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Investigation of the felodipine glassy state by atomic force microscopy

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Abstract

The glassy state of felodipine was prepared by melting crystals of felodipine on a clean glass slide and cooling to room temperature. It has been confirmed that glassy felodipine is a metastable state, and undergoes transformation to the more stable crystalline form. Crystallization occurred slowly and spontaneously at room temperature, below the glassy state transition temperature (T_g). The contact mode of atomic force microscopy was used for topographical imaging of the glassy and crystalline states of felodipine. When the glassy felodipine region next to the recrystallized zone was exposed to controlled mechanical stress through the tip, rapid additional crystallization was observed. This crystallization process can be induced and imaged in real time by atomic force microscopy.

Keywords: Felodipine; Glassy state; Atomic force microscopy; Surface topography; Roughness; Crystallization

1. Introduction

The polymorphism and amorphism of drugs affect their bioavailability and play an important role in the formulation and production of pharmaceutical preparations. When the solids are heated above their melting point and then cooled below it, rapidly as a rule, many of them form a supercooled liquid. This state is commonly called the glass or glassy solid. The glassy state of low molecular weight pharmaceuticals can be used to enhance dissolution rate (Kerč et al., 1990) and drug stabilization (Crystall, 1997). Unfortunately, most glasses are mechanically and thermally unstable (Srčič et al., 1992; Kerč and Srčič, 1995).

Felodipine [Ethyl methyl 4-(2,3-dichlorophenyl)-1,4-dihydro-2,6-dimethyl-3,5-pyridine-dicarboxylate], a Ca^{2+} antagonist, is a member of the dihydropyridine family. Felodipine polymorphism and its glassy state have already been investigated by means of DSC, FTIR, NMR and X-ray diffraction [Srčič et al., 1992]. The glassy state of felodipine is a non-equilibrium state, which at room temperature slowly, or rapidly above the glass transition temperature (T_g for glassy felodip-

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ine is around 43°C), undergoes exothermal transformation to more stable, crystalline forms. By heating the glassy felodipine above T_g, first cold crystallization (at approximately 98°C) and thereafter melting (at approximately 142°C) of the crystals takes place. The existence of different polymorphic forms of recrystallized felodipine has been confirmed (Srčič et al., 1992).

The aim of this work was to observe the topography of the surface of the felodipine glassy state using contact mode atomic force microscopy (AFM) and to investigate the differences between the surface topographies of glassy and recrystallized felodipine. We were interested in the possibilities of this method for investigating the glassy state and the nature of the recrystallization process of glassy felodipine.

AFM is a variation on the method for imaging surfaces with atomic or near-atomic resolution called scanning probe microscopy (SPM). The operating principle of an atomic force microscope is based on the detection of interatomic forces between a sharp probing tip and a surface. The probing tip[1] is attached to an elastic cantilever with a small spring constant ($k \approx 0.1$ N/m). The net force (F) acting on the tip is proportional to the cantilever deflection (Δx) by $F = -k\Delta x$, where k is the spring constant of the cantilever. The forces acting on the probing tip deflect the cantilever, whose bending is usually measured optically by the deflection of a laser beam reflected from the upper side of the cantilever. Prior to imaging, the tip is brought into contact with the surface[2] (contact mode AFM) or just very close to the surface (non-contact and tapping mode AFM). An AFM image is taken by moving the sample horizontally in a regular pattern using a piezoscanner. An image of the surface in a constant deflection regime is acquired by monitoring the vertical movements of the piezoscanner needed to keep the deflection of the cantilever constant as it is scanned across the surface. The image obtained reflects the topography of the surface. For display purposes, the height values and the tip deflection values are usually translated into a linear grey scale, where dark grey values represent low height values while light grey values are assigned to higher areas.

When imaging in ambient air, the sample surface is almost always coated with a thin layer of adsorbed water that forms a meniscus between the tip (the commercial tips are hydrophilic) and the sample and generates a large, attractive capillary force, which can produce artifacts in the image. The problem is detected by the large unexpected curvature of the image in the fast scan direction. It can be overcome using stiffer cantilevers or by imaging in a dry box (dry N_2 atmosphere) (zur Mühlen and Niehus, 1997; Keller, 2000).

Since the invention of AFM, it has become an important tool for imaging the topography of surfaces and for direct measurement of intermolecular forces.

The simplest method for measuring the distance dependence of the force between the tip and the surface is to monitor the deflection of the cantilever as the tip and sample are approached and moved apart. The graphical representation of the distance dependence of the force is called a force plot. Force plots are used to measure tip–sample interactions, determine optimal set points and to calibrate the detector sensitivity (DI, 1993; Keller, 2000).

Imaging in the contact mode can become problematic for soft or weakly adsorbed samples. The force of the tip on the sample can damage or remove the sample and the image is full of artifacts. The development of tapping mode AFM (TM-AFM) has overcome this problem. In tapping mode, the tip oscillates at its resonant frequency. When the tip is within the range of surface forces, its oscillation amplitude changes due to impact with the surface or shift of resonance frequency due to the surface forces. Compared with the contact mode the influence of the tip on the surface is substantially reduced.

It has been demonstrated that tapping mode AFM can be used to distinguish between different polymorphs — the phase images from 50:50 mixed samples of cimetidine polymorphs in the

[1] Apex radius of the commercial tip used is ≈ 15 nm.

[2] It means, that the tip is brought into the region of strong range steric repulsive forces.

form of pressed disks show a two-component surface with dark and light regions (Danesh et al., 1999). This technique (TM-AFM) is also convenient for the investigation of isolated single crystals (Danesh et al., 2000).

The advantages of AFM are high resolution, the ability to measure forces down to 10^{-11} N, simple sample preparation, no requirement for vacuum and a sample, which need not be conductive. AFM also allows the observation of 'in situ' processes occurring at interfaces (zur Mühlen et al., 1996). These properties are most important also for biological applications (Keller, 2000).

2. Materials and methods

2.1. Material

Felodipine, in the form of powder, was used as supplied by Luwitrade, Liechtenstein. It is a very sparingly water-soluble drug and freely soluble in absolute alcohol.

According to DSC measurements, the felodipine sample supplied consists of two polymorphs, II and III. This has also been reported earlier (Srčič et al., 1992). The melting points for the two forms were 141,8 and 144,0°C, respectively, and no other impurities were noticeable.

The contact angle of water on glassy felodipine was approximately 53–60° and on crystalline felodipine approximately 70–75°.

2.2. Sample preparation

The sample for AFM imaging was prepared by heating felodipine crystals on a clean glass slide through the melting temperature at $\sim$150°C. The liquid melt was allowed to cool to room temperature and a thin layer of glassy felodipine was formed.

2.3. Methods

The sample was observed in air using contact mode AFM with a J-type (100 μm scan size) scanner (Nanoscope III, Digital Instruments, USA) immediately, 1, 2 and 7 days after preparation under normal conditions of room temperature, 1 bar pressure and 40% relative humidity. The experiments were conducted in constant force mode. Surface topography images and force distance curves were recorded. Prior to the experiment, the scanner was calibrated using the standard procedure and D1[3] grating (DI, 1993). The grating is a silicon substrate with surface features of precisely known dimensions (DI, 1996).

After AFM experiments, the 'old' samples were additionally observed with polarizing microscopy (under crossed and parallel polarizers) (Nikon, Japan). We used the Hamamatsu digital camera (Hamamatsu Photonics, Germany) for image processing and took several pictures.

DSC measurements were performed with Pyris 1 equipment (Perkin-Elmer, USA). Crystalline felodipine samples (approximately 5 mg) were weighed and non-hermetically sealed in standard aluminium pans. A heating program from 0 to 180°C at scanning rate 1 K/min was used, with nitrogen flow of 20 ml/min.

The sessile drop method with Contact angle meter (Krüss, Germany) was used for contact angle measurements of water on felodipine glassy and crystalline states. In the latter case, 200 mg of felodipine was pressed (80 kN for 10 s) in a plate.

3. Results and discussion

For AFM imaging the glassy felodipine sample was prepared and the distance dependence of the force between the tip and the surface measured. Immediately after sample preparation, a long-range attractive force contribution was present between the tip and the sample, as is evident from the force plot (Fig. 1). However, 1 day later, the force plot was different and no long-range attraction was observed. The magnitude of the observed difference at a separation of 20 nm between the tip and the surface was 0.6 nN (Fig. 1). Such a specific, long range attractive force, is usually attributed to electric charges on the surface. It is

[3] Digital Instruments.

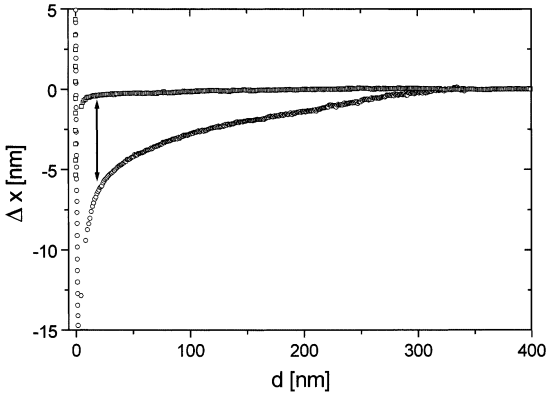

Fig. 1. Comparison between force plots on a felodipine sample immediately after glass preparation (circles) and 1 day later (squares). The difference in attractive force at a separation of 20 nm is marked with an arrow. Δx is the deflection of the cantilever and d is the separation between the tip and the sample.

possible that this Coulomb attraction was neutralized by the adsorption of water vapor and other gaseous contaminants, because the glassy state is highly energetic. After this relatively drastic change, the force plot did not change further. We also wanted to find out if the tip–sample interactions on glassy and recrystallized regions are different. No important differences were observed.

The possible influence of the capillary force on the images has been checked by performing experiments in a dry environment (dry N_2 atmosphere), which showed that the capillary force did not affect our measurements.

The AFM images were taken at a constant force of approximately 4 nN. Immediately after the preparation of the felodipine glassy surface, it was found to be very smooth and homogenous, and the maximum height difference was approximately 3–4 nm (Fig. 2). Then 1 day later, some new structures were observed on the surface. After 1 week at room temperature, macroscopic yellow crystallized circular spots appeared on the glassy sample. Crystallization occurred slowly and spontaneously at room temperature, i.e. below the glassy state transition temperature. The size of the appearing crystalline regions was not uniform (typical size (2r) was ~ 1 mm). Optical microscopy (magnification five times) was used to observe these spots (Fig. 3). During the observation no change of spot size or form took place. The crystallized regions were birefringent, as checked with a polarizing microscope under cross polarizers. The region appears to be made up of several crystallites. In contrast, the glassy region was completely dark under crossed polarizers, and therefore, optically homogenous (Fig. 4).

On further investigation of the felodipine glassy state, the AFM tip was placed on the border area

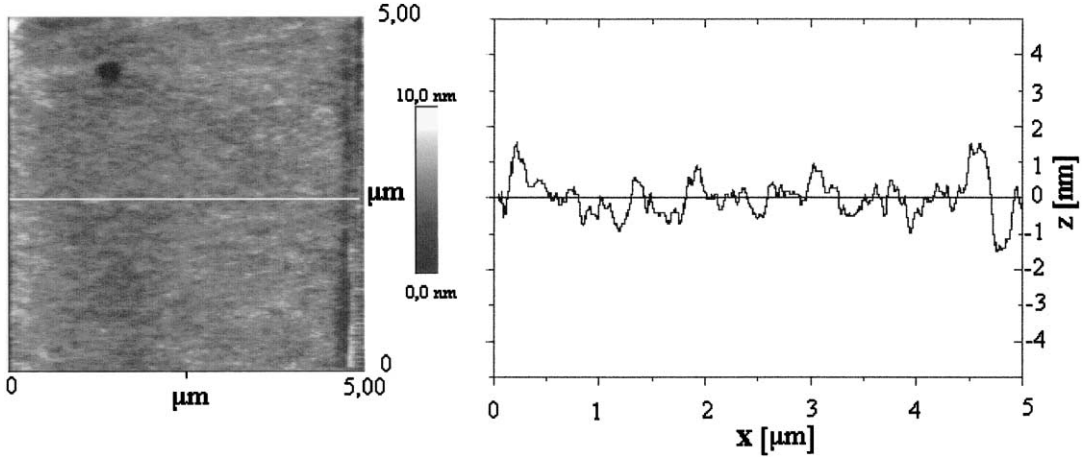

Fig. 2. Left image: The contact mode AFM image of glassy felodipine surface topography (5×5 μm) observed immediately after sample preparation. Right image: Section analysis — the maximum height difference was approximately ± 2 nm. The RMS amplitude of the surface roughness of glassy state felodipine is 0.6 nm.

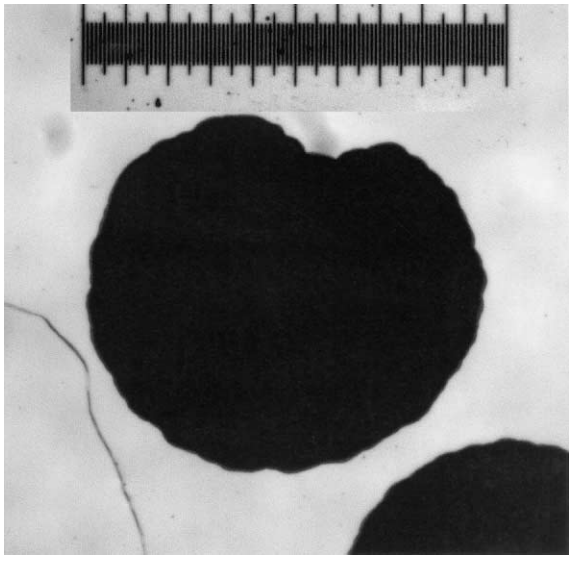

Fig. 3. Macroscopic spot on a sample of glassy felodipine observed with optical microscopy (magnification 5 times). The spot size is approximately 1 mm.

Fig. 4. The crystalline region on a 7-day-old felodipine sample observed by polarizing microscopy under crossed polarizers (magnification 20 times). Note that the glassy region is dark and the crystallized region is birefringent.

between the glassy and recrystallized zones and the image displayed in Fig. 5 was taken. The contrast between the glassy felodipine and yellow spot zones is shown to be the different roughness of regions A and B. The border between the two regions is very sharp. The glassy region A is flat and smooth, but the crystalline region B is coarse and rough (Fig. 6). The RMS[4] amplitude of the roughness of the crystallized region is approximately 60 times larger than that of the glassy zone. On a 3-D image, it can be seen that the glassy felodipine changed to the crystalline state in depth and not just on the surface. Sharp edged regular shapes resembling crystallites were observed in region B after imaging it at a smaller scan size.

When the glassy felodipine region at the border between A and B was exposed to the mechanical stress of the AFM tip during contact mode imaging, additional crystallization was observed. The mechanical stress was produced by increasing the AFM set point (and therefore, increasing the force), scan rate and instrument gain. We estimate that the force of the AFM tip to sample was 40 nN with this set of parameters. This force produced a local pressure of about 30 MPa (300 bars). We scanned the sample at this force for 20–30 min at scan size 20 μm. After that, the force of the tip was reduced back to 4 nN, the scan size increased to 50 μm and the deformed region scanned. Additional crystallization was observed at the place scanned with high pressure. The 'tip induced' crystalline region on Fig. 7 is denoted by arrows. The instantaneous crystallization consequent on this mechanical stress can be explained by the instability of the glassy state. During the period of observation, the whole glassy area under observation crystallized and became rough.

When, in contrast to the above experiment, fresh sample was used and the glassy felodipine was exposed to the mechanical stress produced by the tip, recrystallization did not occur. The topography image of glassy felodipine, with normal instrument settings, can always be taken without any sample damage.

In conclusions, we can say, that other microscopic techniques do not allow the roughness of glassy and recrystallized felodipine surfaces to be

[4] The roughness was determined with Root Mean Square procedure (RMS) (DI, 1993).

estimated and the differences between those two regions to be visualized on the nanoscale. It is, however, not possible with AFM to determine in which polymorphic form, the recrystallized felodipine resides (it is possible with DSC technique).

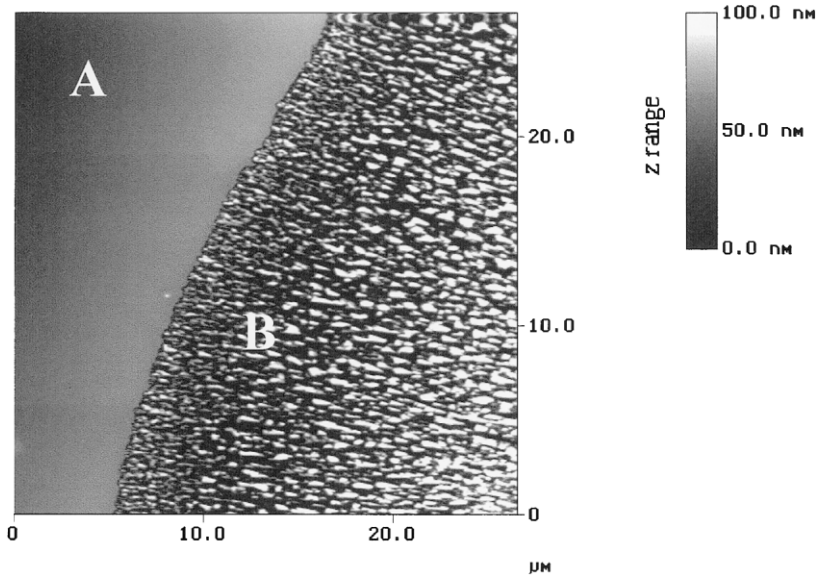

Fig. 5. The topography of felodipine surface (25 × 25 μm) 7 days after glass preparation. Region A is glassy felodipine and region B is crystallized felodipine. Note that the z range is 100 nm, in contrast to the Fig. 2, where the z range is 10 nm.

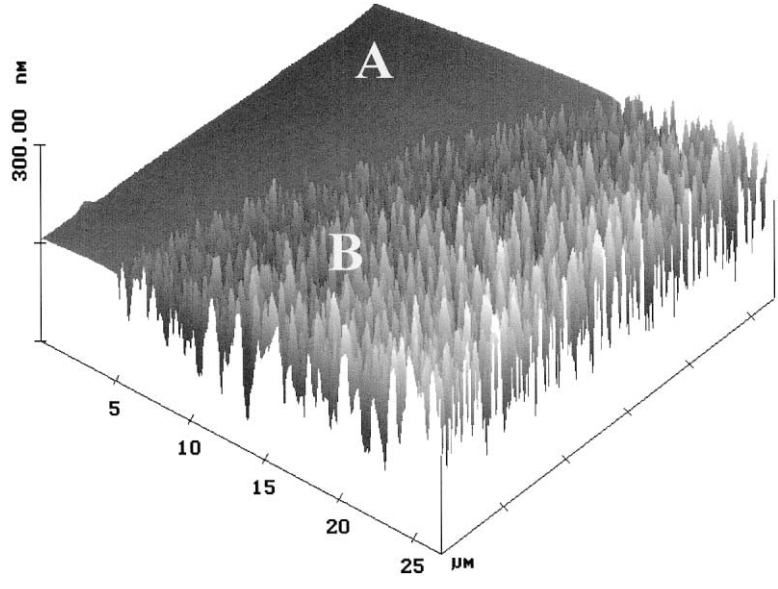

Fig. 6. Fig. 5 displayed as a 3-D image. Note that the crystalline region, which sprang spontaneously from glassy felodipine is not restricted to the surface.

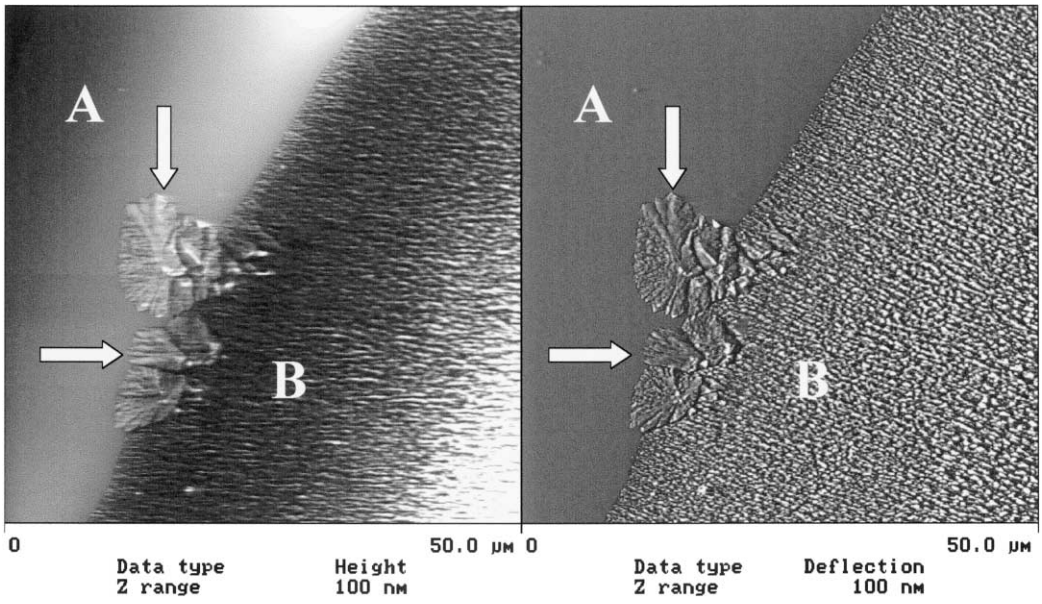

Fig. 7. Topography of a felodipine surface (50 × 50 μm) after mechanical stress was applied with the tip (the area of the scan encompasses the scan region of Fig. 5). Arrows denote the appearance of 'stress induced' crystalline areas. The local pressure under the AFM tip was approximately 30 MPa. The RMS amplitude of the surface roughness of the crystalline region is 36 nm.

The main advantage of the AFM technique in this study proved to be the online monitoring of the crystallization processes and the ability to induce additional crystallization. We conclude that AFM is an appropriate and valuable method for studying glassy and crystalline states of small molecules like felodipine and transitions between them.

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