Characterisation of Drug Nanoparticles by Atomic Force Microscopy

N A Bailey, J Booth and B C Clark

AstraZeneca Pharmaceuticals, PAR&D, Macclesfield, Cheshire, SK10 2NA, England
Nicola.Bailey@AstraZeneca.com

ABSTRACT

Nanoparticles of a bicalutamide nanosuspension were imaged using Atomic Force Microscopy (AFM) to gain some insight into particle size and aggregation. Particles of bicalutamide in a PVP-stabilised suspension were imaged and the data analyzed. The particles were observed to be uniform in size at down to 160 nm in diameter although some larger aggregates were observed. Sample roughness was analyzed and found to be highly dependent on the level of image processing that had occurred; therefore it was important that the calculations for area roughness be noted at each stage of processing so they could be compared. The relative area coverage was analyzed by characterizing the fractal index and the potential for this type of calculation to investigate nanoparticles stability discussed. Ideas for future work are also presented.

Keywords: nanoparticles, atomic force microscopy, particle size

1 INTRODUCTION

Drug nanoparticles are among the most exciting and promising approaches to the challenge of delivering poorly soluble drugs [1]. Because a large proportion of compounds now under investigation by the pharmaceutical industry have low aqueous solubility, interest in drug nanoparticles has rapidly grown, as reflected by the growing availability of processing technologies and published literature in this area.

A key consideration when developing a drug nanoparticle formulation, particularly when intended for parenteral administration, is the control of particle size, shape and solid state, which in turn impact on drug solubility, dissolution kinetics and Ostwald ripening rate. These factors, in turn, determine the safety, shelf life and pharmacokinetic performance of such dosage forms. Consequently, applying meaningful approaches to the characterisation of drug nanoparticles is a prerequisite to the development of viable formulations.

Atomic Force Microscopy has emerged as an important technique for studying particles in the colloidal size range. AFM allows detailed analysis of the dimensions and architecture of individual particles and is therefore complementary to "bulk" particle-sizing techniques such as light scattering, which are applied routinely to the characterisation of nanoparticles. The technique has been further developed to enable real time imaging in order to track spherulite growth in polymers [2]. AFM also enables investigation of the effect of different additives, surfactants, and methods of formulation on crystal growth. There is a diverse range of literature in this area with AFM proving to be a key technique for characterization at the nano-scale.

In situ analysis is proving an increasingly popular method of analysis by AFM as it allows for real time processes to be followed and the kinetics for the process to be determined. In this way dissolution properties have been determined [3-4] as well as growth [5], or a combination of both processes [6]. Further, others have compared the effect of additives [7-8], acidic with alkaline conditions [9] and the effect of habit modifiers on the crystallization process [10] all in situ.

The focus for this research was whether the technique could provide a method for studying compounds early in development where less material is available. If so, this would find wide application within the pharmaceutical industry.

2 EXPERIMENTAL

2.1 Nanoparticle preparation

Crystalline nanosuspensions of bicalutamide, a poorly water-soluble anti-cancer agent, were prepared by a controlled precipitation technique. Nanosuspensions were prepared in a PVP solution, then pipetted onto a metal stub and allowed to air dry. The resulting film was analyzed using AFM.

2.2 AFM imaging

The samples were imaged in either contact or tapping mode using a TA Instruments 2990 µ-TA system with a silicon nitride 1660-00 probe. Samples were initially

imaged over a wide area and then areas of interest identified and imaged at higher resolution. Topography, forward and reverse sensor and z-piezo images were collected simultaneously, with forward and reverse sensor compared to try and identify and potential artifacts in the images. Images were processed with the SPM Lab software.

2.3 Roughness analysis

The calculated roughness values are derived from the SPM Lab software package. The average roughness (Ra) is defined as the arithmetic mean of the deviations in height from the image, Eq. (1), and the root mean square roughness (RMS) defined as the square root of the mean value of the squares of the distance of the points from the image mean value, Eq. (2), with the average height being the sum of all height values divided by the number of data points as given in Eq. (3)

$$R_a = \frac{1}{N}\sum_{i=1}^{N}\left|z_i - \bar{z}\right| \quad (1)$$

$$RMS = \sqrt{\frac{1}{N}\sum_{i=1}^{N}\left(z_i - z\right)^2} \quad (2)$$

$$\text{Avg. Height} = \frac{1}{N}\sum_{i=1}^{N} z_i \quad (3)$$

2.4 Fractal analysis

The calculated fractal analysis values are derived from the SPM Lab software package, which takes its algorithm for the calculations from the Gomez-Rodriguez publication [11]. The fractal dimension of the 3-D surface can be calculated from a log L vs. log A plot, where L is the perimeter and A the area for each lake.

3 RESULTS AND DISCUSSION

Images of the nanoparticles were collected so that the homogeneity and aggregation of the particles could be examined with a typical image of a deposited bicalutamide nanosuspension shown in Figure 1.

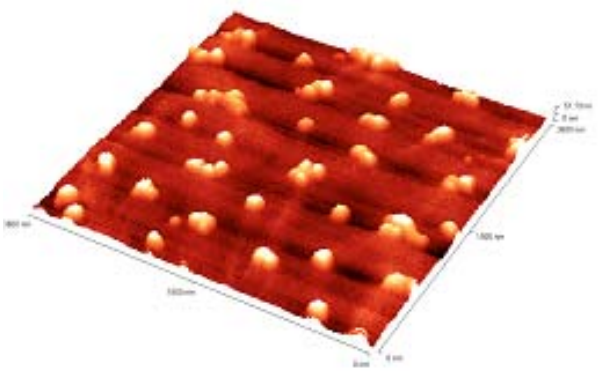


Figure 1: Tapping Mode image of nanoparticles dispersed in a PVP solution

To ensure that the images collected were valid an area on the same stub but away from the sample was imaged for comparison and lacked the defined structure of the sample area, implying that we were genuinely imaging the nanoparticles. Further a blank PVP solution was also imaged to show that the nanoparticles observed weren't a result of a contaminant in the PVP. Once it was established that the images observed weren't artifacts of the method of preparation or imaging, the particle size was examined by applying a line analysis at different points in the image and measuring the particle diameter (see Fig. 2). Particle sizes down to approximately 160nm were measured although some larger particles were also present. These particle sizes were consistent with particle size data from dynamic light scattering experiments (not shown). This was expected as the use of AFM as a method for determining particle size has been considered in the characterization of Ferro-fluid's and the data found to be comparable to traditional techniques such as TEM [12] and therefore validated the analysis conditions. The presence of larger particles may be due to itinerant particles formed during the precipitation process or from aggregation. The height of the nanoparticles by this technique was measured to be only 60nm as shown in the line analysis (Figure 2b), which may imply non-spherical particles. However, more likely the nanoparticles are partially imbedded in the PVP suspension media and therefore the height analysis data should be treated with caution.

The surface roughness was also calculated as a method of comparing the dispersion of nanoparticles in different environments, based on the area of the whole image. The data was calculated after different levels of leveling and very different results obtained, see Table 1. As the image underwent additional processing the surface roughness dramatically decreased, illustrating the importance of comparing images, which have been processed in similar ways. This has also been reported by others who have commented that when doing these calculations the image is not corrected for the tilt in the plane of the data and therefore fitting or flattening the image will alter the data [13]. For the roughness measurements to have any

significance they should be repeated over a number of regions of the sample and compared to samples prepared in different media/ conditions. In the context of the data presented here, it is meant to qualitatively demonstrate the significance of data processing in terms of interpreting resultant data.

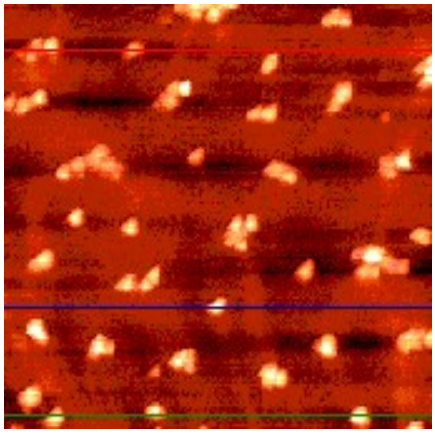


Figure 2a: Image showing points for line analysis as taken from AFM image in Figure 1

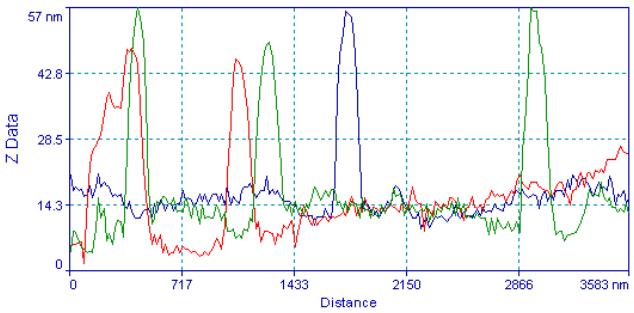
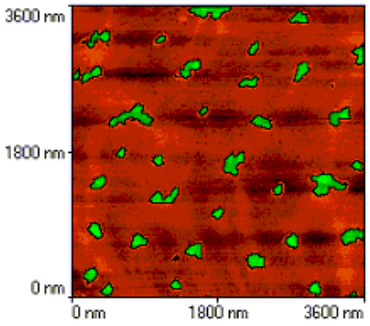


Figure 2b: Using line analysis to get an indication of particle size

	Raw Data	**Quick Leveling**	**Horizontal Leveling**
Area Ra	380.86	73.04	5.61
Area RMS	447.70	87.00	8.70
Average Height (nm)	1016	255.7	67.4

Table 1: Roughness calculation comparison under different processing conditions

The relative coverage area of the particles was calculated from the fractal index of the nanoparticles as determined using the SPM lab software (see Figure 3). . For the initial sample this was calculated to be 8.49% based on the area imaged - see Figure 3. Assuming the concentration is constant, it is assumed that samples taken at different times would therefore be representative of any change with time, with an increase in coverage area potentially being an indication of ripening or a decrease in coverage area of dissolution. No change in coverage area may be an indication of stability in solution.



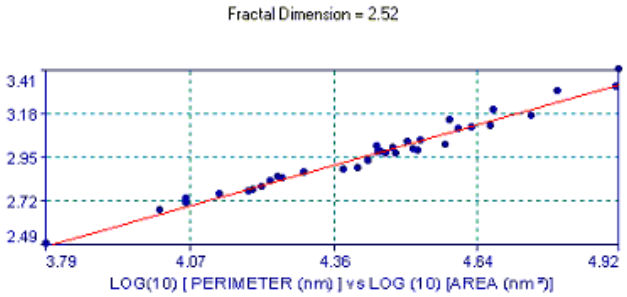


Figure 3: Using the fractal index to calculate percentage coverage area

In future, by monitoring the evolution of particle coverage and size distribution as a function of time this approach will be used to measure particle ripening and dissolution kinetics in parallel with topographical imaging. By imaging under liquid this will enable correlation with real time dissolution data. In this way the physical stability of nanoparticles can be studied in a direct manner. However this would only be a viable method of measuring the kinetics if they were sufficiently slow that the image doesn't change in the time taken to collect an image of sufficient resolution. Otherwise a real-time imaging method such as that presented by Hobbs et al. [2] would need to be considered.

4 CONCLUSIONS

AFM has been shown to be a powerful tool in visualizing the morphology and measuring the particle size of nanoparticles. This technique provides direct, real time, high-resolution spatial information about particle aggregation and dissolution phenomena, and is therefore a useful technique to underpin the design and optimization of nanoparticle formulations for the delivery of poorly soluble drugs.

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