An *In Situ* Dissolution Study of Aspirin Crystal Planes (100) and (001) by Atomic Force Microscopy

Ardeshir Danesh,[1] Simon D. Connell,[1] Martyn C. Davies,[1] Clive J. Roberts,[1,3] Saul J. B. Tendler,[1] Phillip M. Williams,[1] and M. J. Wilkins[2]

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Purpose. To observe *in situ* and on individual aspirin crystal faces the comparative rates and processes of dissolution of the dominant faces.
Methods. The kinetics of the dissolution rate of two aspirin crystal planes (001) and (100) under 0.05M HCl are studied *in situ* at room temperature using Atomic Force Microscopy. The dissolution process of each crystal plane was followed by observed changes in topographic features.
Results. The results revealed that crystal plane (001) dissolves by receding step edges, and has a dissolution rate of 0.45 nm s^{-1}. Conversely, plane (100) displays crystal terrace sinking at an average rate of 2.93 nm s^{-1}. Calculated intrinsic dissolution values (g s^{-1} cm^{-2}) for planes (001) and (100) are 1.37×10^{-7} g s^{-1} cm^{-2} and 8.36×10^{-7} g s^{-1} cm^{-2}, respectively.
Conclusions. These values indicate that the rate of flux of material from plane (100) is approximately six times greater than that from plane (001), under 0.05M HCl. Interpretation of the data, based upon intrinsic dissolution rates and dissolution rate velocities, correlate with reported variations in the dissolution behavior of commercial aspirin products. These observations illustrate the suitability of the technique for characterizing the dissolution behavior of crystalline drugs.

KEY WORDS: aspirin; atomic force microscopy; AFM; dissolution; crystal.

INTRODUCTION

The processes surrounding the dissolution and growth of crystals are basic chemical and physical phenomena with importance across a range of fields, including natural processes such as mineral dissolution and industrial applications such as crystal growth in pharmaceuticals (1). The understanding of fundamental factors which influence crystal growth and dissolution have proved important (2), particularly within the pharmaceutical industry where the characterisation of such processes may help devise more desirable re-crystallization procedures (by controlling the crystal size and habit during growth) and provide an insight into the dissolution behavior of drug crystals both *in vitro* and *in vivo*.

The emerging application of Atomic Force Microscopy (AFM), for *in situ* investigation of crystal growth and dissolution has revealed its ability for monitoring and measuring information at a microscopic level from various types of materials (2–7). By monitoring the evolution of suitable features in real time, such as translation of steps across the surface, the rate of reaction/ dissolution may be inferred and approximate estimations of the corresponding rates can be made (8). Thus far, AFM studies on the dissolution of materials have concentrated mostly on macromolecular organic crystals for example proteins such as insulin (9,10,11), catalase (12), lysozyme (12) and naturally occurring minerals such as calcite (13). Although some recent studies have reported on the dissolution profile of salicylic acid (2,14), the dissolution rate studies of pharmaceutical products have in most parts relied on the conventional approaches that use macroscopic solid particles often in the form of a pressed pellet, where quantities such as weight loss and surface area of the particle or the concentration of elements eluted into a contacting fluid are measured. However, such approaches suffer from several inherent drawbacks that originate from the use of large solid particles, thus obscuring our understanding of dissolution phenomena (15). Here, to overcome these drawbacks we employ AFM for direct microscopic level observation of the surface of aspirin crystal planes subjected to dissolution. Aspirin is an example of a substance where *in vitro* dissolution rate studies of drugs are important in order to provide an insight into their behavior *in vivo*. Investigations of different commercial aspirin tablets have illustrated that *in vitro* dissolution rates vary between preparations and are proportional to *in vivo* absorption rates (16). Morphological investigations of aspirin crystals reveal that their dissolution rates depend on the extent of exposure of different crystal planes. Needle shaped crystals (where crystal plane (100) has had optimum growth, as a result of re-crystallization from hexane) are much faster in dissolving than plate shaped crystals (where plane (001) has had optimum growth, as a result of re-crystallization from ethanol). Conventional dissolution rate experiments have indicated that crystal plane (100) has a dissolution rate at least twice as fast as the dissolution rate of plane (001) in pure water (17). These observations are however not due to any essential differences in crystallographic properties or as a result of polymorphism (18) among the commercial preparations, but caused by a difference in crystal habits observed (19). In this experiment, we examine the dissolution behavior of each of the two crystal planes at a microscopic level and calculate their intrinsic dissolution rates.

MATERIALS AND METHODS

Sample Preparation

Aspirin crystals were obtained from re-crystallization of U.S.P specification aspirin at room temperature from a hot solution of 95 percent ethanol. The crystals were immobilized on AFM sample stubs using an epoxy resin based adhesive (Araldite Rapid, Bostik Ltd, U.K.). Two kind of samples were prepared, one exposing crystal plane (001) and the other revealing plane (100) at their surface.

AFM Analysis

Images were obtained using a ThermoMicroscopes Explorer AFM (ThermoMicroscopes, U.K.) utilizing oxidized

[1] School of Pharmaceutical Sciences, University Park, The University of Nottingham, Nottingham NG7 2RD, U.K.
[2] SmithKline Beecham Pharmaceuticals, New Frontiers Science Park, Harlow, Essex, U.K.
[3] To whom correspondence should be addressed. (e-mail: clive.roberts@nottingham.ac.uk)

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sharpened silicon nitride probes. Experiments involved the addition of a few drops of freshly prepared dissolution medium onto the surface of a sample followed by continuous imaging. The acquisition speed of the images was determined by the rate of change of features in the images. Three solutions were investigated as dissolution mediums in the following order; pure water, 0.1M HCl, and 0.05M HCl. However only the later proved suitable for our study, as the dissolution under water was too slow and 0.1M HCl proved too rapid to be imaged. Data was typically recorded with a scan frequency of 40Hz for plane (001) and 20Hz for plane (100). This is as opposed to a scanning frequency of 2Hz typically employed. Since the volume of dissolution liquid (~ 1 ml) is large in comparison to a single crystal of aspirin, it is assumed that the concentration of solute in the dissolution medium is negligible compared to the concentration dissolving and therefore sink conditions apply. This assumption is explained using the Noyes-Whitney equation (formula 1) (20), where the rate of dissolution is expressed as the rate of change of concentration of the dissolution medium with respect to time (dc/dt).

$$dc/dt = k(c_s - c) \quad (1)$$

Where c is the concentration of dissolved solute at time t, c_s is the concentration of saturated solution of the solute in dissolution medium, and k is a constant. In these experiments, even when assuming the complete dissolution of a whole 350 μg crystal, c would be no greater than 0.35% of c_s.

RESULTS AND DISCUSSIONS

Typical images (10 μm × 10 μm) from the surface of crystal plane (001) exposed to 0.05M HCl are displayed in Figure 1a–f. The images acquired consecutively illustrate the translation of step edges in two directions; bottom to top and right to left. The black arrow that appears in all the images illustrates the position of a step edge with time as the crystal plane erodes. To ensure the observed effect was not due to horizontal drift of the instrument, reference is made to the indentation at the bottom corner of the images (indicated by the white arrow and highlighted by insets on images a and f). This indentation remains stationary while the step edges translate across the image. The observed step edges have velocities in the range of 16 nm s^{-1}–19 nm s^{-1} that are independent of the step height that varies from 1–41 nm. Thus, the dissolution rate of crystal plane (001) based on an average step velocity of 17 nm s^{-1} calculated using the following equation (formula 2) (20) is 0.45 ± 0.15 nm s^{-1}.

$$\text{Dissolution rate of a crystal plane (ns s}^{-1}) = \frac{\text{Step height (nm)} \times \text{Step velocity (nm s}^{-1})}{\text{Step spacing (nm)}} \quad (2)$$

A series of images (5 μm × 5 μm) obtained from the crystal plane (100) are demonstrated in Figure 2a–f. Through the dissolution process it can be seen that the rugged topography of plane (100) gradually erodes over time. The plane background of these images is as a result of the z-piezo reaching its maximum extended level. This provides a fixed reference point against which measurements in height can be made. The acquisition of 10 μm × 10 μm images from the surface of plane (100) proved problematic because of large variations in the z-range of the rougher surface offered by the (100) plane.

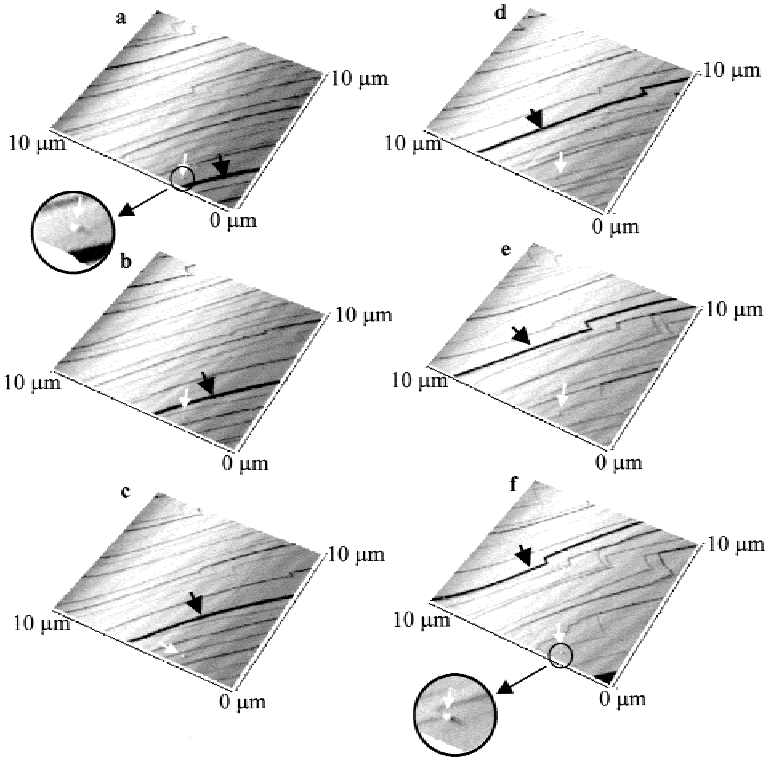

Fig. 1. 10 μm × 10 μm images from the surface of aspirin crystal plane (001), a) at time (t) = 0 seconds (s), b) at t = 66 s, c) at t = 132 s, d) at t = 197 s, e) at t = 263 s, f) at t = 329 s. Images are displayed as if artificially illuminated from the left. Magnified views of the small fixed feature identified by the white arrow are given in a) and f).

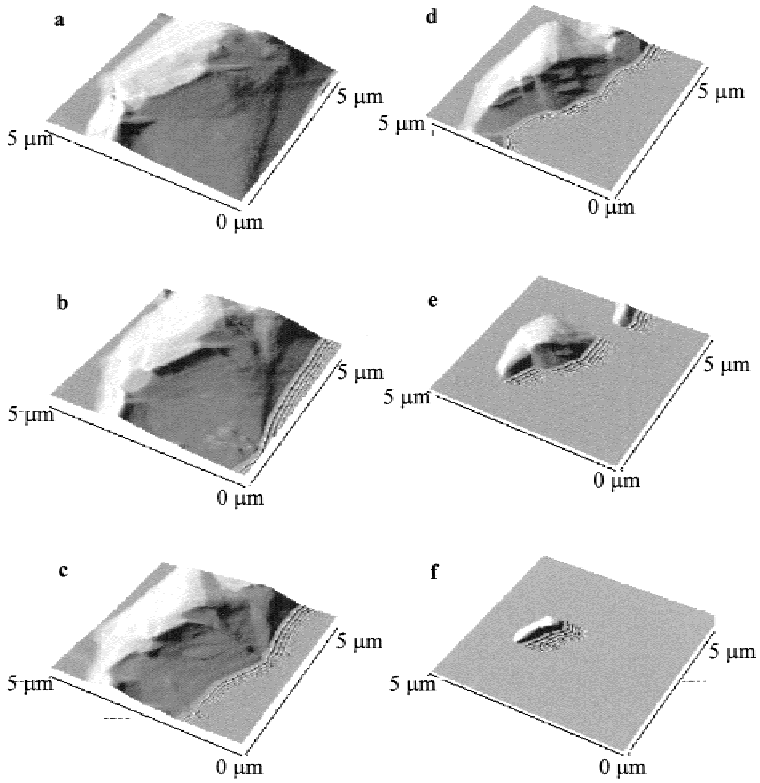

Fig. 2. 5 μm × 5 μm images from the surface of aspirin crystal plane (100), a) at time (t) = 0 seconds (s), b) at t = 89 s, c) at t = 178 s, d) at t = 267 s, e) at t = 356 s, f) at t = 444 s. Images are displayed as if artificially illuminated from the left.

In contrast to crystal plane (001) the images in Figure 2 illustrate the dissolution of plane (100) to occur via the sinking of crystal terraces at an average rate of 2.93 nm s^{-1} calculated by measuring the decrease in vertical height of a point at the peak of the image with respect to time.

In addition to velocity analysis, the volume of solute dissolving for each plane was calculated. For plane (001) the volume dissolving from the recession of step edges was considered to be the volume of a slab, calculated by using the step edge length (l), height (h) and horizontal shift (w) as the three corresponding parameters of a "rectangular cube" (Figure 3). In case of plane (100) the volume was measured using the software available on the ThermoMicroscopes AFM instrument, which calculated the volume of substance present above the fixed reference point. Since aspirin has a unit density (ρ) value of 1.4 g cm^{-3}, the mass (g) of solute dissolving for each plane as a function of time (dm/dt) was calculated. Crystal plane (001) and (100) have average dm/dt values of 6.78×10^{-15} g s^{-1} and 1.28×10^{-13} g s^{-1}, respectively. To allow a direct comparison between the dissolution rates of the two planes in terms of mass dissolving, their intrinsic dissolution rates (G) were calculated using the following equation (formula 3) (20);

$$G = \frac{dm/dt}{A} \tag{3}$$

where dm/dt, is the change in mass with time (g s^{-1}) and A, is the exposed area (cm^2)

The area (A) for plane (001) was the area of the whole image, i.e. 10 μm^2. Where as the area for plane (100) was calculated using the software available on the Topometrix instrument, which measures the area available above the fixed reference point.

The calculated G values for the crystal plane (001) and (100) are 1.35×10^{-7} g s^{-1} cm^{-2} ($\pm$ 1.51×10^{-7}), and 8.36×10^{-7} g s^{-1} cm^{-2} ($\pm$ 3.21×10^{-7}), respectively. Both methods of calculation indicate that under 0.05M HCl, plane (100) is six times faster in dissolving than plane (001).

The observed differences in the mechanism and rate of dissolution for the two planes of aspirin may be due to the combination of a number of factors. If we consider the surface molecular structure of the two dissolving planes (Figure 4), plane (001) Figure 4a has benzene rings exposed, which are not readily liable for attack. However, the expression of the ester groups at its step edges provides a potentially reactive

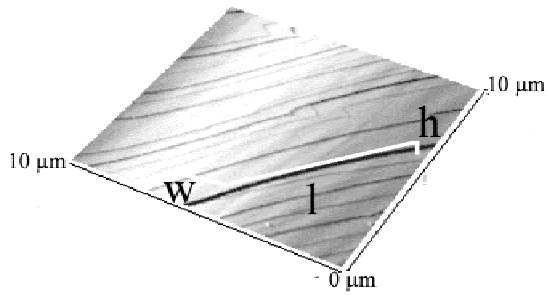

Fig. 3. An image of plane (001) demonstrating the parameters height (h), length (l) and width (w), used in the calculation of volume and area of dissolution from the receding step edges. The image is displayed as if artificially illuminated from the left.

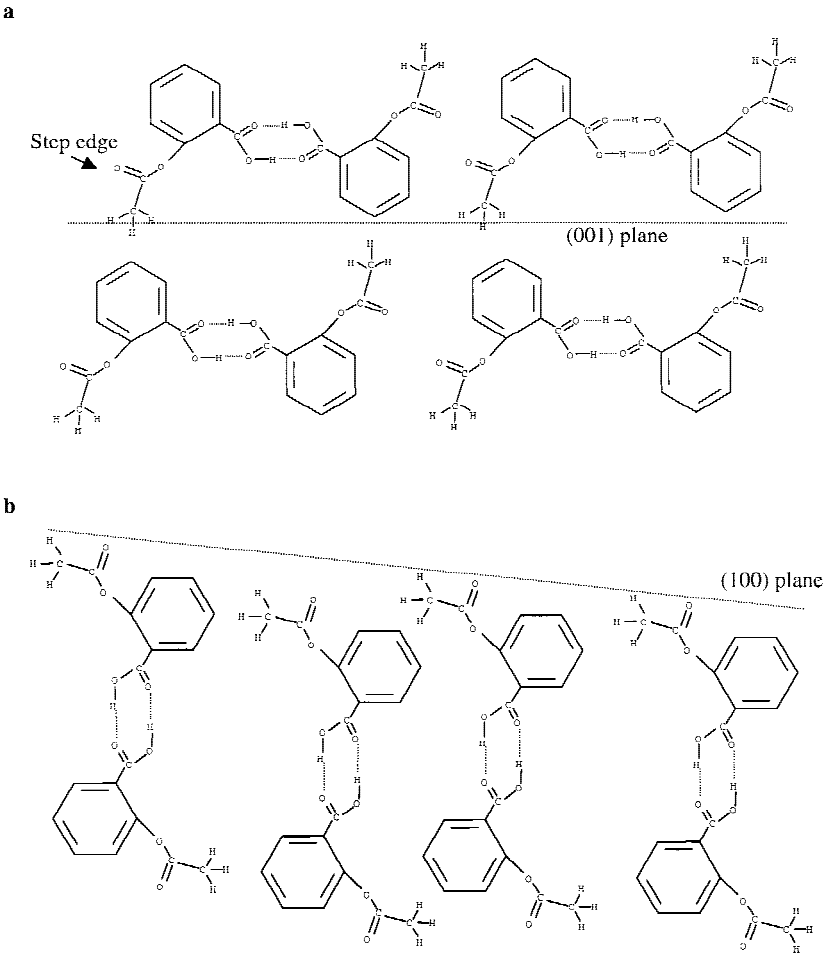

Fig. 4. Schematic representations of the surface molecular structure of aspirin crystal planes a) (001) and b) (100).

site for attack by the dissolution medium, hence the observance of retreating step edges. The lack of correlation between the height and velocity of these retreating steps may be an indication of an acid catalysed hydrolysis of the ester of acetyl salicylic acid. In case of plane (100) (Figure 4b) the expression of the ester group at its surface makes all points uniformly accessible for attack by the dissolution medium. This thus explains the homogenous decline in the height of the features on plane (100) as the surface erodes. The observed results may therefore demonstrate a reactive dissolution, which is responsible for the larger differences between the rates of dissolution of the two planes. Earlier studies by Kim and co-workers (17) demonstrated that the dissolution velocity of plane (100) was twice that of plane (001). The larger difference observed in this study may well be due to the reactive nature of the dissolution process.

Another contributing factor to the different rates of dissolution may be the greater surface roughness of crystal plane (100), which leads to a larger surface area and increased dissolution. Surface roughness has previously been identified to have a significant effect upon dissolution rate (21–23). The rougher surface of plane (100) observed, correlates with the previously reported disordered nature of the surface on crystal plane (100) compared to (001) (24). It should also be noted that AFM itself combined with fractal analysis has been used as technique to determine surface roughness of pharmaceutical powders and granules (25). Finally, the differences in hydrophilic and hydrophobic nature of the two surfaces (26), may lead to easier wetting of the more hydrophilic surface plane (100) in aqueous environment and hence its higher rate of dissolution.

Undoubtedly, it is the commercial tablets of aspirin containing a significant amount of needle shaped crystals (where plane (100) has had optimum growth), which have faster dissolution and absorption rates. Therefore, the control of crystallization conditions will help obtain the crystal habit that provides the optimum dissolution characteristics for intended use. Thus, a further potential use of AFM if used in combination with a liquid flow cell (2,14) may be to enable the dynamic alteration of *in situ* dissolution or growth conditions. This would allow for the *in situ* monitoring of the effect of different parameters on the crystal habits forming or dissolving at a microscopic level. This type of investigations may provide the pharmaceutical industry with a tool that will lead to the design of more efficient crystallization and dissolution procedures on a larger scale.

CONCLUSION

AFM investigations demonstrated the profile of dissolution for two aspirin planes (001) and (100). The results re-

vealed that crystal plane (001) dissolves by receding step edges, and has a dissolution rate of 0.45 nm s^{-1}. Conversely, crystal plane (100) displays crystal terrace sinking at an average rate of 2.93 nm s^{-1}. Calculated intrinsic dissolution values (G) (g s^{-1} cm^{-2}) for crystal plane (001) and (100) are 1.35×10^{-7} g s^{-1} cm^{-2} and 8.36×10^{-7} g s^{-1} cm^{-2}, respectively. These values indicate that the rate of flux of material from plane (100) is approximately six times greater than that from crystal plane (001), under 0.05M HCl. These observations demonstrate the ability of the technique to monitor and measure the progress of processes at a microscopic level from a single crystal, which can be directly related to the reported variations between their commercial preparations. We therefore believe that AFM could play a significant role in the characterization of growth and dissolution behavior of crystalline pharmaceutical drugs.

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