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Improvement of the dissolution rate of artemisinin by means of supercritical fluid technology and solid dispersions

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Abstract

The purpose of this study was to enhance the dissolution rate of artemisinin in order to improve the intestinal absorption characteristics.

The effect of: (1) micronisation and (2) formation of solid dispersions with PVPK25 was assessed in an in vitro dissolution system [dissolution medium: water (90%), ethanol (10%) and sodium lauryl sulphate (0.1%)]. Coulter counter analysis was used to measure particle size. X-ray diffraction and DSC were used to analyse the physical state of the powders.

Micronisation by means of a jet mill and supercritical fluid technology resulted in a significant decrease in particle size as compared to untreated artemisinin. All powders appeared to be crystalline. The dissolution rate of the micronised forms improved in comparison to the untreated form, but showed no difference in comparison to mechanically ground artemisinin. Solid dispersions of artemisinin with PVPK25 as a carrier were prepared by the solvent method. Both X-ray diffraction and DSC showed that the amorphous state was reached when the amount of PVPK25 was increased to 67%. The dissolution rate of solid dispersions with at least 67% of PVPK25 was significantly improved in comparison to untreated and mechanically ground artemisinin.

Modulation of the dissolution rate of artemisinin was obtained by both particle size reduction and formation of solid dispersions. The effect of particle size reduction on the dissolution rate was limited. Solid dispersions could be prepared by using a relatively small amount of PVPK25. The formation of solid dispersions with PVPK25 as a carrier appears to be a promising method to improve the intestinal absorption characteristics of artemisinin.
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Keywords: Artemisinin; Micronisation; Supercritical fluid technology; Solid dispersions; PVPK25; Dissolution

1. Introduction

Artemisinin, a sesquiterpene lactone endoperoxide (Fig. 1), is the active anti-malarial moiety isolated in 1972 from the Chinese medicinal herb qinghao, Artemisia annua L (Luo and Shen, 1987). Artemisinin is a potent blood schizontocide with a minimum inhibitory concentration of 10^{-7} M (De Vries and Dien, 1996). The peroxide group is essential for its anti-malarial activity (Li and Wu, 1998). Artemisinin was the first compound of a completely deviant class of drugs, which may be of significant importance in the combat of malaria. Artemisinin can be used

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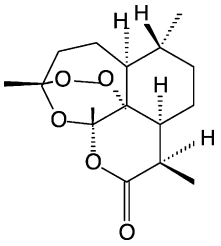


Fig. 1. Chemical structure of artemisinin.

for the treatment of multidrug resistant *Plasmodium vivax* and *P. falciparum* infections, including the cerebral form. The parasite and fever clearance rates with artemisinin are higher than with any other anti-malarial drug (Titulaer et al., 1991). Moreover, until now no resistance has been described in patients (Meshnick, 1998).

Pharmacokinetic data on artemisinin are limited due to the difficulties in developing selective, sensitive and specific analytical methods to determine the concentrations in biological fluids. Artemisinin does not possess a fluorescent or ultraviolet light absorbing chromophore. Despite these inherent difficulties, several methods have been developed for the measurement of artemisinin (and derivatives) in biological fluids. These include colorimetric methods, thin layer chromatography, gas chromatography–mass spectrometry, high performance liquid chromatography with electrochemical detection, and pre- or post-column derivatisation with ultraviolet detection (Dhingra et al., 2000). An additional problem in pharmacokinetic studies with artemisinin is the fact that the absolute bioavailability of artemisinin is not known because of the lack of an intravenous formulation for human use. Nevertheless, a few pharmacokinetic studies indicate that artemisinin is incompletely absorbed after oral intake. The relative bioavailability in comparison with an intramuscular injection of a suspension in oil is estimated to be about 32% (Navaratnam et al., 2000). A study carried out by Ashton et al. (1998) showed high interindividual variability in plasma concentrations after both oral and rectal administration. The low bioavailability after oral intake can be due to (1) a low transepithelial transport across the intestinal mucosa or (2) to the poor dissolution characteristics of artemisinin in the intestinal fluids.

Previous studies using the Caco-2 model (an in vitro cell culture mimicking the intestinal mucosa) showed that transepithelial transport is probably not a limiting factor for the absorption of artemisinin after oral intake (Augustijns et al., 1996). Since artemisinin may have a dissolution-limited absorption, improvement of the dissolution characteristics can be useful to enhance intestinal absorption. According to the Noyes–Withney equation (Eq. (1)), this can be established by particle size reduction and/or by increasing saturation solubility:

$$\frac{\mathrm{d}m}{\mathrm{d}t} = \frac{AD}{h}(C_\mathrm{s} - C_t) \tag{1}$$

where $\mathrm{d}m/\mathrm{d}t$ is the dissolution rate, A the specific surface area of the drug particle, D the diffusion coefficient, h the diffusion layer thickness, C_s the saturation solubility and C_t is the concentration at time t.

In this study, particle size reduction was obtained by: (1) mechanically grinding in a mortar, (2) jet milling and (3) by means of supercritical fluid (SCF) technology. Supercritical fluids can be valuable for particle size reduction in that they combine the dissolving power of a liquid with high compressibility. Rapid expansion of SCF solutions (RESS) containing dissolved substances yields relatively small particles and has been used as an alternative to traditional milling. Carbon dioxide (CO_2) was chosen among the supercritical fluids because it is non-toxic, non-flammable, and inexpensive and it has a relatively high dissolving power for artemisinin. Moreover, its critical parameters are relatively low (T_c = 31.1 °C, P_c = 73.8 bar) which makes CO_2 attractive for heat-sensitive pharmaceuticals (Ghaderi et al., 1999).

Wong and Yuen (2001) have recently shown that the solubility of artemisinin can be increased through inclusion complexation with β- and γ-cyclodextrins. Another way for saturation solubility enhancement could be the formation of solid dispersions. Solubility increases due to the micro-environment created by the carrier. Moreover, the presence of a carrier prevents aggregation of individual drug particles exhibiting high solid–liquid surface tension. The use of a hydrophilic carrier will lead to an improved wettability of the drug substances. A major drawback in the use of solid dispersions is the physical and chemical

instability. Indeed, the amorphous state often obtained by the formation of solid dispersions is a metastable state, so that recrystallisation is inevitable. A key parameter is the glass transition temperature. It indicates the borderline between high and low molecular mobility (Van den Mooter et al., 2001). By using PVPK25 as a carrier, a polymer with a high glass transition temperature (T_g) (177 °C), the T_g of the mixture will rise and so the molecular mobility will decrease, reducing the tendency to crystallise (Taylor and Zografi, 1997).

In this paper, we report the improvement of the dissolution rate of artemisinin by means of particle size reduction and by formation of solid dispersions. The different powder forms were characterised by coulter counter for particle size, by powder X-ray diffraction and by differential scanning calorimetry to examine the physical state. The pharmaceutical performance of the different powder forms was evaluated by means of an in vitro dissolution method.

2. Materials and methods

2.1. Materials

Artemisinin was obtained from Mediplantex (Hanoi, Vietnam) with a purity of 99.87%. All other chemicals were of analytical or reagent grade [sodium lauryl sulphate (Federa, Brussels, Belgium), sodium hydroxide and sodium chloride (BDH Laboratory Supplies, Poole, England), polyvinylpyrrolidone (PVPK25; Kollidon® K25) (BASF, Ludwigshafen, Germany) and dichloromethane (Merck, Darmstadt, Germany)].

2.2. Artemisinin assay

Concentrations of artemisinin were measured according to a slight modification of the method described by Zhao and Zeng (1985), in which artemisinin is transformed into a UV absorbing degradation product, with an absorbance maximum at 290 nm, by heating the solution with sodium hydroxide.

After appropriate dilution with 0.2% sodium hydroxide and heating for 30 min at 50 °C, the concentration of artemisinin was determined at 290 nm with a UV spectrophotometer (Pye Unicam SP6-550, Philips, Eindhoven, The Netherlands).

2.3. Dissolution experiments

Dissolution profiles of the different artemisinin powder forms were obtained according to the USP XXIII paddle method, carried out in a Hanson SRII Dissolution Test Station: 75 rpm, 600 ml of dissolution medium, $T = 37 \pm 0.1$ °C, sink conditions ($C < 0.2C_s$). Samples were taken until approximately 15% of saturation concentration. The dissolution medium consisted of water (90% v/v), ethanol (10% v/v) and sodium lauryl sulphate (0.1% w/v). Ethanol was added to the dissolution medium to improve solubility so that sink conditions could be maintained in a relatively small amount of dissolution medium. For every dissolution experiment, an amount of powder equivalent to 20 mg artemisinin was used.

At predetermined time intervals, samples of 13 ml were taken and replaced with the same volume of fresh solvent. The samples were filtered over a cellulose acetate filter of 0.2 μm (Sartorius AG, Goettingen, Germany). 10 ml of the filtered solution was assayed after carrying out the alkali reaction. Sodium lauryl sulphate and PVPK25 did not interfere with the UV analysis at 290 nm.

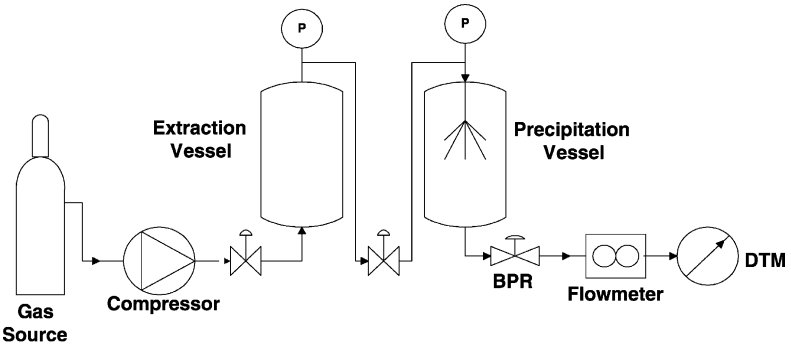
2.4. Particle size reduction

2.4.1. Jet mill

Micronisation of artemisinin was obtained using a Gem T jet mill (Trost Mills, Basel, Switzerland). This mill operates on the principle of impact and attrition (collisions between particles) induced by a high velocity air stream. Particles leave the mill as soon as their size is reduced to the size of a classifying exit, thus resulting in a narrow particle size distribution.

2.4.2. SCF technology

The supercritical fluid nucleation process exploits the high supersaturation ratios that are achieved when a solution of a compound dissolved in a supercritical fluid is rapidly reduced in pressure. (The process has been given the acronym RESS, for rapid expansion of supercritical solutions.)



BPR – Back Pressure Regulator
DTM – Dry Test Meter (measures total volume of gas)

Fig. 2. RESS equipment schematic.

Fig. 2 is a schematic diagram of a laboratory apparatus configured in RESS mode. A gas (CO_2 in this study) is supplied from a manifold, raised in pressure via a compressor and passed through a vessel that has been filled with the compound (artemisinin).

The artemisinin is dissolved by the CO_2 (at conditions of 300 bar, 80 °C) and the gaseous solution is passed through a valve downstream of the extraction vessel and decreased virtually instantaneously to a lower pressure, e.g. 30 bar, resulting in the formation of particles of about 0.5–10 μm in diameter. (The size of the particles varies with different pressure and temperature conditions.) Particles are collected on a filter positioned at the outlet of the precipitation vessel, and the now lower pressure gas passes through a back pressure regulator and in series to a flow meter and dry test meter for volume integration. At the end of a test, the system is depressurized and the particles harvested and examined by optical microscopy.

2.5. Preparation of solid dispersions and physical mixtures

Co-evaporated systems containing from 17 to 50% w/w of artemisinin (initial concentration) were prepared by dissolving the drug and PVPK25 in a minimum amount of dichloromethane. The solvent was then removed rapidly by evaporation under reduced pressure at 45 °C. The dispersions were stored in an oven at 40 °C for 48 h and afterwards ground in a mortar.

Physical mixtures were prepared by mixing artemisinin and PVPK25 thoroughly during 3 min in a mortar until a homogeneous mixture was obtained.

2.6. Particle size measurement

A coulter counter (Multisizer II, Coulter Electronic Ltd., Luton, Great Britain) fitted with an orifice of 100 μm (SCF-treated artemisinin, solid dispersions) or 400 μm (untreated artemisinin, mechanically ground artemisinin, physical mixture of artemisinin and PVPK25) was used for the determination of the particle size distribution. A volume of 2 ml was sampled. The suspension was made up in 0.9% NaCl saturated with artemisinine. The procedure was repeated three times for each sample.

2.7. X-ray powder diffraction

The crystalline state of artemisinin in the different samples was evaluated with X-ray powder diffraction. Diffraction patterns were obtained on a Philips PW 1050 diffractometer (Bragg–Brentano principle, Eindhoven, The Netherlands), with a radius of 173 mm. The Cu Kα radiation (Kα = 1.54184 A) was Ni filtered. A system of diverging, receiving and anti-scattering slits of 1°, 0.2 mm and 1°, respectively, was used. The pattern was collected with 45 kV of tube voltage and 20 mA of tube current in the angular range 4–65° in step-scan mode (step width, 0.04°; counting time, 1 s per step).

2.8. Differential scanning calorimetry

Differential scanning calorimetry measurements were carried out using a Perkin-Elmer DSC-7 differential scanning calorimeter (Perkin-Elmer, Norwalk, CT, USA) equipped with a liquid nitrogen subambient accessory.

Pure water, cyclohexane and indium were used to calibrate the DSC temperature scale; the enthalpic response was calibrated with indium. Data were treated mathematically using the Pyris software version 3.03 (Perkin-Elmer).

The anti-plasticising effect of PVPK25 was evaluated scanning the solid dispersions from 293 to 453 K at 20 K/min.

3. Results and discussion

Particle size reduction of artemisinin was achieved by mechanically grinding, by jet milling and by using SCF technology. Best results for micronisation were achieved by using the jet mill. The median diameter decreased from 75.8±4.8 μm for the untreated powder to 4.1 ± 0.2 μm for the powder micronised by the jet mill. Powder obtained by SCF technology was characterised by a median particle size of 10.6 ± 0.5 μm. Median particle diameter after mechanically grinding amounted to 27.4 ± 4.6 μm. Particle size distributions of the micronised powder forms are shown in Fig. 3a.

The particle size of artemisinin in solid dispersions with 50, 67, 75 and 83% of PVPK25 was

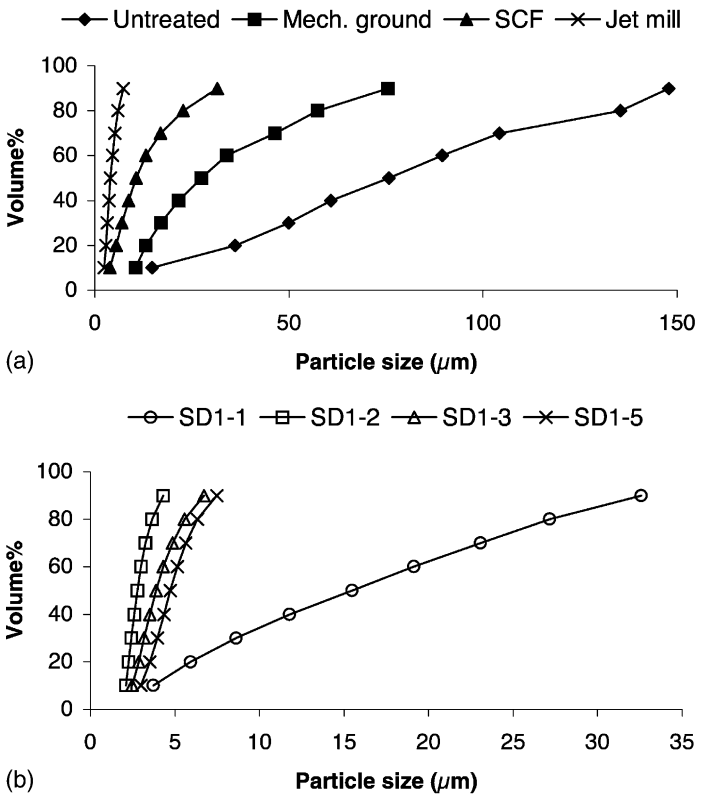


Fig. 3. (a) Particle size distribution of untreated artemisinin, mechanically ground artemisinin, artemisinin micronised by means of a jet mill and artemisinin treated by SCF technology. Cumulative volume percentage is expressed in function of particle diameter. (b) Particle size distribution of artemisinin in solid dispersions with different percentages of PVPK25, i.e. 50% (SD1-1), 67% (SD1-2), 75% (SD1-3) and 83% (SD1-5) of PVPK25. Cumulative volume percentage is expressed in function of particle diameter.

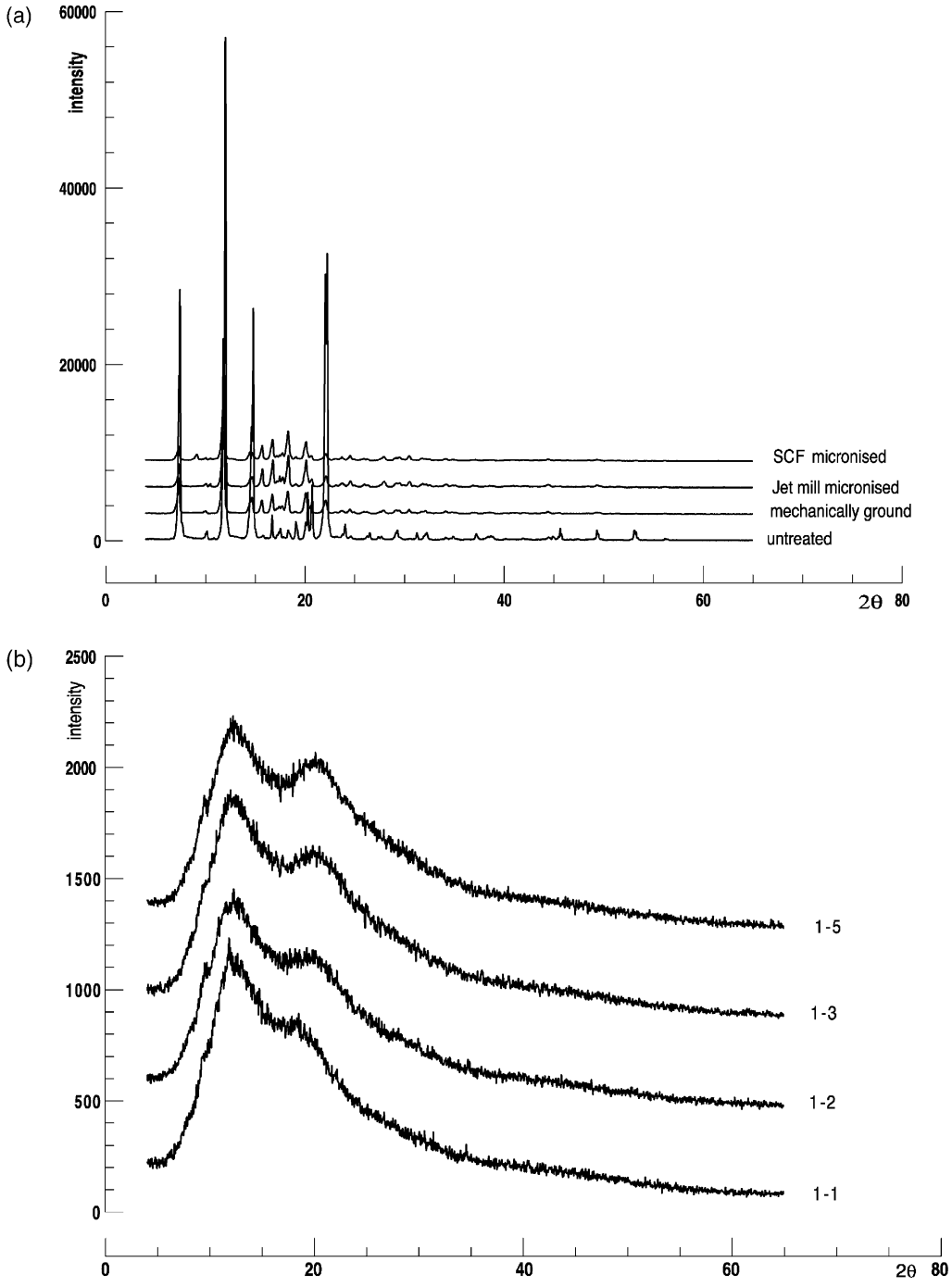


Fig. 4. (a) X-ray diffraction data of untreated artemisinin, mechanically ground artemisinin, artemisinin micronised by means of a jet mill and artemisinin treated with the SCF technology. The intensity of the emitted radiation is plotted against the diffraction angle (2θ). (b) X-ray diffraction data of artemisinin in solid dispersions with different percentages of PVPK25, i.e. 50% (SD1-1), 67% (SD1-2), 75% (SD1-3) and 83% (SD1-5) of PVPK25. The intensity of the emitted radiation is plotted against the diffraction angle (2θ).

also measured. A decrease in particle size and spread could be observed when the amount of PVPK25 was increased from 50 to 67%. Median diameters are 15.5 ± 1.0 and $2.8 \pm 0.1\,\mu m$, respectively. Further increase of the amount of PVPK25 did not result in a further decrease of particle size (Fig. 3b).

In addition to the particle size, the physical state of a powder is an important determinant of dissolution rate. Therefore, the physical state was investigated by both X-ray diffraction and DSC studies. X-ray diffraction studies showed that mechanically ground artemisinin, artemisinin micronised by means of a jet mill and artemisinin micronised by SCF were still crystalline (Fig. 4a). On the other hand, the solid dispersions with 50, 67, 75 and 83% of PVPK25 did not show any crystallinity (Fig. 4b). This result implies that artemisinin is present in an amorphous form in all the solid dispersions made. X-ray diffraction data of artemisinin in a physical mixture with PVPK25 still shows crystallinity, which means that the mere presence of PVPK25 in the physical mixture has no influence on the physical state of artemisinin (data not shown).

DSC analysis, an alternative method to investigate the physical state of powders, was carried out to confirm the results obtained by X-ray diffraction. Melting peaks which can be observed in the DSC plots of mechanically ground artemisinin, artemisinin micronised by means of a jet mill and artemisinin micronised by SCF technology, confirmed the crystalline state of the powder (data not shown). The DSC plot of the solid dispersion with 50% of PVPK25 (Fig. 5a) shows a melting peak, which indicates a crystalline fraction in this mixture. This crystalline fraction could not be detected by means of X-ray diffraction. This may be the result of a higher sensitivity of the DSC apparatus in comparison with the X-ray diffractometer. In the solid dispersions with a higher percentage of PVP, no melting peak could be observed anymore (Fig. 5b), which illustrates the amorphous state of artemisinin in these mixtures. The glass transition temperature, a key parameter in amorphous solid dispersions, increased upon increasing the fraction of PVPK25 in the mixture: 86, 107 and 117 °C for solid dispersions with 67, 75 and 83% of PVPK25, respectively. This increase can be explained by the high glass transition temperature of PVP (177 °C).

The ultimate pharmaceutical performance of the different powders was investigated by determining their dissolution profile. After 60 min, the amount of artemisinin dissolved when using powder micronised by means of a jet mill was about twice as high as the dissolved amount of the untreated form. A similar

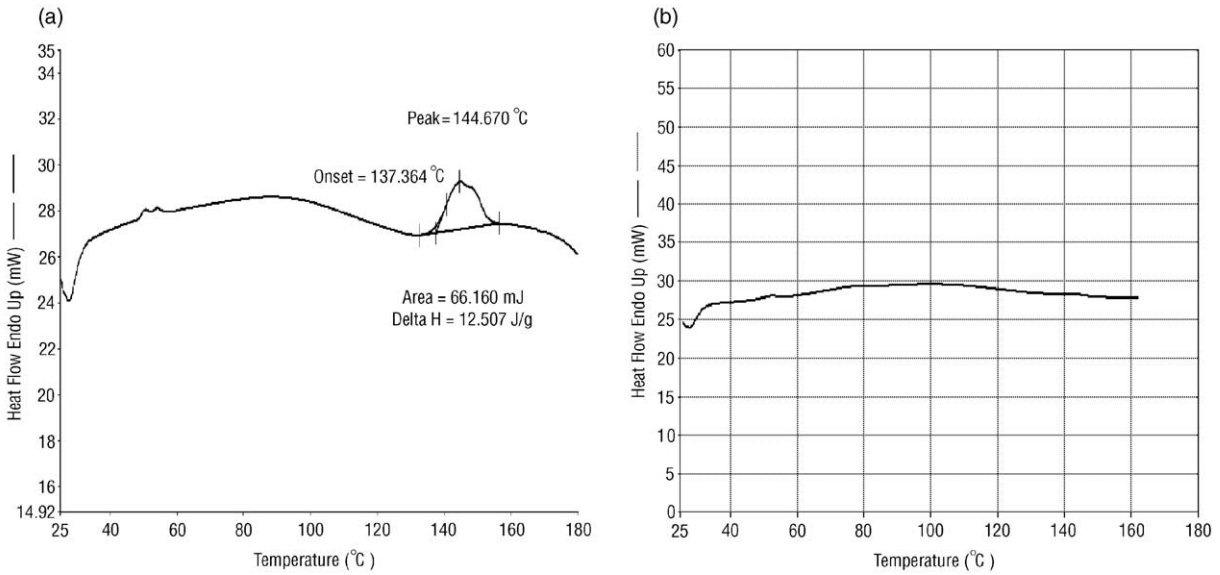


Fig. 5. DSC plots of solid dispersions of artemisinin with 50% of PVPK25 (a) and with 67% of PVPK25 (b).

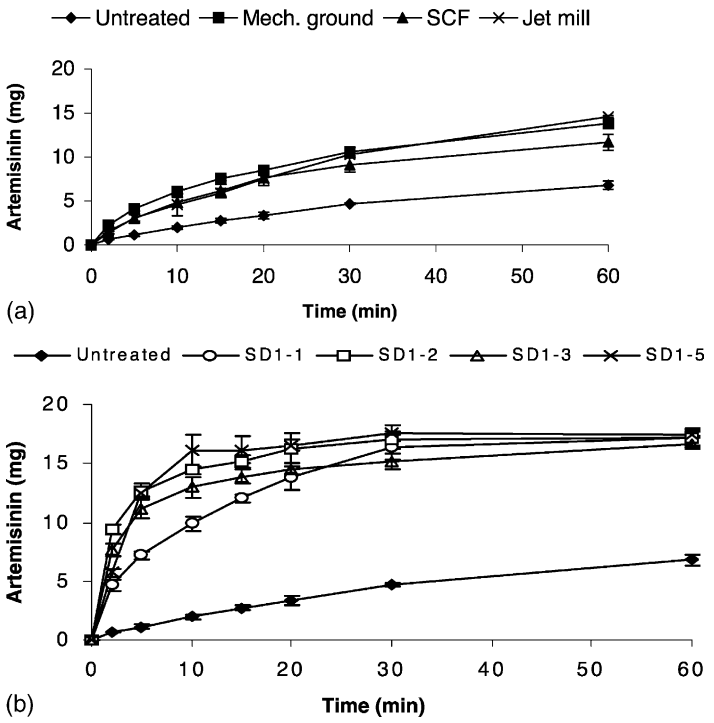


Fig. 6. (a) Dissolution profile of untreated artemisinin, mechanically ground artemisinin, artemisinin micronised by means of a jet mill and artemisinin treated by SCF technology. The amount of powder used is equivalent to 20 mg of artemisinin. Results are expressed as the amount of artemisinin dissolved in the dissolution medium ±S.D. ($n = 4$) in function of time. (b) Dissolution profile of untreated artemisinin and artemisinin in solid dispersions with different percentages of PVPK25, i.e. 50% (SD1-1), 67% (SD1-2), 75% (SD1-3) and 83% (SD1-5) of PVPK25. The amount of powder used is equivalent to 20 mg of artemisinin, based on the initial concentration. Results are expressed as the amount of artemisinin dissolved in the dissolution medium ±S.D. ($n = 4$) in function of time.

dissolution profile was obtained for the mechanically ground powder; in spite of the large difference in particle size there is no difference in dissolution rate between artemisinin micronised by a jet mill and mechanically ground artemisinin. Artemisinin obtained by SCF technology also resulted in a limited dissolution enhancement, illustrating that size reduction may not be a major determinant of the dissolution characteristics of artemisinin. These results are graphically presented in Fig. 6a.

The dissolution rate of artemisinin in the solid dispersions is highly increased in comparison with untreated and mechanically ground artemisinin (Fig. 6b). It is important to point out that saturation solubility of the solid dispersion made in this study is not significantly increased (data not shown).

4. Conclusion

The effect of (1) micronisation and (2) formation of solid dispersions with PVPK25 was evaluated in an in vitro dissolution system.

Micronisation by means of supercritical fluid technology resulted in a significant decrease in particle size as compared to untreated artemisinin. All micronised powders appeared to be crystalline. The dissolution rate of the micronised forms was improved in comparison to the untreated form, but showed no difference in comparison to mechanically ground artemisinin. Solid dispersions of artemisinin with PVPK25 as a carrier were prepared by the solvent method. Both powder X-ray diffraction and DSC showed that the amorphous state was reached when the amount of

PVPK25 was increased to 67%. The dissolution rate of solid dispersions containing at least 67% of PVPK25 was significantly improved in comparison to untreated and mechanically ground artemisinin.

Improvement of the dissolution rate of artemisinin was obtained by both particle size reduction and formation of solid dispersions. The effect of particle size reduction on the dissolution rate was limited. Solid dispersions could be prepared by using a relatively small amount of PVPK25. The formation of solid dispersions with PVPK25 as a carrier appears to be a promising method to improve the intestinal absorption characteristics of artemisinin.

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