective in other trematode and cestode infections (El-Subbagh and Al-Badr, 1998). For its efficacy, safety and comparative cost-effectiveness characteristics, PZQ is included in the World Health Organization Model list of Essential drug (web site WHO).

As regards to its biopharmaceutics properties, PZQ is classified in Class II in the Biopharmaceutics Classification Systems (BCS) (Lindenberg et al., 2004); this means that PZQ has very low water solubility and high permeability, the dissolution thus is the absorption rate limiting factor (Amidon et al., 1995). The enhancement of PZQ dissolution rate is therefore an important and challenging aspect in formulation development. Quite surprisingly, up till now only a few studies have had focus on this aspect: El-Arini and Leuenberger (1998) and De La Torre et al. (1999) investigated the possibility of improving PZQ dissolution rate by the preparation of drug-polyvinylpyrrolidone coprecipitates and physical mixtures, while El-Arini and Leuenberger (1996) and Becket et al. (1999) studied the dissolution and physicochemical properties of PZQ and β-cyclodextrin and PZQ and α-, β- and γ-cyclodextrin complex, respectively.

The aim of this research was to evaluate other approaches to enhance the dissolution rate of PZQ, with particular regards to technologies, which could be rapid, easily scaled-up, industrially applicable and low expensive. Two techniques have been evaluated: melt granulation and spray congealing. Both these technologies involve the use of a substance, which

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melts at relatively low temperature. In the melt granulation this substance is added in the molten form or it melts due to the heat of friction or to the heating chamber and then it acts as a liquid binder, whilst in the spray congealing the low melting carrier is melted and then atomised. Neither melt granulation nor spray congealing, consequently, requires the use of organic or aqueous solvents and hence both techniques are environmentally friendly and less consuming in terms of time and energy compared to the more famous wet granulation and spray drying, respectively. Previous studies have shown that, by selecting the suitable material, both melt granulation and spray congealing can be used to prepare fast release systems (Passerini et al., 2002a; Seo et al., 2003; Passerini et al., 2002b; Perissutti et al., 2003).

As a continuation of our research, in this work the suitability of melt granulation and spray congealing for improving the dissolution rate of PZQ is evaluated. In the first part of the study, the production of granules by melt granulation in high shear mixer is considered, while in the second part the preparation of lipid microparticles by the ultrasonic spray congealing technique is examined. The in vitro dissolution rate of the drug from all the systems (granules and microparticles) was investigated and the morphology, particle size and drug loading were studied. In the case of microparticles, their physicochemical properties were also examined using DSC, HSM, FT-IR and XRD analysis.

2. Materials and methods

2.1. Materials

(±) Praziquantel (PZQ) was kindly supplied by Ascor Chimici (Forlì, Italy). For the preparation of granules, α-lactose monohydrate (90% less than 355 μm) (Polichimica s.r.l., Italy) was used as a diluent, while poloxamer 188 (Lutrol® F68, kindly supplied by BASF, Italy) and polyethylene glycol 4000 (PEG 4000, Polichimica s.r.l., Italy) were used as meltable binders.

As a carrier for the microparticles, Gelucire 50/13 E.P. grade, kindly supplied by Gattefossé-Italia (Milano, Italy), was employed.

2.2. Preparation of the samples

2.2.1. Preparation of the granules

The granules were prepared in a laboratory scale high-shear mixer (Rotolab®, Zanchetta s.r.l. Lucca, Italy), equipped with an electrically heated jacket; the volume of the vessel was 2 l and the batch size was 300 g. The granulation process and the formulations were selected on the basis of preliminary trials; the final formulations contained PZQ 10%, lactose monohydrate 53.3% and melting binder (PEG 4000 or poloxamer 188) 36.7% (w/w). The drug and the diluent were mixed in the high-shear mixer for 10 min, using an impeller speed of 120 rpm; then the mixture was heated up by heating the jacket to 50 °C. Poloxamer 188 or PEG 4000 were heated separately to 65 °C, and then added in the molten form to the mass. During the massing phase the impeller speed was set at 500 rpm for 5 min. At the end of the granulation process, the granules were cooled at room temperature by spreading them out on trays, collected and sieved as described in a following section.

Binary physical mixtures of PZQ and PEG 4000 or poloxamer 188 at different weight ratios were prepared to determine the solubility of the PZQ in the melted binders, while for the dissolution tests a physical mixture was prepared by mixing in a Turbula mixer for 10 min PZQ, lactose monohydrate and poloxamer 188 in the same weight ratios as the granules.

2.2.2. Preparation of the microparticles

The microparticles were produced by the ultrasonic spray congealing process (Fig. 1). Gelucire 50/13 was heated at a temperature of 10 °C above the carrier melting point. The drug (5, 10, 20, and 30%, w/w) was then added to the molten carrier and magnetically stirred to obtain a suspension (A), which was then loaded into the thermo-stated reservoir (B), kept at 70 °C to avoid the solidification of the suspension. Once in contact with the sonotrode (C) (type UIP 250, Hielscher, Berlin, Germany), whose surface vibrated at the ultrasonic frequency (the frequency was 25 kHz and the power output was 250 W), the ultrasonic energy atomised the liquid suspension, converting it into small molten droplets (D), which then solidified during the fall in the chamber (E) at room temperature. Finally, the microparticles were collected and stored in a vacuum desiccator at room temperature.

For comparison purposes, physical mixtures were prepared by mixing in a Turbula mixer for 10 min PZQ and empty Gelucire

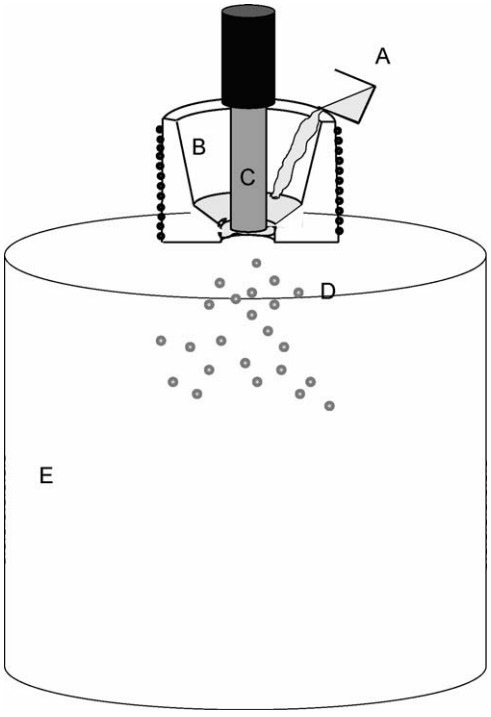

Fig. 1. Schematic representation (not in scale) of the ultrasonic spray congealing process: (A) drug + molten carrier, (B) thermostated reservoir, (C) sonotrode (atomizer), (D) molten droplets, (E) solidifing chamber.

50/13 microparticles (obtained by US spray congealing) in the same weight ratios as the microparticles.

2.3. Characterisation of granules and microparticles

2.3.1. Morphology

The shape of the granules was examined by an optical microscope (Nikon Elipse E 400) connected through a camera (Nikon DN 100) to an image acquisition system.The shape and surface characteristics of the microparticles were observed by SEM. Samples were sputter-coated with Au/Pd using a vacuum evaporator (Edwards, Milano, Italy) and examined using a scanning electron microscope (Model XL30, Philips, Eindhoven, The Netherlands) at 10 kV accelerating voltage using the secondary electron technique.

2.3.2. Size distribution

The size distribution of granules and microparticles was evaluated by sieves analysis, using a vibrating shaker (Octagon Digital, Endecotts, London, UK) and five or seven standard sieves (Scientific Instruments s.r.l., Italy) in the range 75–1400 μm (granules) or 75–500 μm (microparticles). The fractions were then collected, stored in a desiccator at 25 ± 2 °C and used for the further studies.

2.3.3. Determination of drug content

2.3.3.1. Granules. The analysis of the PZQ content in each size fraction was carried out by dissolving 200 mg of granules in 100 ml of water/ethanol 1:1 (w/w) solution; the amount of drug was then spectrophotometrically determined (UV2 Spectrometer, Unicam) at 263.0 nm. Each analysis was carried out in triplicate.

2.3.3.2. Microparticles. The determination of the drug content in the microparticles was determined using a procedure previously reported (Passerini et al., 2002a,b) and conveniently modified according to the PZQ properties (solubility, wavelength). Briefly, a known amount of microparticles (containing theoretically 20 mg of drug) was accurately weighed and then added to 100 ml of water/ethanol 1:1 (w/w) solution. The sample was heated up at 40 °C to soften the carrier and then shaken for 24 h. Finally, the solution was filtered and the drug content was assayed spectrophotometrically (UV–vis spectrophotometer mod. UV2, Unicam, Cambridge, UK) at 263.0 nm. Each sample was analysed in triplicate.

2.3.4. In vitro dissolution studies

In vitro dissolution tests were performed using the USP 28 paddle method (Pharmatest, Germany) rotating at 100 rpm. As a dissolution medium, 900 ml of deionised water containing 0.02% (w/v) Tween 20 (polysorbate 20) were used at a temperature of 37 °C. Samples of pure drug, physical mixtures, granules or microparticles, containing 30 mg of PZQ to assure sink conditions ($C < 0.2$ Cs), were added to the dissolution medium. The aqueous solution was filtered and continuously pumped (12.5 ml/min) to a flow cell in a spectrophotometer (UV2 Spectrometer, Unicam). The amount of drug dissolved was analysed at 263.0 nm. The dissolution tests were performed at least in triplicate. Finally, the analysis of variance (one-way ANOVA test) was carried out on dissolution data, comparing the mean value of each sample to its relative standard deviation.

2.3.5. Determination of PZQ solubility in PEG 4000, poloxamer 188 or Gelucire 50/13 aqueous solutions

Solubility measurements of PZQ were preformed at 25 °C in aqueous solutions containing various concentrations (0–20%, w/v) of PEG 4000 or poloxamer 188 or 1% (w/v) of Gelucire 50/13. An excess of drug was added into each of the polymeric solutions; the samples were magnetically stirred at 25 °C for 72 h, then the suspensions were filtered through 0.20 μm membrane filters and the filtrates were analyzed spectrophotometrically at 263.0 nm. The measurements were performed in triplicate.

2.3.6. Differential scanning calorimetry (DSC)

The DSC analyses were performed using a Perkin-Elmer DSC 6 with nitrogen as purge gas (20 ml/min). The instrument was calibrated for temperature using indium and lead and for enthalpy using indium. The experiments were performed in non-hermetically sealed aluminum pan; the weight of each sample was 8 ± 1 mg and the heating rate was 10 °C/min. The peak and the onset temperature and the enthalpy of fusion reported are the mean of three determinations.

2.3.7. Hot stage microscopy (HSM)

A hot plate (FP 52 Mettler, Greifensee, Switzerland), connected to a temperature controller (FP 5 Mettler) was used. A little amount of each sample (pure drug, atomised Gelucire 50/13, microparticles and corresponding physical mixtures) was placed on a glass slide and heated at 10 °C/min in the temperature range of 30–140 °C. The changes in the samples were monitored via an optical microscope (Reichert Biovar, Wien, Austria) (magnification 10×).

2.3.8. X-ray powder diffraction (XRD) analysis

Single components, microparticles and corresponding physical mixtures were studied by X-ray powder diffraction technique using a X'Pert PRO (PANanalytical, Almelo, NL) diffractometer with Cu Kα radiation (λ = 1.5418 Å). The voltage was 40 kV and the current 20 mA. The scanning angle ranged from 5 to 35° of 2θ, steps were of 0.05° of 2θ and the counting time was of 2 s/step.

2.3.9. Fourier transform infrared spectroscopy (FT-IR)

FT-IR spectra were performed using an IR spectrometer (Jasco FT-IR 200) using the KBr disc method. The samples were mixed with KBr and compressed into a tablet (10 mm in diameter and 3 mm in thickness) using a hydraulic press (Perkin-Elmer, USA) at 300 kg/cm for 1 min. The scanning range was 650–4000 cm^{-1} and the resolution was 1 cm^{-1}.

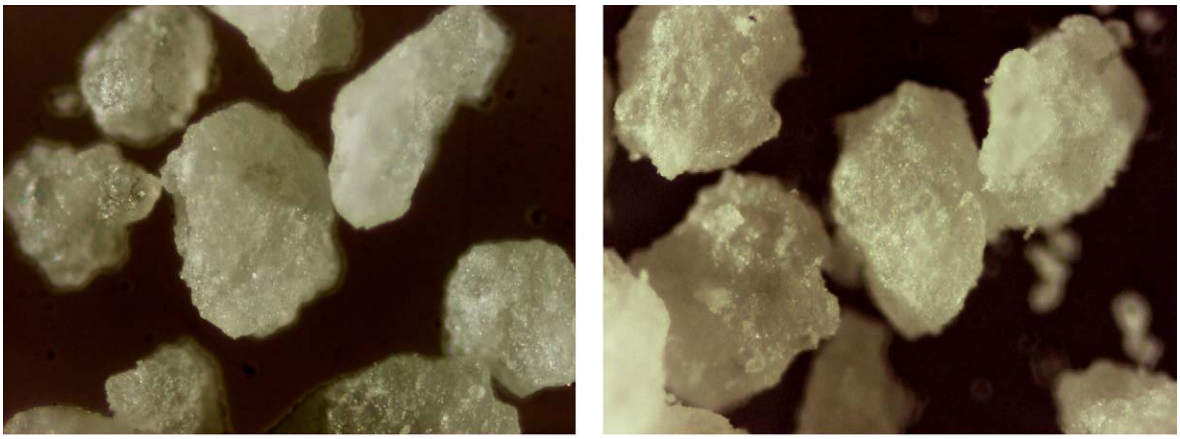

Fig. 2. Optical microscopy images of formulation A (a) and formulation B (b) granules. The magnification is 40×.

3. Results and discussion

3.1. Preparation of granules by melt granulation

Previous papers (Passerini et al., 2002a; Seo et al., 2003; Perissutti et al., 2003) have shown that melt granulation could be a viable means to improve the dissolution rate of poorly water soluble drugs, therefore, the first approach to enhance the dissolution rate of PZQ has been the preparation of melt granules. Two formulations were examined, differing each others only in the hydrophilic low melting material used as a binder: PEG 4000 (formulation A), the most used hydrophilic binder in melt granulation, and poloxamer 188 (formulation B), recently successfully employed to enhance the dissolution rate of ibuprofen (Passerini et al., 2002a). Therefore, both carriers appear very promising for achieving the goal of the study. After preliminary tests using placebo formulations to select the operating conditions, melt granules containing 10% (w/w) of PZQ, 37.6% of PEG 4000 or poloxamer 188 as binder and 53.3% of lactose monohydrate as a filler were successfully prepared.

To determine the solubility of the PZQ in the melted binders, DSC scan of binary physical mixtures of the drug and each of the binders were performed and the phase diagrams were constructed (Seo et al., 2003). The results indicated that PZQ is completely soluble in both the liquid melt of PEG 4000 and poloxamer 188 up to a concentration of 10% (w/w), therefore, the concentration of PZQ in the granules is above its maximum solubility in the melted binders.

3.2. Characterisations of the granules

Images obtained by an optical microscope (Fig. 2a and b) showed that using both formulations quite regularly shaped granules were obtained. Moreover, the results of the granule size analysis (Fig. 3) demonstrated that the parameters of the granulation process were correctly selected: in fact, regardless of the binder, the amount of fine powder (size < 75 μm) was very scarce and big lumps (size > 1400 μm) were absent. For both formulations, the main size fraction was 200–500 μm and more than 65% of the granules had a size in the range 75–750 μm.

Table 1 reports the PZQ content in each granule size fraction for formulation A and B: the drug is quite uniformly distributed in the particles and its content was in most cases slightly higher than the theoretical one (10%, w/w). This fact could be attributed to the loss in melting binder during the binder addition phase. In fact, both PEG 4000 and poloxamer 188 are primarily melted and then added to the powder mixture, therefore, a certain amount of molten binder can remain adhered to the vessel.

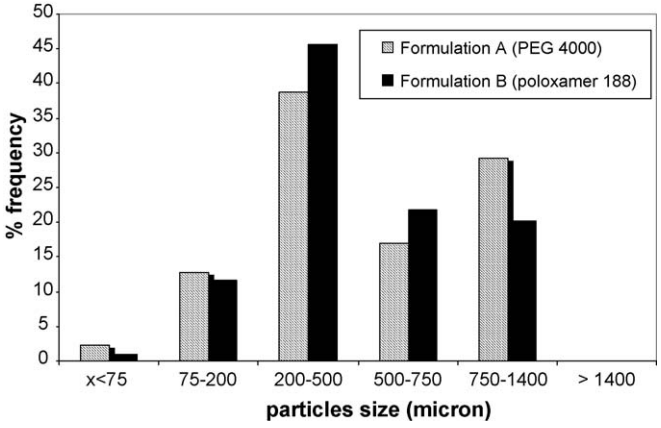

Fig. 3. Size distribution of granules A and B obtained by melt granulation.

Table 1
Evaluation of the drug content in each granule size for formulation A and B

Granule fraction (μm)	Drug content (% ± S.D.)	
	Formulation A (PEG 4000)	Formulation B (poloxamer 188)
75–200	11.1 ± 0.2	11.3 ± 0.3
200–500	11.8 ± 0.3	10.7 ± 0.2
500–750	11.7 ± 0.3	11.1 ± 0.2
750–1400	11.3 ± 0.5	9.8 ± 0.4

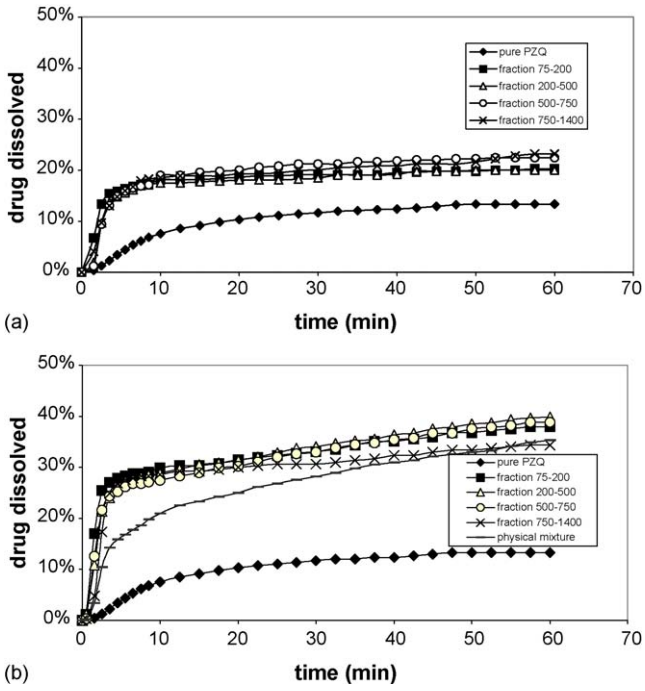

Fig. 4. (a) In vitro dissolution profiles of different size fraction of formulation A and (b) formulation B granules (the S.D. did not exceed 5%).

In order to assess if the goal of improving the dissolution rate of PZQ preparing melt granules was reached, in vitro dissolution profiles of each size fraction of PEG and poloxamer 188 granules were compared to that of pure drug (Fig. 4a and b). The dissolution rate of pure PZQ was very low, being the percentage of pure drug dissolved less than 8 and 15% in 10 min and in 1 h, respectively. The preparation of PEG granules increases these values: the amount of PZQ dissolved from PEG granules is about 18% in 10 min and is more than 20% in 1 h; however, the analysis of variance showed that dissolution profiles of PEG granules were not significantly different ($P = 0.05$) from that of pure PZQ. Moreover, the granule particle size had no effect on the dissolution rate; the dissolution profiles of different fractions were almost superimposable (Fig. 4a). On the contrary, replacing PEG 4000 by poloxamer 188 resulted in a significant ($P = 0.05$) enhancement of PZQ dissolution rate (Fig. 4b): 27–30% and 35–40% of the drug is released from the granules in 10 min and 1 h, respectively. The dissolution profiles of the physical mixture is higher than the pure drug and lower with respect to the granules; however, the analysis of variance showed that the dissolution profiles of the physical mixture was not significantly different from that of the granules, confirming the results obtained by Vilhelmsen et al. (2005) for Lu-X and poloxamer 188 granules prepared by melt agglomeration. As for PEG 4000 granules, the dissolution profiles of different poloxamer 188 granule size were very similar.

Table 3
Actual drug content in the different microparticle size

Microparticle fraction (μm)	Actual PZQ content (% ± S.D.)			
	5%	10%	20%	30%
75–150	6.1 ± 0.3	10.4 ± 0.5	21.7 ± 0.4	31.0 ± 0.2
150–250	6.2 ± 0.4	11.2 ± 0.1	22.4 ± 0.2	31.3 ± 0.2
250–355	7.4 ± 0.2	10.2 ± 0.3	22.2 ± 0.3	34.1 ± 0.3

The solubilities of PZQ in aqueous solutions at various polymer concentrations were then measured to clarify the different dissolution profiles of PEG 4000 granules compared to poloxamer 188 ones. The results (Table 2) show that the solubility of the drug linearly increases as the water concentration of both polymers increased ($R^2 = 0.9928$ for PEG 4000 and $R^2 = 0.9749$ for poloxamer 188); however, poloxamer 188 shows a better effect on PZQ solubility with respect to PEG 4000, increasing the value from 30.4 mg/100 ml to 84.8 mg/100 ml at 25 °C. The reason of the better performance of poloxamer 188 compared to PEG 4000 could be correlated to its chemical structure. In fact both binders are very water-soluble carriers, therefore, their effect on the PZQ dissolution rate is due to an improvement of wettability and solubility of the drug. In addition, poloxamer 188 is a block copolymer, consisting of ethylene oxide (EO) and propylene oxide (PO) blocks organized in the basis $(EO)_x$–$(PO)_y$–$(EO)_x$ structure, where x is 80 and y is 27. This arrangement results in an amphiphilic structure, which has the properties to self-assemble into micelles in aqueous solution (Kabanov et al., 2002); the hydrophobic core (PO block) can act as reservoir for the drug, while the hydrophilic portion (EO) acts as interface between the aqueous medium and the drug. The increased dissolution rate of poloxamer 188 granules with respect to PEG ones could be thus explained with its better performance as solubilizing agent, hypothesising the formation of polymeric micelles able to solubilise the drug.

The results of the first part of this study show that the preparation of melt granules with PEG 4000 did not significantly increase the dissolution rate of PZQ compared to pure drug, while poloxamer 188 significantly enhances the dissolution of PZQ. However, with the aim to further increase the dissolution rate of the drug, in the second part of the work the preparation of lipid microparticles by the ultrasonic spray congealing technique is examined.

Table 2
Solubility of PZQ (mg/100 ml) in aqueous solutions containing various concentrations of PEG 4000 or poloxamer 188 at $T = 25$ °C

Polymer	Concentration (%, w/v)				
	0	5	10	15	20
PEG 4000	30.4 ± 0.9	38.7 ± 1.4	50.6 ± 0.8	60.0 ± 1.2	66.9 ± 0.9
Poloxamer 188	30.4 ± 0.9	49.3 ± 1.6	64.7 ± 1.3	75.2 ± 0.9	84.8 ± 1.0

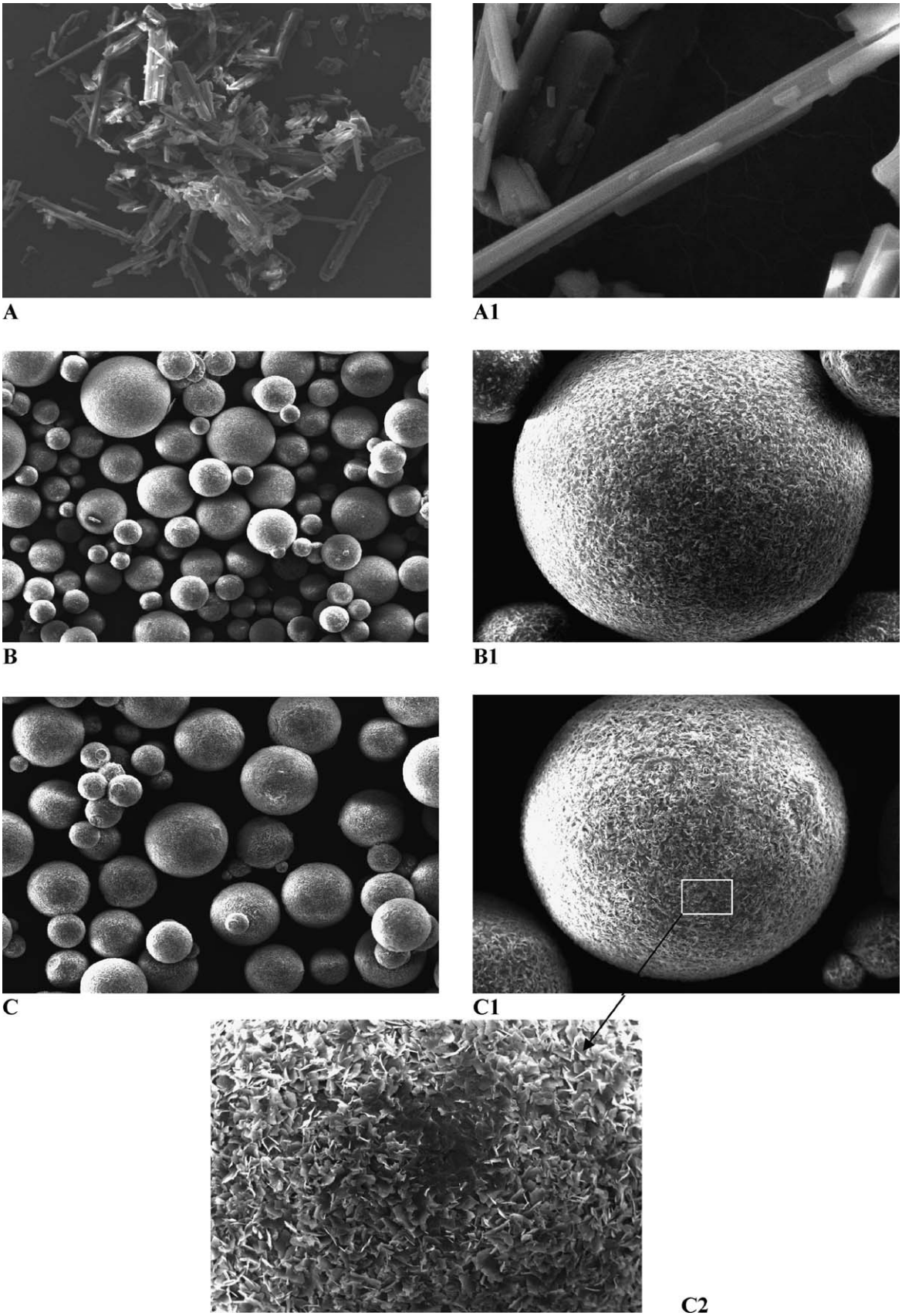

Fig. 5. Scanning electron microscopy of PZQ (A1 and A2), of drug free microparticles (B at 100× and B1 at 400×) and of microparticles containing different amount of PZQ (C–F at 100×, C1–F1 at 400× and C2 and F2 at 1000×).

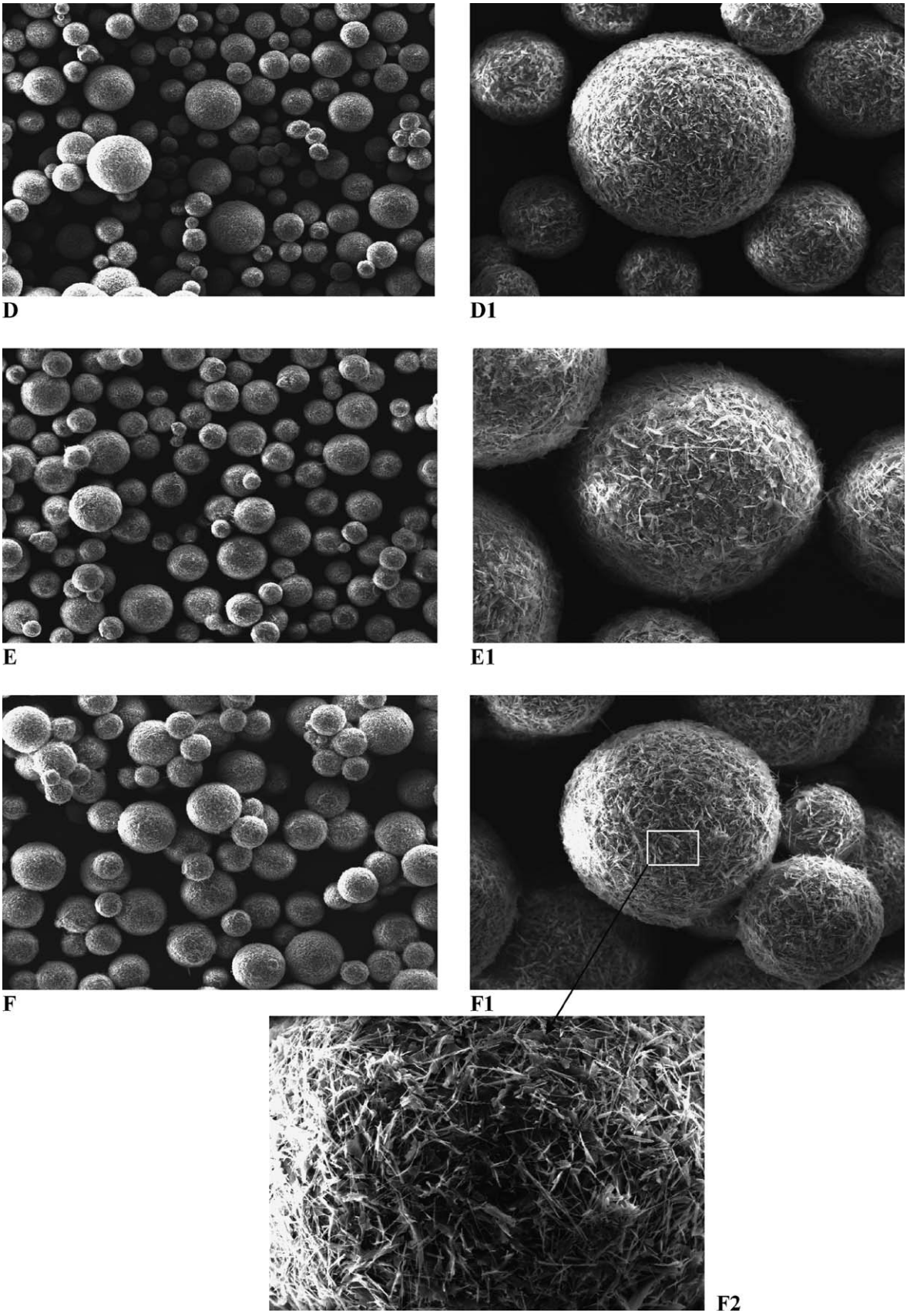

Fig. 5. (*Continued*).

3.3. Preparation of microparticles by the ultrasonic spray congealing technique

A number of low melting point hydrophilic carriers can be suitable for the spray congealing technique, included PEG 4000 and poloxamer 188 that have been utilised in the first part of this study to prepare melt granules. However, we experienced that PEG 4000 and poloxamer 188 to be suitable in the spray congealing process need a cooling system to allow the solidification of the microparticles. On the contrary, Gelucire 50/13 can be an alternative low melting hydrophilic carrier that permits to overcome this inconvenience. In fact, Gelucire 50/13 microparticles quickly solidify at room temperature and the cooling accessory is not necessary. This way, the spray congealing equipment is less expensive, and thus more suitable to be proposed in the developing countries where PZQ is widely used. Gelucire 50/13 was then selected as carrier for the preparation of the microparticles. This part of the research started with the preparation of microparticles with the same theoretical drug loading as the granules (10%, w/w). Subsequently, microparticles with theoretical PZQ content of 5, 20 and 30% (w/w) were performed.

3.4. Characterisation of the microparticles

SEM analysis was then performed to examine the shape and morphology of raw PZQ and of the spray congealed microparticles (Fig. 5). Raw PZQ appeared as acicular-shaped crystals (Fig. 5A and A1). Images at low magnification (100×) of the microparticles showed that non-aggregated microparticles with a regular spherical shape were obtained for drug free microparticles (Fig. 5B) and for all the PZQ loaded systems (Fig. 5C–F). Images at high magnification (400×) revealed that the surface of drug free systems was quite irregular with a "sponge-like" structure (Fig. 5B1). The surface of 5% (Fig. 5C1 and C2) and 10% (D1) PZQ loaded microparticles was analogous to that of drug-free ones and no drug crystals were evident. On the contrary, on the surface structure of 20% (Fig. 5E1) and 30% (Fig. 5F1 and F2) PZQ loaded microparticles, a number of small acicular structures could be recognised, suggesting that at PZQ concentrations >10% the carrier is not enough to completely coat the drug.

The results of particle size analysis (Fig. 6) showed that more than 80% of the microparticles ranged between 75 and 355 μm with a prevalent fraction in the range 150–250 μm. The amount of drug in the microparticles did not seem to influence their dimensions.

As regards to the drug loading of the microparticles, the data in Table 3 evidenced that PZQ was uniformly distributed in the different fractions and that the actual drug content was slightly higher than the theoretical one in all samples; this fact could be explained, as previously described for the melt granules, considering that a certain amount of molten Gelucire 50/13 could stick to the reservoir of the ultrasonic atomiser during the process.

Fig. 7a shows the dissolution profiles of different sizes of 10% PZQ microparticles compared to that of PZQ alone; the microparticles exhibited a significant higher dissolution rate

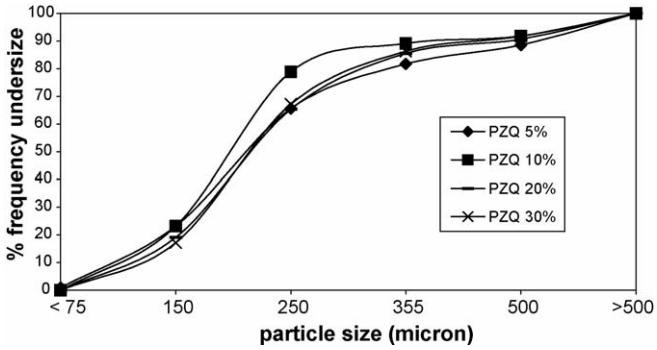

Fig. 6. Particle size distribution of microparticles containing different amount of PZQ.

($P = 0.05$) compared to the pure drug, ranging about 20% the amount of PZQ dissolved from the microparticles after 10 min and raising the 60% in 1 h. As previously observed in the granules, the microparticle size did not influence the dissolution profiles of 10% PZQ-loaded microparticles. Therefore, the prevalent size fraction (150–250 μm) was selected for further dissolution tests. Moreover, the ANOVA test carried out comparing the dissolution data of poloxamer 188 granules and Gelucire 50/13 microparticles having same size and drug content showed that the dissolution rate of the microparticles was significantly higher with respect to the corresponding granules, highlighting that the preparation of microparticles by spray congealing using Gelucire 50/13 is the most suitable strategy to achieve the aim of the study. The PZQ solubility measurement in aqueous solution containing Gelucire 50/13 showed that at 1% (w/v) of Gelucire

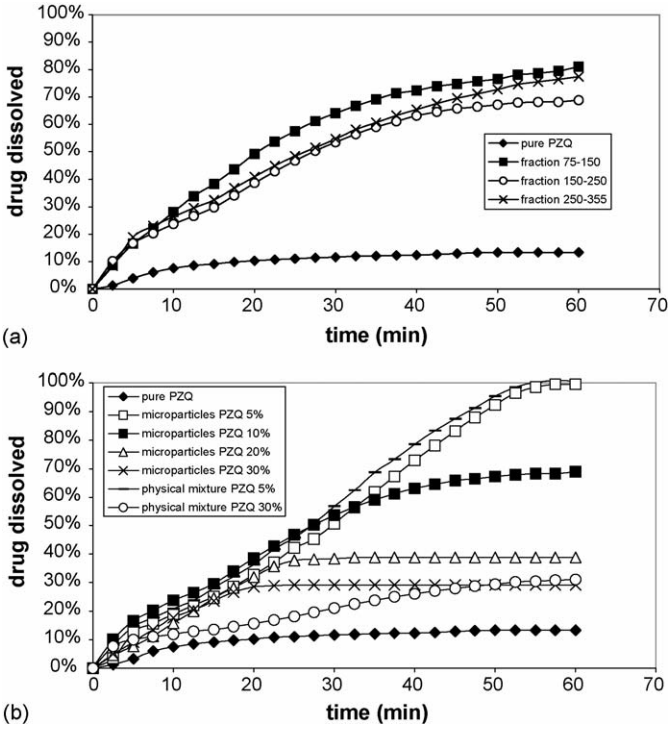

Fig. 7. (a) Effect of the microparticle's size on the dissolution profiles of 10% PZQ microparticles and (b) effect of the amount of PZQ on the dissolution profiles of 150–250 μm microparticles (the S.D. did not exceed 5%).

50/13, the increase in solubility of the drug was about three fold compared to the increase obtained with 20% poloxamer solution, evidencing a significant higher solubilisation effect of Gelucire 50/13 on PZQ compared to poloxamer 188.

Once established that the preparation of PZQ loaded Gelucire 50/13 microparticles significantly ($P = 0.05$) enhanced the dissolution rate of PZQ, the following step involved the preparation of microparticles having different drug content (5, 20 and 30%, w/w) in order to evaluate if the PZQ concentration affected the dissolution profiles of the microparticles; moreover, the dissolution profiles of the physical mixtures at the lower (5%) and at the higher (30%) drug content were tested (Fig. 7b). The results showed that all the microparticles had a higher dissolution rate respect to pure PZQ; however, the increase of the PZQ content considerably decreases the dissolution rate of the drug and this effect was particularly evident in the second part of dissolution profiles. In fact, till 20 min all the microparticles revealed a similar improvement of drug dissolution compared to PZQ, while after 20 min, 5 and 10% PZQ loaded systems showed dissolution significantly enhanced ($P = 0.05$) compared to 20 and 30% PZQ microparticles. The dissolution profiles of the 5 and 30% PZQ physical mixtures were not significantly ($P = 0.05$) different with respect to the microparticles having the same PZQ content. However, it is important to underline that the direct mixing of the drug with the carrier is impossible because commercially available Gelucire 50/13 is in the form of semispherical beads with a size of 6–8 mm, thus it needs a preliminary grinding step using a cooled mill to prevent the melting or an atomisation process (in this work the physical mixtures were prepared by mixing PZQ and empty Gelucire 50/13 microparticles obtained by US spray congealing). The atomisation of the molten mixture of PZQ and the carrier to obtain the microparticles is preferred over the physical mixture because microparticles prevent the segregation, ensuring the manufacturing of a uniform system.

Mechanisms responsible for the improved dissolution rate of a drug in the spray congealed microparticles could be different: besides to the improved wetting and solubilisation of PZQ by the hydrophilic carrier evidenced by the solubility measurements, the reduction of the drug particle size, the transformation of the solid state of the drug from a crystalline form into the amorphous state or from a polymorphic form to a different one could be involved. With the aim of trying to clarify the mechanism involved in the PZQ dissolution rate improvement in the microparticles and to explain the effect of PZQ loading, a physico-chemical characterisation of the systems has been performed using DSC, HSM, XRD and FT-IR analysis.

Fig. 8 reports the DSC scans of pure PZQ, Gelucire 50/13 empty microparticles, their physical mixture at 5 and 30% of PZQ and microparticles containing 5 and 30% of drug. The DSC curve (curve a) of PZQ showed only a single endotherm peak at 143.14 °C ($\Delta H = 98.3$ J/g), in agreement with the melting point and enthalpy of fusion of the racemic form of the drug (El-Arini et al., 1998; Liu et al., 2004; De La Torre et al., 1999). The DSC scan of Gelucire 50/13 empty microparticles (curve b) exhibited a very small pre-transition around 35 °C and a main transition at 49.2 °C, indicating that the carrier is in the stable form I′ (Perissutti et al., 2000). DSC trace of 5% PZQ physical mixture

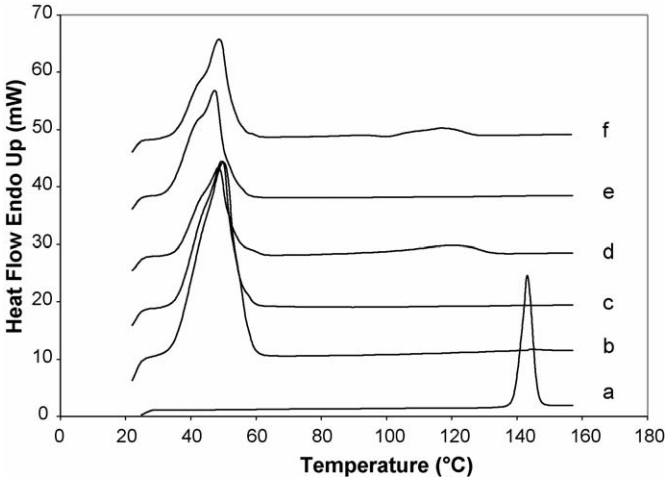

Fig. 8. DSC curves of PZQ (a), Gelucire 50/13 empty microparticles (b), 5% PZQ physical mixture (c), 30% PZQ physical mixture (d), 5% PZQ loaded microparticles (e) and 30% PZQ loaded microparticles (f).

(curve c) showed only an endothermic peak at 49.0 °C, due to the melting of Gelucire 50/13, while the DSC curve of 30% PZQ physical mixture (curve d) revealed, besides the melting peak of the carrier at 47.6 °C, a weak broad endotherm between 105 and 120 °C attributable to the drug. The DSC curves of 5% (curve e) and 30% PZQ microparticles (curve f) were analogous to that of corresponding physical mixture. In both cases the disappearance of the PZQ melting peak in 5% drug loaded samples could be explained hypothesising that, at low drug concentration, PZQ crystals dissolved into the molten Gelucire 50/13 during DSC analysis, as suggested by Damian et al. (2000) for UC-781/PEG 6000 systems, while at 30% PZQ concentration the amount of drug exceeded its solubility in the molten carrier, therefore, the endotherm at around 110 °C may be due to the melting of the un-dissolved crystalline drug. The lowering and broadening of the drug melting point can be due to the presence of the carrier in the molten state, as suggested by Craig (2002).

Subsequently, as previously described for the granules, the maximum solubility of PZQ in melted Gelucire 50/13 was determined using the phase diagram. The results indicated that PZQ is completely soluble in the melted carrier up to a concentration of 15% (w/w), therefore, the different dissolution behaviour of 5 and 10% PZQ microparticles with respect to 20 and 30% could be correlated to the drug solubility in Gelucire 50/13.

To confirm these assumptions, physical changes in the samples on heating were monitored performing hot stage microscopy (HSM) studies. Pure PZQ appeared as non-aggregated needle shaped crystals undergoing melting between 137 and 139 °C, depending on their length. After melting, the recover of crystal structure at room temperature appeared dramatically delayed. Gelucire 50/13 microparticles were spherical particles with an almost transparent core surrounded by dark boundaries. They started to melt from 48 °C, then the fusion of some microparticles together became visible and a transparent clear fuse resulted at 51 °C. With reference to the 5% microparticles, at room temperature the identification of drug particles was not possible but their core appeared darker com-

pared to that of empty microparticles. After the fusion of the polymer, commencing at 44 °C and being completed at 52 °C, PZQ was easily recognised as rare acicular crystals dispersed throughout the molten base. It was necessary to melt up to 77 °C in order to obtain the complete dissolution of the drug into the molten carrier. As the amount of PZQ (e.g. 30%, w/w) increased, the microparticles core became progressively darker. Once the carrier melted (at about 58 °C), dense shadowy aggregates of drug crystals came into view and progressively dissolved into the molten polymer. The massive dissolution underwent in the 95–125 °C temperature range. In the physical mixtures (containing 5 and 30% of drug) we could clearly distinguish between spherical microparticles of Gelucire 50/13 and acicular crystallites of PZQ. After the fusion of the polymer, only the drug particles in close contact with the carrier started a slow dissolution in the molten carrier, whilst the others remained unchanged till their melting point around 140 °C.

Additional information on the solid state structure of the microparticles were obtained by XRD analysis; Fig. 9 shows the XRD patterns of pure PZQ, Gelucire 50/13, 5 and 30% microparticles and corresponding physical mixtures. PZQ raw material is crystalline, as demonstrated by sharp and intense diffraction peaks (a); its XRD pattern corresponded to that of PZQ racemate, as reported by Liu et al. (2004), both in the diffraction angles and in the intensity of the peaks. XRD pattern of Gelucire 50/13 (b) exhibited typical signal of triglycerides between 16° and 25° of 2θ, called "short spacing" and corresponding to the shortest distances between the fatty acids carbon chain (Perissutti et al., 2000). In the 5% PZQ microparticles (c) a dramatic reduction of drug peaks intensity was obtained. To evaluate whether this phenomenon was due to the carrier dilution effect or to an actual reduction of PZQ crystallinity, the microparticles were compared to the corresponding physical mixture (d) that revealed an analogous low-intensity peaks pattern where only the Gelucire 50/13 peaks are evident. In both 30% drug-loaded systems (e and f), crystalline PZQ was still detectable, with a slightly higher intensity in the physical mixture (f), suggesting only a little amorphisation of the drug after spray congealing process. Further, the peak diffraction angles in both microparticles formulation were substantially identical to that of physical mixtures, and only little variations in the intensity of the signals were noticed, indicating a lack of a well-defined interaction between the drug and the carrier in their solid state.

Finally, FT-IR analysis were performed with the aim to detect interactions between the drug and the carrier (Fig. 10);

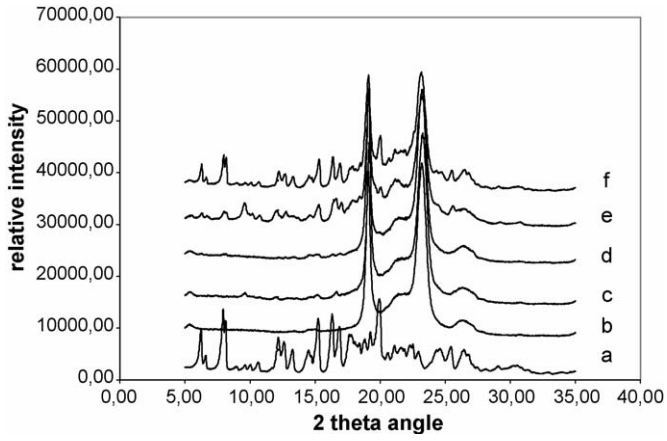

Fig. 9. X-ray diffraction patterns of PZQ (a), Gelucire 50/13 empty microparticles (b), 5% PZQ loaded microparticles (c), 5% PZQ physical mixture (d), 30% PZQ loaded microparticles (e) and 30% PZQ physical mixture (f).

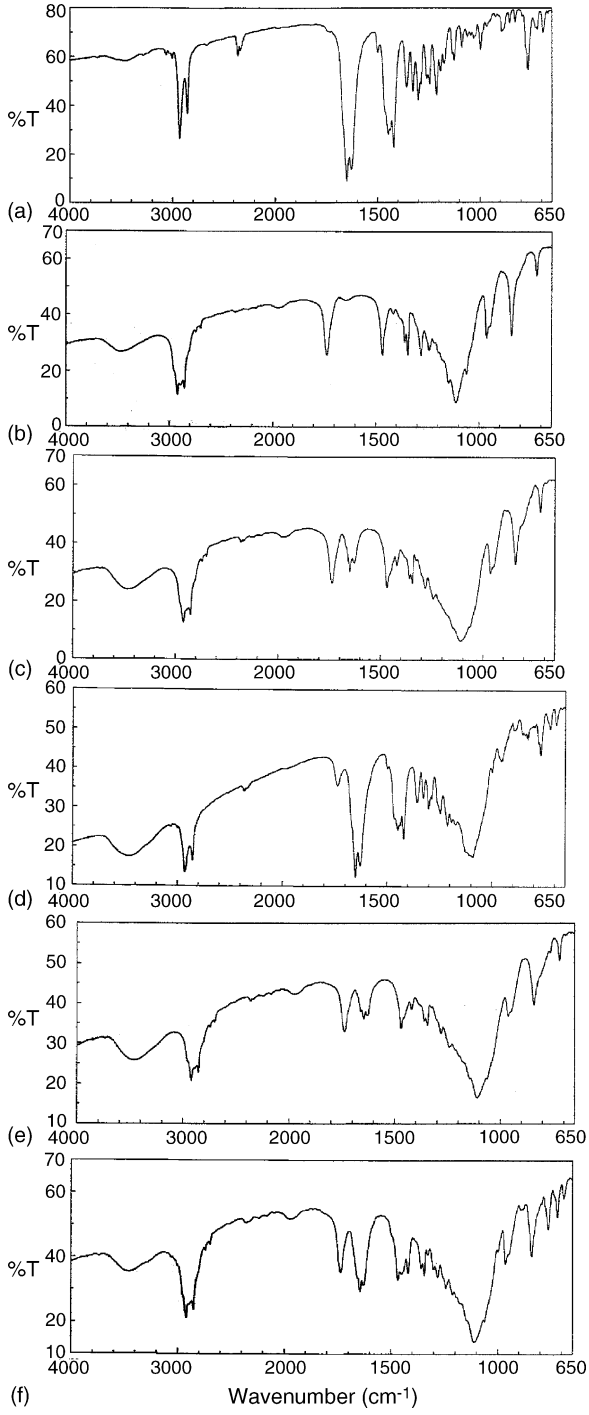

Fig. 10. FT-IR spectra of PZQ (a), Gelucire 50/13 (b), 5% PZQ physical mixture (c), 30% PZQ physical mixture (d), 5% PZQ loaded microparticles (e), and 30% PZQ loaded microparticles (f).

the chemical structure of PZQ and Gelucire 50/13 suggests that possible interaction would be an hydrogen bond between the hydroxyl group of the polymer and the carbonyl functions of PZQ. The FTIR spectrum on PZQ (a) shows characteristic peaks at 2930 and 2852 cm^{-1}, due to the C–H and $C–H_2$ stretching vibration, and at 1649–1627 cm^{-1}, due to the amide stretching vibrations; moreover, the peaks in the region 3300–3700 cm^{-1} and in the fingerprint region below 1500 cm^{-1} confirms the racemic form of the drug (Liu et al., 2004). Gelucire 50/13 spectrum (b) presents a large band in the region 3650–3100 cm^{-1}, due to the free O–H stretching vibration of the COOH groups, and a peak at 1737 cm^{-1} for the C=O vibration. The FTIR spectra of 5% (c) and 30% (d) PZQ/Gelucire 50/13 physical mixtures and of the corresponding microparticles (e, f) are almost superimposable; no shifts of all the characteristic peaks of the drug and of the carrier are evident, suggesting the lack of significant interactions. Moreover, the permanence of the drug in its original form was confirmed by this analysis.

The results of the microparticle's characterisation evidenced the absence of both modifications of the solid state of PZQ and of significant interactions between the drug and the carrier; the improvement of wettability of the drug and the solubilisation of the PZQ by Gelucire 50/13 at the diffusion layer are thus the main mechanisms proposed to be responsible for the PZQ enhanced dissolution rate.

4. Conclusions

The results of this study showed that both poloxamer 188 granules and especially Gelucire 50/13 microparticles evidenced a significantly enhancement of PZQ in vitro dissolution rate. Moreover, the physicochemical characterisation of the microparticulate systems, performed using DSC, HSM, XRD and FT-IR analysis, suggested the absence of modifications of the solid state of the drug and of significant interactions between PZQ and the carrier. Melt granulation and ultrasonic spray congealing could thus be proposed as solvent free, rapid and low expensive manufacturing methods to increase the dissolution rate of this low water soluble anthelminthic drug, very important for developing countries.

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References

Amidon, G.L., Lennernas, H., Shah, V.P., Crison, J.R., 1995. A theoretical basis for a biopharmaceutic drug classification: the correlation of in vitro drug product dissolution and in vivo bioavailability. Pharm. Res. 12, 413–420.

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.

Craig, D.Q.M., 2002. The mechanisms of drug release from solid dispersion in water-soluble polymers. Int. J. Pharm. 231, 131–144.

Damian, F., Blaton, N., Naesens, L., Balzarini, J., Kinget, R., Augustijns, P., Van Der Mooter, G., 2000. Physicochemical characterisation of solid dispersions of the antiviral agent UC-781 with polyethylene glycol 6000 and Gelucire 44/14. Eur. J. Pharm. Sci. 10, 311–322.

De La Torre, P., Torrado, S., Torrado, S., 1999. Preparation and characterisation of praziquantel solid dispersions. Chem. Pharm. Bull. 47, 1629–1633.

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

El-Arini, S.K., Leuenberger, H., 1996. Dissolution properties of praziquantel-β-cyclodextrin systems. Pharm. Dev. Technol. 1, 307–315.

El-Arini, S.K., Leuenberger, H., 1998. Dissolution properties of praziquantel-PVP systems. Pharm. Acta Helv. 73, 89–94.

El-Subbagh, H.I., Al-Badr, A.A., 1998. Praziquantel. In: Florey (Ed.), Analytical Profiles of Drug Substances and Excipients, vol. 25. Academic Press Inc., New York, pp. 463–500.

Kabanov, A.V., Batrakova, E.V., Alakhov, V.Y., 2002. Pluronic block copolymers as novel polymer therapeutics for drug and gene delivery. J. Contr. Rel. 82, 189–212.

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 characterisation and enantioseparation of the chiral compound praziquantel. J. Pharm. Sci. 93, 3039–3046.

Passerini, N., Albertini, B., Gonzalez-Rodriguez, M.L., Cavallari, C., Rodriguez, L., 2002a. Preparation and characterisation of ibuprofen-poloxamer 188 granules obtained by melt granulation. Eur. J. Pharm. Sci. 15, 71–78.

Passerini, N., Perissutti, B., Moneghini, M., Voinovich, D., Albertini, B., Cavallari, C., Rodriguez, L., 2002b. Characterisation of carbamazepine-Gelucire 50/13 microparticles prepared by a spray-congealing process using ultrasounds. L. Pharm. Sci. 91, 699–707.

Perissutti, B., Rubessa, F., Moneghini, M., Voinovich, D., 2003. Formulation design of carbamazepine fast-release tablets prepared by melt granulation technique. Int. J. Pharm. 256, 53–63.

Perissutti, B., Rubessa, F., Princivalle, F., 2000. Solid dispersions of carbamazepine with Gelucire 44/14 and 50/13. S.T.P. Pharma. Sci. 10, 479–484.

Seo, A., Holm, P., Kristensen, H.G., Schaefer, T., 2003. The preparation of agglomerates containing solid dispersion of diazepam by melt agglomeration in a high shear mixer. Int. J. Pharm. 259, 161–171.

Vilhelmsen, T., Eliasen, H., Schaefer, T., 2005. Effect of a melt agglomeration process on agglomerates containing solid dispersion. Int. J. Pharm. 303, 132–142.