ELSEVIER

International Journal of Pharmaceutics 111 (1994) 159–170

international journal of pharmaceutics

Increased metastable solubility of milled griseofulvin, depending on the formation of a disordered surface structure

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Received 31 January 1994; modified version received 25 April 1994; accepted 2 May 1994

Abstract

The effect of mechanical processing on the solubility of griseofulvin was studied. Milling of griseofulvin produced an increased metastable solubility. The milling operation did not change the material's bulk thermal properties identifiable by DSC, nor did it alter the surface area or the individual primary particle size, to fully explain the apparent increase in solubility. However, the thermodynamic evaluation of milled griseofulvin showed an increase in free energy and a reduction in the heat of solution. This suggests that the increase in solubility was due to disordering of the solid structure, estimated to be about 5.8% w/w of the total mass and limited only to an external assumed surface layer 40–50 nm in thickness. The initial solubility levels obtained were found to be directly related to the solid solute particle surface structure. The slow decrease from the initially high metastable solubility level to the stable, low equilibrium solubility seemed to be controlled by a surface reaction mechanism, probably a solid-state rearrangement process and not by a solute molecular diffusion in the bulk solution.

Key words: Disordered surface layer thickness; Mechanical activation; Molar heat of solution; Particle surface structure; Surface molecule rearrangement; Solubility

1. Introduction

Technological and processing operations can cause mechanical activation (Hüttenrauch, 1978, 1983, 1988; Hüttenrauch et al., 1985). The activation can be manifested throughout the entire crystal where an amorphous (non-crystalline) structure is produced, or affect only the surfaces of crystals and perhaps even then, be restricted to localised points on that surface. Whilst an almost totally disordered structure is easily identified by a variety of techniques, the mechanically induced surface effects, limited only to a few nanometers into the crystal, might go unnoticed. The surface mechanical activation induced by milling has been suggested to form a thin amorphous surface layer on particles (Hersey and Krycer, 1980; Hüttenrauch, 1983).

Mechanically induced physico-chemical modifications of solid drug substances, to increase bioavailablity, have been frequently reported in the literature. An increase in surface energy, formation of an amorphous state or amorphisation

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SSDI 0378-5173(94)00132-O

of the surface layer by mechanical manipulation were suggested to lead to higher dissolution rates (York, 1983). The 'activation' of digoxin by milling (Florence et al., 1974; Florence and Salole, 1976; Chiou and Kyle, 1979) and of calcium gluceptate (Suryanarayanan and Mitchell, 1985) has been shown to influence the solubility or dissolution rate of the drugs. Since disordered (amorphous) regions are high energy sites, less activation energy is needed for dissolution at these points relative to the more structurally ordered, lower energy sites, at the particles surface.

The solubility of a solid in a given solvent is the amount of the solid that goes in solution at a given temperature. If the solubility limit is exceeded, solid particles of the solute may be present, and the solution phase will be in equilibrium with the solid (Florence and Attwood, 1988). Since solubility is an equilibrium phenomenon, variations and increases in solubility exist as metastable equilibria (Buckton and Beezer, 1992). The mechanisms behind such elevated thermodynamically unstable solubilities (Buckton and Beezer, 1992) have not been described in any detail. The explanations for the relatively slow process of re-establishing the lower, stable solubility level (Higuchi et al., 1979) and the influence of the so-called epitaxi phenomenon, in this context, are seldom discussed.

The aim of this study was to evaluate a hypothesis that an increased metastable solubility is due to the formation of a disordered (amorphous) surface layer, around an ordered (crystalline) core. It was, thus, of interest to identify both the position and amount of disordered material. Another aim was to find an explanation for the slow decrease from an initially high metastable solubility level, to the lower stable level.

2. Experimental

2.1. Materials

Griseofulvin, microsize (Glaxo, U.K.), was used as a model fine particulate, practically insoluble material, supplied in an agglomerated form. This quality of griseofulvin will be referred to throughout this paper as the unmilled material or form (I).

Milled griseofulvin was prepared by milling about 1 g of griseofulvin using a mortar and pestle for 15 min and is sometimes referred to as form (II).

Quenched griseofulvin was prepared by melting unmilled griseofulvin at about 225°C followed by rapid cooling in liquid nitrogen and is sometimes referred to as form (III). This form was used as a model of a totally disordered (amorphous) griseofulvin.

The water used in the solubility experiments was distilled water filtered through an ultra-pure water system (Milli-Q UF Plus, Millipore, France).

2.2. Methods

2.2.1. Primary characterisation of the test materials

2.2.1.1. Apparent particle density. The density was assessed with an air comparison pycnometer (model 930, Beckman, U.S.A.) ($n = 3$).

2.2.1.2. Particle size. The particle size was determined with a Coulter Counter (model TAII, Coulter, U.S.A.) fitted with a 30 μm aperture tube and calibrated with 8.06 and 2.02 μm latex spheres. A stock saturated griseofulvin solution in distilled water containing 0.9% sodium chloride and 0.01% polysorbate 80 was prepared, and passed through a 0.22 μm Millipore filter. To this solution amounts of unmilled and milled griseofulvin forms were added to make 30 mg/ml suspensions. De-agglomeration of the particles was performed by ultrasonic treatment in a water bath for 5 min.

For the particle size determinations, a known volume of the prepared suspensions was added to the griseofulvin-electrolyte solution and stirred in the TAII at 800 rpm. The number of individual primary particles in 14 size classes was recorded for both the griseofulvin-electrolyte solution

(background count) and the test suspensions. The particle median volume diameter by weight were calculated using a Hewlett Packard 9825T computer ($n = 3$).

2.2.1.3. Gas adsorption surface area. The specific surface area was determined by the BET method using nitrogen gas (Flowsorb 11 2300 surface area analyser, Micromeritics, U.S.A.) ($n = 3$).

2.2.1.4. Differential scanning calorimetry (DSC). DSC scans of the different forms of griseofulvin were obtained using a Mettler TC 3000 differential scanning calorimeter, DSC (Hightstown, NJ, U.S.A.) in the temperature range 25–250°C, and at a scanning rate of 10°C/min. The sample weight was 4.5–5.5 mg ($n = 3$).

2.2.2. Solubility measurements

2.2.2.1. Analytical procedure. The solubilities of the different forms of griseofulvin were determined by adding amounts of the test materials ranging between 60 and 960 mg/l to purified water in a 500 ml volumetric flask. The suspensions were stirred over a magnetic stirring board at a rate of 300 rpm (±50). 7.5 ml aliquot samples were withdrawn periodically into 10 ml centrifugation tubes and centrifugated using a Wifug laboratory centrifuge (Lamor-M, U.K.) at a speed of 4500 rpm for about 5 min. After centrifugation, 5 ml samples were transfered to 25 ml volumetric flasks and the volumes were made up with water. The amount of griseofulvin dissolved was measured spectrophotometrically (Zeiss PM6, Germany) at 295 nm. ($n = 3$).

2.2.2.2. Definition of the initial solubility. For the milled and quenched griseofulvin samples an initially high solubility level was reached. This solubility peak value corresponds to the maximum solubility level attainable under the experimental conditions used (amount of material added, temperature, agitation, etc.). The term 'initial solubility' will be used throughout this paper to describe the peak solubility values obtained for the griseofulvin samples.

3. Results and discussion

3.1. Evaluation of the concentration-time (solubility) profiles

The concentration-time profiles of griseofulvin in water at 21°C (Fig. 1) showed a steady increase in the amount of the unmilled sample, form I, dissolved in water, to reach a plateau concentration after about 8 h. The plateau concentration agreed with earlier reported solubility values of griseofulvin (Nyström et al., 1985; Sjökvist et al., 1991).

The milled sample, form II, showed an initially high solubility peak value after about 6 h followed by a decline to a lower plateau. The plateau observed in this case is higher than that obtained for the unmilled sample. The profile produced by the milled sample resembled the characteristic concentration-time profile of a metastable polymorph (Lin, 1972) and that of anhydrous material (Rodrigues-Hornedo et al., 1992), except that in the latter cases, the plateau concentrations reached with time were not at elevated levels. In other cases, the plateau concentration was stabilised at the peak concentration for a prolonged time period (Chiou and Kyle, 1979).

The quenched griseofulvin sample, form III, produced a concentration-time profile similar to that of the milled sample except that the peak

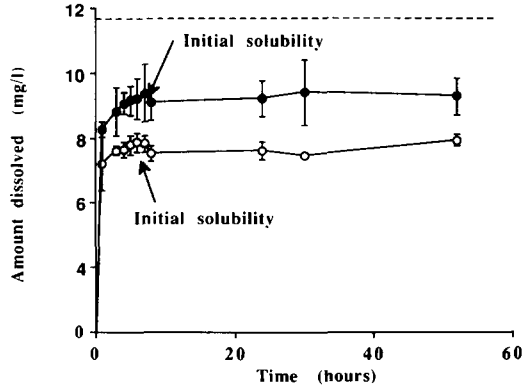

Fig. 1. Concentration-time profiles of griseofulvin in water at 21°C, 60 mg/l and 300 rpm (bars = 95% confidence interval of the mean): (○) (I) unmilled material; (●) (II) milled material; (dotted line) (III) quenched material initial level.

Table 1
Initial solubilities of different samples and treatments of griseofulvin at 23°C, 60 mg/l and 300 rpm

Sample		Solubility (mg/ml)	n
Form I	unmilled material	8.624 (±0.36)	10
Form II	milled for 15 min	10.047 (±0.40)	8
	milled for 30 min	10.205 (±0.60)	8
Form III	quenched material	12.325 (±1.89)	5

value was much higher, as represented by the dotted line in (Fig. 1) and the time required to reach the peak was much longer (more than 40 h), which thus did not allow the presentation of the whole profile in Fig. 1. The long time required by the quenched samples to reach the peak value is probably due to the small surface area taking part in the dissolution process, as quenching of griseofulvin produced smooth, dense and round spheres.

The increase in solubility attained by the milled griseofulvin, form II (Fig. 1 and Table 1) may correspond to the solubility of an 'activated state'. The relatively fast (6 h compared to 8 h) dissolution process to reach this solubility level may depend on either an increased effective surface area for dissolution (S_c) of the solid drug by de-agglomeration of the starting agglomerates, or be due to an improved dissolution process at the particle surfaces. The latter could be due to increased solubility of drug molecules undergoing dissolution (C_s) as described by the equation based on the theories of Noyes and Whitney (1897), Nernst (1904) and Brunner (1904);

$$(dc/dt) = (D/Vh) \cdot S_c \cdot (C_s - C_t) \quad (1)$$

where D is the diffusion coefficient, h denotes the thickness of an assumed stagnant diffusion boundary layer, V is the volume of solvent and C_t the concentration of the solute in the main part of the solvent.

As activated states are high-energy metastable states by definition (Hüttenrauch, 1985; Buckton and Beezer, 1992), they tend to resume more thermodynamically stable, lower energy, states. The actual peak solubility value of milled griseofulvin might be higher than the measured peak during the experiments, since the change back to a more stable crystalline form of griseofulvin, by a precipitation/crystallisation process, might compete with the dissolution of the milled material during the early dissolution phase (Chiou and Kyle, 1979; Rodrigues-Hornedo et al., 1992). This might explain the concentration-time profile of the milled griseofulvin, where the initial phase of the profile is dissolution-dominated until the highest possible level of supersaturation is reached, i.e., the peak, while the second phase of the profile is dominated by a transition back to a more thermodynamically stable form of griseofulvin, shown by a decline from the peak value. However, during the relatively short observation period in Fig. 1 no statistically significant reduction was seen.

3.2. Primary characterisation of the test materials

3.2.1. Particle shape and size

Microscopic examination of milled griseofulvin revealed a flattened, elongated and irregular agglomerated particles, while unmilled samples consisted of a mixture of spherical and elongated particles. It seemed that although the milling might have affected the particle shape, it did not lead to any de-agglomeration, since only agglomerates and not individual primary particles were observed.

This is supported by the individual primary particle size analysis using Coulter counter (Table 2), which showed individual primary particle sizes of 3.8 and 4.6 μm for the unmilled and milled samples, respectively, indicating that the milling

Table 2
Apparent particle density, surface area and particle size of griseofulvin test samples

Sample	Density (cm^3/g)	Coulter counter particle size [a] (μm)	BET	
			Weight (g)	Surface area (m^2/cm^3)
Umilled	1.45	3.8	0.5	2.4
			2.0	2.2
Milled	1.44	4.6	0.5	2.4
			1.8	2.5

[a] Median volume diameter by weight measured with a Coulter Counter TAII.

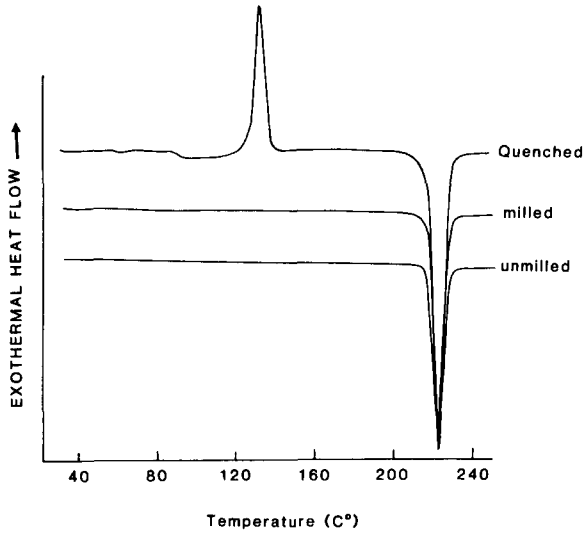

Fig. 2. DSC thermograms of griseofulvin: (I) unmilled material; (II) milled material; (III) quenched material.

process did not reduce the size of the individual primary particles.

3.2.2. Surface area

Nitrogen gas adsorption surface area measurements were performed to study the effect of milling on surface area. The BET surface area was not affected by the milling of griseofulvin. The measured BET surface area values were 2.4 m^2/cm^3 for both the unmilled and milled samples (Table 2).

3.2.3. Differential scanning calorimetry (DSC)

The DSC scans of the unmilled, milled and quenched griseofulvin showed that the unmilled and milled forms produced scans typical of crystalline materials (Fig. 2b–c), with a melting point of ~219°C and a heat of fusion, ΔH_f, of 117 J/g. The quenched griseofulvin, form III, showed a typical amorphous behaviour (Fig. 2a), with a glass transition temperature, T_g, at ~85°C and a crystallisation temperature, T_c, at ~133°C. The scans presented in Fig. 2 are consistent with previously published DSC scans of griseofulvin (Grant et al., 1986; Ford, 1987). If the samples of forms I and II were scanned for a second time, after cooling to about room temperature, scans typical of noncrystalline materials were produced, with identifiable T_g, and T_c corresponding to the quenched sample, form III.

Since no differences were noted between the unmilled and milled forms, it seemed that these samples were thermodynamically equivalent. However, if milling of griseofulvin produces activation occurring only at the particle surfaces, the total amount of disordered material may be too low to be detected by DSC, which measures the bulk and not the surface properties of the sample (Hersey and Krycer, 1980).

From the above characterisation results, it appears that milling of griseofulvin did not produce changes in the bulk thermal properties identifiable by DSC, nor did it increase the BET surface areas (S_c) or the individual primary particle size, to fully explain the apparent increase in solubility and in the initial dissolution rate of the milled griseofulvin, form II.

3.3. Thermodynamic evaluation

3.3.1. Free energy

For increased solubility to occur, there should be a thermodynamic transformation of molecules at the particle surface or of the entire bulk solid, to a higher energy state. During milling, changes in surface free energy (Dialer and Kussner, 1973; Buckton et al., 1988; Grant, 1991) and in instantaneous (corresponding here to 'initial') solubility (Grant, 1991), have been reported. Processing increases the free energy, i.e., $G_{II} > G_I$, for the milling process, whereas the crystallisation of amorphous material is spontaneous, i.e., has a negative ΔG value.

A simplified attempt to calculate the free energy of the milled material compared to the crystalline was made by using the solubility terms C_s:

$$\Delta(\Delta G) = \Delta G_I - \Delta G_{II} = RT \ln(C_{S(I)}/C_{s(II)}) \quad (2)$$

where $C_{s(II)}$ is the initial solubility of the processed material (II) and $C_{s(I)}$ represents the solubility of the untreated material (I) (Grant, 1991). Since no 'perfect' crystal, having a ΔG value of 0, is attainable, comparative measurements could be performed by assuming the unmilled material as having a ΔG value of 0. In this case, the milled

Table 3
Effect of milling on some thermodynamic properties of griseofulvin (I, unmilled griseofulvin; II, milled griseofulvin for 15 min)

	Form I	Form II
ΔG [a] (J/mol)	0 [c]	−376
ΔH_s [b] (kJ/mol)	26.7	14.3

[a] Calculated using Eq. 2.
[b] From van't Hoff plot in Fig. 3.
[c] ΔG = O by definition for a perfect unmilled crystalline material.

test material had a free energy value of −376 J/mol (Table 3).

3.3.2. Molar heat of solution

The effect of temperature on the initial solubility of the unmilled and the milled griseofulvin was evaluated. By use of the van't Hoff relationship between the log initial solubility values and the reciprocal of the absolute temperature (Fig. 3) a linear correlation was established between the initial solubility values for both forms and the reciprocal absolute temperature (correlation coefficent 0.99 and 0.98). The solubility values of the unmilled showed a greater sensitivity to changes in temperature than the milled samples in the temperature range studied. The slope of the straight line in Fig. 3 permitted the calculation of the molar heats of solution, ΔH_s, of 26.7 and 14.3 kJ/mol for the unmilled and milled forms, respectively.

These findings might provide the explanation for the increased solubility of the milled griseofulvin, as they show that less energy is required for the molecules of the milled samples to leave the solid surface and to be molecularly dispersed in the liquid surroundings. The reduction of the molar heat of solution is thought to be due to the increased disordering of the material induced by grinding, i.e., mechanical activation. The heat of solution has earlier been used as a mean of characterising the crystallographic states of solid pharmaceuticals (Hendriksen, 1990).

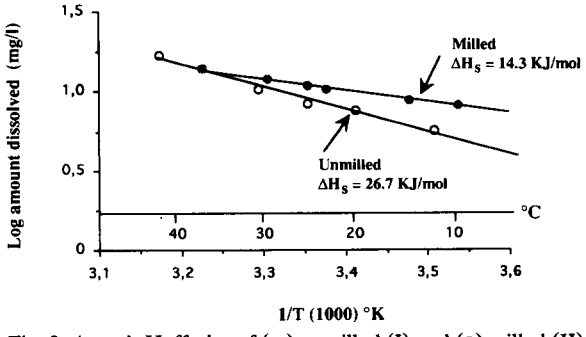

Fig. 3. A van't Hoff plot of (○) unmilled (I) and (●) milled (II) griseofulvin; 60 mg/l and 300 rpm.

Thus, the process-induced increase in free energy will correspond to a general increase in reactivity and disorder (Davies and Rideal, 1963; Nelson, 1972), i.e., an increased molecular mobility at the particle surface, as well as exposure of more reactive chemical groups to the solvent (Ahlneck and Zografi, 1990). The free energy could be measured by a reaction-related equilibrium property, e.g., the instantaneous solubility (Grant, 1991) (Table 3), or a decreased enthalpy which reduces the heat of solution, ΔH_s. Here, the energy required for bond breakage during dissolution is reduced which depends on both the enthalpy and the entropy of the process.

3.4. Evaluation of the amount and position of the disordered (amorphous) material

The results obtained so far indicated that milling of griseofulvin particles caused activation, and thus disordered, at least parts of the solid structure. To study further the amount and position of such a disordered fraction, several specific experiments were performed. Due to the type of milling procedure and since the attainment of the initial solubility level is an interfacial phenomenon, it was assumed that the disordered (amorphous) fractions of the milled griseofulvin samples exist as an external surface layer, around a more or less crystalline core. This hypothesis was the basis for the design of the following experiments.

3.4.1. Effect of the amount of griseofulvin used in the solubility experiment

The initial solubility values of the unmilled griseofulvin, form I, were more or less the same

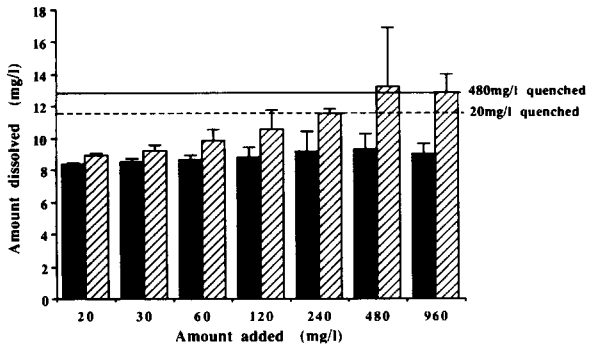

Fig. 4. Effect of amount of griseofulvin added on the initial solubility for unmilled (filled bars) and milled (hatched bars) griseofulvin at 21°C and 300 rpm (vertical bars = 95% confidence interval of the mean).

regardless of the amount of material added (Fig. 4). This is to be expected for a perfectly ordered crystalline material, where a thermodynamically stable solubility level is reached which is in agreement with the Noyes-Whitney equation (Eq. 1), i.e., the increase in surface area affects only the dissolution rate and not the solubility. In the experiments to collect the data used in Fig. 4, the above statement was justified by quicker attainment of the peak, when the amount of material added to the dissolution medium was increased. The slight increase in the initial solubility values for the unmilled material (Fig. 4) is probably due to the presence of a small number of defects in the unmilled material, an absolute number that increases when increasing the amount added. The use of a large excess of sparingly soluble drug in solubility measurements was reported to greatly aggravate the effects of impurities and the energetic heterogeneity of the crystal (Higuchi et al., 1979; Grant and Higuchi, 1990).

The milled material, form II, showed a proportional increase in the initial (peak) solubility values when increasing the amount added to the dissolution medium, until it reached the level of the quenched material (represented by lines in Fig. 4). A further increase in the amount added did not produce any increase in the initial solubility values.

For the quenched griseofulvin, form III, increasing the amount of the quenched material did not produce any significant increase in the initial solubility values, as represented by the small difference between the two inserted lines in Fig. 4.

The differences between the milled and unmilled forms, shown in Fig. 4, can be explained by the fact that milling causes preferential disordering of the surface structure rather than the entire bulk structure. Therefore, when low amounts of the milled griseofulvin close to the saturation level were used, a substantial volume fraction of the added griseofulvin particles was dissolved, and thus, exposed a new solid liquid interface consisting mainly of crystalline solid structure. The initial solubility level obtained will, then, be determined mainly by the relative contribution of the total amount of the disordered material in the sample used. When larger amounts of the milled material are added, the greater relative contribution of the disordered material in the sample will increase the initial solubility level progressively until the solution phase reaches a saturation level of disordered material at 480 mg/l, where the initial solubility level equals that of the totally amorphous quenched sample.

3.4.2. Repeated solubility measurements of a milled griseofulvin sample

Repetitive solubility experiments were performed using a large amount of the milled material, form II. Here, after each measurement, the supernatant solution was withdrawn, assayed and the particles were re-suspended for a new measurement, repeated 7 times. The mean initial solubility values (Fig. 5) decreased gradually each time until a plateau level was reached, comparable to the one obtained using the same amount of the unmilled material.

This finding indicates that by re-suspending the same particles, the amount of activated, highly energetic and easily soluble material at the particle surfaces progressively decreases with each repeat, until a more crystalline surface was reached, thus supporting the assumption that milling of griseofulvin creates a surface layer or regions of disorder at the particles surface.

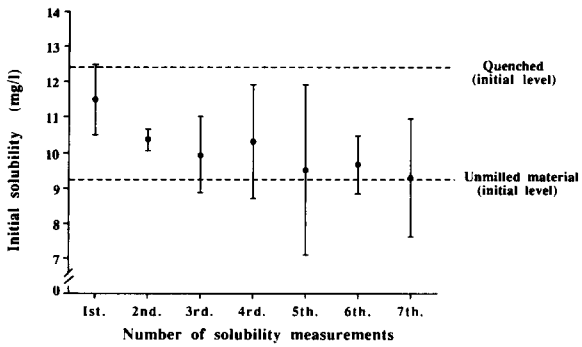

Fig. 5. The initial solubility of ground griseofulvin (II) as a function of the number of repetitive solubility measurements at 21°C, 300 rpm and 480 mg/l (bars = 95% confidence interval of the mean).

3.4.3. Calculation of the amount of disordered material

Using the data presented in Fig. 5, the total amount of disordered material was calculated as outlined in Table 4. The amount of milled griseofulvin added in the first solubility experiment was 480 mg/l. This amount was considered high enough (Fig. 4) to enable saturation of the solution phase with the disordered material, while still maintaining a disordered interfacial solid structure. Thus, the entire amount dissolved can be regarded as originating from the disordered fraction.

In the last, seventh solubility experiment, since the initial solubility level was close to that produced by an equal amount of unmilled material, it was argued that no disordered material was left on the particles surface. By addition of the amounts of disordered material dissolved from the particles surface in the repeated solubility experiments, the total calculated amount of disordered material was estimated to be 28.1 mg (Table 4), corresponding to a total volume fraction of disordered material of 5.8%.

Table 4
Calculation of the amount of disordered material from the data of the repeated solubility measurement of milled griseofulvin

Experiment	Solubility (mg/l)	Increase in solubility from unmilled level (mg/l)	Calculated amount of disordered material (mg)
1	11.5	2.3	2.3/2.3×11.5 = 11.5
2	10.4	1.2	1.2/2.3×10.4 = 5.4
3	9.9	0.7	0.7/2,3× 9.9 = 3.0
4	10.3	1.1	1.1/2.3×10.3 = 4.9
5	9.5	0.3	0.3/2.3× 9.5 = 1.2
6	9.7	0.5	0.5/2.3× 9.7 = 2.1
7	9.2	0.0	0.0/2.3× 9.2 = 0
			Σ 28.1

[a] Amount of milled griseofulvin used was 480 mg/l.
[b] Solubility value of 480 mg/l of unmilled griseofulvin was 9.2 mg/l.

3.4.4. Calculation of the thickness of an assumed disordered surface layer

Consider a hypothetical spherical milled particle 4.6 μm in diameter (Fig. 6). Assuming that milling produces a complete and uniform external layer of disordered material around a crystalline core, and that the milled particle and the crystalline core have a similar density. From the value of 28 mg total amount of disordered material obtained above, the thickness of the assumed disordered surface layer was calculated as follows:

$$\text{mass of original particles} = 480 \text{ mg} = nd_1(\pi/6)d_1^3$$

$$\text{mass of crystalline core} = (480 - 28) \text{ mg} = nd_2(\pi/6)d_2^3$$

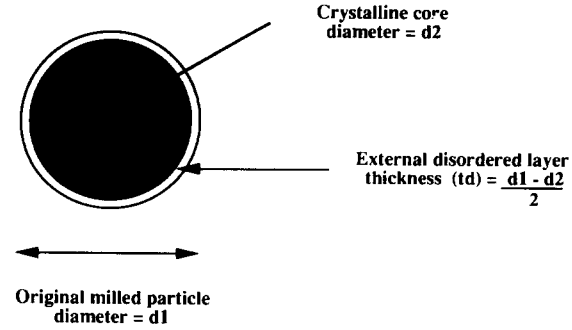

Fig. 6. Simplified geometrical illustration of an assumed disordered surface layer around a crystalline core of a milled griseofulvin particle.

external disordered minimum layer thickness (t_d)

$$= (d_1 - d_2)/2 \text{ (Fig. 6)}$$

$$= d_1 \left[\frac{1 - \left(\frac{480 - 28}{480} \right)^{1/3}}{2} \right]$$

The disordered layer thickness (t_d) = 46 nm.

3.4.5. *Effect of the particle surface structure on the initial solubility*

The interpretation of the results presented so far suggested that the surface structure of griseofulvin particles determines the initial solubility level reached. It is thus of interest to determine further whether a mixture of both crystalline and disordered materials will deliver initial solubility levels reflecting the composition of the external surface structure, i.e., if a replacement of the solid material interface would be shown directly by a change in the initial solubility.

Two suspensions were prepared using 960 mg/l of forms I and II of griseofulvin. After attainment of the initial solubility levels, the two suspensions were centrifuged to remove the solid particles. To the supernatant solutions obtained, fresh (new) solute particles equivalent to 960 mg/l of form I, form II and a mixture of the two forms (75:25, 50:50, 25:75%) were added. The change in the amount of griseofulvin in solution was then followed with time, with continuous stirring.

For the solutions obtained using form I particles (Fig. 7a), the addition of fresh form I particles did not produce any change in the amount of griseofulvin in solution. However, the addition of form II particles led to a substantial initial increase in the amount of griseofulvin in solution, followed by a gradual decrease, in much the same way as presented earlier (Fig. 1). The mixtures of forms I and II particles added produced a stepwise initial increase in the amount of griseofulvin in solution, proportional to the increased portion of form II used, followed by a gradual decrease as in the case of pure form II added particles. These findings indicates that the addition of a mainly amorphous interface changes the pesudo-solubility equilibrium so that more molecules are released from the solid amorphous interface and goes into solution.

For the solutions obtained using form II particles (Fig. 7b), the addition of fresh form I particles led to a sharp and rapid drop in the amount of griseofulvin in solution. The mixture containing 75% form I particles behaved in much the same way, but the drop was of a much lower extent than the 100% form I sample. It seemed that the presence of form I particles favoured the precipitation/crystallisation process, manifested by a decrease in the amount of griseofulvin in

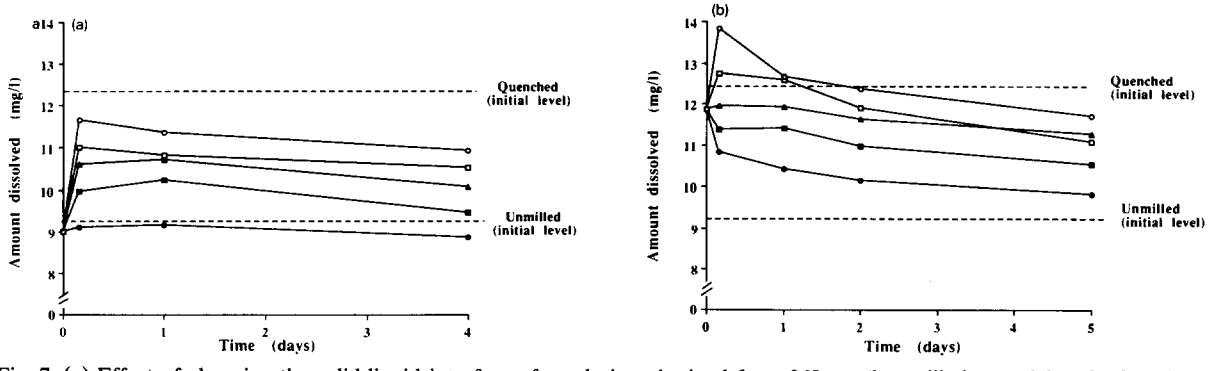

Fig. 7. (a) Effect of changing the solid-liquid interface of a solution obtained from 960 mg/l unmilled material at 21°C and 300 rpm: (●) 100% unmilled, (■) 75% unmilled + 25% milled, (▲) 50% unmilled + 50% milled, (□) 25% unmilled + 75% milled, (○) 100% milled. (b) Effect of changing the solid-liquid interface of a solution obtained from 960 mg/l milled material at 21°C and 300 rpm. (●) 100% unmilled, (■) 75% unmilled + 25% milled, (▲) 50% unmilled + 50% milled, (□) 25% unmilled + 75% milled, (○) 100% milled.

solution. This can be explained assuming that the precipitation/crystallisation process led to the formation of a solid having a structure similar to that of the solid interface introduced, i.e., epitaxi phenomenon (Rodrigues-Hornedo et al, 1992). It would follow, then, that the crystalline surface layer, or interface added, will induce precipitation of a solid having a crystalline structure. Such a precipitation releases more exothermic energy than an amorphous precipitate. Therefore, when form I particles were added to the form II solution, the new suspension formed was supersaturated, and the precipitation/crystallisation of the solute molecules decreases their concentration in the solution phase.

The addition of 100% form II particles produced a further initial increase in the amount of griseofulvin dissolved followed by a decrease. The mixture sample containing 75% form II particles showed the same trend as the 100% sample, but the increase was relatively smaller. Here, it seem that the supersaturation in the local micro-environment of the mainly amorphous interface added is greater than that in the bulk solution. Accordingly, the concentration gradient causes the molecules to diffuse away from the particle micro-environment into the solution phase, and thus, increase the concentration of solute molecules in solution.

The 50:50% mixture of forms I and II added to form II solution did not show any change in the amount of griseofulvin dissolved. It seems that the tendency to decrease of the amount of griseofulvin dissolved due to the presence of form I particles, is compensated by an equal and opposite tendency to increase, produced by the presence of form II particles.

These findings support the view that the initial solubility level of milled griseofulvin is a true and direct reflection of the solid state structure of the particle surface.

3.5. Evaluation of the metastable nature of the increased solubility

It has been argued throughout this paper that the increased solubility of the milled griseofulvin, form II, is metastable. Therefore, the activated

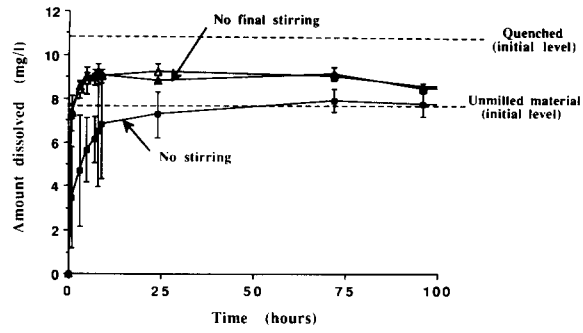

Fig. 8. Effect of stirring on the concentration-time profile of milled griseofulvin at 21°C, 60 mg/l and 300 rpm (bars = 95% confidence interval of the mean): (○) continuous stirring, (▲) stirring only in the initial dissolution phase, (■) no stirring.

molecules in solution ought to lose their acquired high-energy states and precipitate or crystallise out of solution in a structurally more ordered, thermodynamically more stable and less soluble form, to ultimately reach an equilibrium, i.e., the equilibrium solubility. To investigate the reasons why this did not happen during the observation time in Fig. 1, the effect of molecular diffusion on the concentration-time profile was investigated. Here, experiments were performed with and without stirring throughout the whole profile. In a third experiment, stirring was performed only until the initial (peak) solubility was reached, and stirring was not continued beyond that point.

The concentration-time profiles of the three sets (Fig. 8) revealed that the unstirred samples showed a slow increase in the amount of griseofulvin dissolved to reach a plateau at about 80 h. The stirred samples demonstrated a relatively faster increase in the amount of griseofulvin dissolved until the peak value was reached, followed by a gradual decrease. The third sample showed behaviour identical to that of the stirred sample, and both samples showed a gradual, slow and equal decrease in the amount of griseofulvin in solution after reaching the peak value, which was not affected by stirring.

The above findings indicated that the rate-limiting step in the portion of the concentration-time profile before the attainment of the peak is controlled by solute molecular diffusion. However, after the attainment of the peak, the transi-

tion to a lower energy state was not affected by the solute molecular diffusion, and the rate-limiting step seemed to be a surface-controlled growth mechanism, probably a slow solid-state rearrangement process.

4. Conclusions

Milling of griseofulvin particles produced an increased metastable solubility, which might correspond to the solubility of an activated state. The milling operation did not change the materials bulk thermal properties identifiable by DSC, nor did it increase the BET surface area or affect the individual primary particle size to explain the apparent increase in solubility. However, thermodynamic evaluation of the test materials showed an increase in the free energy and a decrease in the enthalpy of solution of the milled griseofulvin, suggesting that the increase in solubility was due to disordering of the solid structure.

The disordering of the particles was estimated to be about 5.8% w/w of the total mass. The relatively small total disordering produced by this mechanical process limits its impact on the total dissolution rate and hence, its bioavailability. The disordering is, probably, limited only to an external assumed surface layer (40–50 nm). Procedures to estimate both the amount and position of the disordering caused by the milling operation have been described.

The surface structure of griseofulvin particles seemed to determine the initial solubility level reached, and the replacement of the solid material interface exposed to the dissolution medium, would directly be refleced by a change in the initial solubility.

The decrease from the initially high metastable solubility level to the stable equilibrium solubility level reflects the transition of the disordered material to a more ordered (crystalline) form. This process is slow, and the rate-limiting step seems to be a surface reaction mechanism, probably a solid-state rearrangement process, and not a process controlled by solute molecular diffusion in the solution phase.

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