

# STRUCTURAL CHARACTERIZATION OF DRUGS: FROM THE NANOSCALE TO DRUG RELEASE.

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## Introduction

Elucidation of the molecular conformation of a biomolecule is an important step in determining the relationship between physical structure and biochemical function. The conformation of any biological macromolecule governs the physical, chemical and mechanical properties of the molecule either individually or collectively, as aggregates.

For this reason, there has been a great deal of effort in the development of techniques that can directly resolve the structures of individual biological macromolecules within the nanometric scale. Foremost among these techniques has been atomic force microscopy (AFM), whose potential for investigating biological samples on a scale ranging from living cells to single molecules has been recognized since its introduction almost two decades ago [1]. The advantage of AFM is not so much resolution (nanometers) but the fact that molecules can be imaged in their native environment (in buffer, room temperature, etc.).

The introduction of *intermittent-contact mode* or *tapping mode* AFM (TMAFM) is a key advance in the AFM technique. This potent tool allows medium resolution but very robust imaging conditions in most media of materials that are difficult to image by other AFM operation modes. TMAFM overcomes problems associated with friction and shear forces by alternately placing the tip in contact with the surface to increase the resolution, and then lifting it off to avoid dragging and damaging the sample surface.

With all these characteristics, Tapping Mode technique is useful because delicate samples can be imaged without severe distortion. By this means single molecule observations [2-4] and surface roughness measurements [5] of pharmaceutical systems have been reported.

A powerful extension of TMAFM is phase imaging, that provides nanometer-scale information about surface viscoelasticity properties often not revealed by other techniques. Phase images

show the phase lag of the cantilever oscillation relative to the excitation signal sent to the cantilever's piezo driver as it interacts with the sample surface. Accordingly, phase imaging goes beyond simple topographical mapping to detect differences in the interaction of the tip with the different materials on the studied surface, giving rise to properties such as composition, adhesion and viscoelasticity. Besides, since phase imaging highlights differences in viscoelasticity hence detecting different materials on the sample surface, feature edges are also highlighted. Consequently, this imaging mode is not affected by large-scale height differences, thus providing for clearer observation of fine features, which can be obscured by rough topography.

The rapidly growing list of phase imaging applications includes characterizing the components of composite materials, mapping of surface friction and adhesion, and identification of surface contamination. On the other hand, since the difference in phase shift partially depends on the local ionic concentration, the surface charge on biological macromolecules has been directly observed by this means [6]. Finally, in phase imaging molecules can be resolved at higher scan rates and lower forces than in topographic imaging. Higher scan rates make it possible to image faster processes, and therefore allows for a better imaging of dynamic processes. In this fashion, Argaman *et al.* showed high quality images of moving DNA and DNA complexes in aqueous buffers [7].

## Objectives

The final goal of this work is to provide a structural interpretation of the interaction capabilities of dexchlorpheniramine maleate (DCPM) within the nanometric scale. To this end, DCPM samples are thoroughly characterized by means of TMAFM.

## Experimental

AFM studies were performed in Tapping Mode with an Extended Multimode microscope, and controlled by a Nanoscope IIIa electronics (Digital Instruments, Santa Barbara, CA).

The AFM probes were ultrasharp silicon tips (NT-MDT, Moscow, Russia), manufactured in monocrystalline silicon with a nominal tip radius of about 10 nm (also checked by SEM), pyramidal shape, a spring constant of approximately 35 N/m and a resonant frequency of about 300 KHz.

Samples were studied in both highly oriented pyrolytic graphite, HOPG (Advanced Ceramics, Cleveland, OH) [8,9], and in muscovite mica (Asheville-Schoonmaker Mica Co., Newport News, VA) [10].

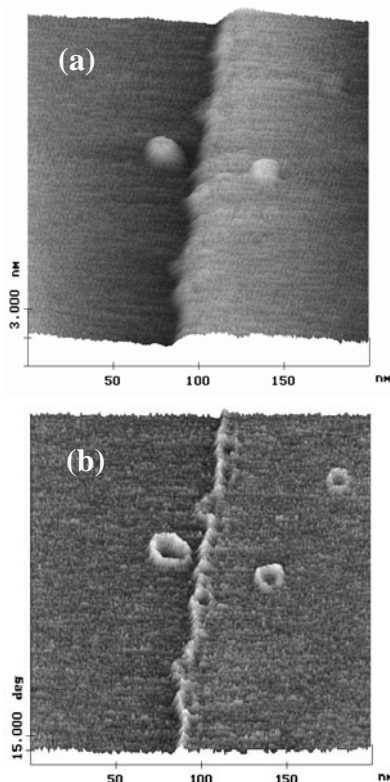
Details on sample preparation, deposition onto substrate and drying protocol have already been described in previous works [11].

## Results and Discussion

AFM characterization revealed a lot of useful information about DCPM nanostructure. But the knowledge about the chemical properties of the drug and a rather high degree of familiarity with the technique were essential for a correct interpretation of the experimental data.

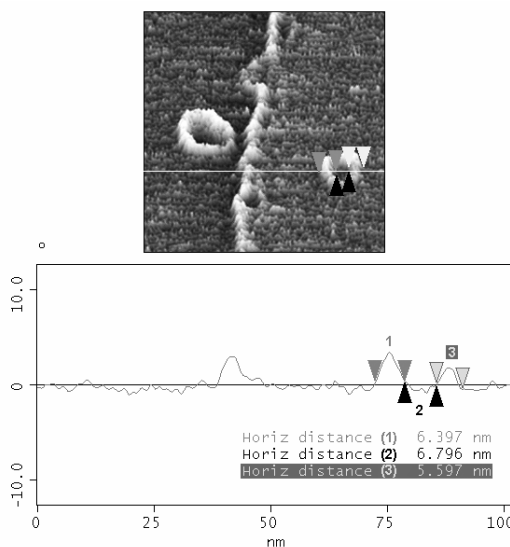
Topographic AFM images of DCPM molecules adhered to an HOPG surface show highly homogeneous spheroidal particles randomly distributed on HOPG terraces (*Fig. 1a*). Moreover, several irregularities can be observed on the graphite step, suggesting the presence of molecules. Step edges are usually preferential deposition sites since they provide higher interactions with the substrate. No further interpretation of DCPM structure could be obtained from topographic data.

A clearer understanding of DCPM structure was achieved from phase images: Viscoelastic changes provided a better definition of feature edges, since we got strong hardness differences between the molecules and the substrate. Ring-shaped molecular structures were observed. Besides, those molecules "attached" to the HOPG steps mostly appear in an extended way, thus identifying those HOPG irregularities (*Fig. 1b*).



*Figure 1. Characterization of DCPM by Topographic AFM (a) and Phase Imaging (b)*

Section analysis of DCPM molecules had therefore to be performed from both topographic data for height measurements, and from phase data for lateral dimensions (*Fig. 3*).

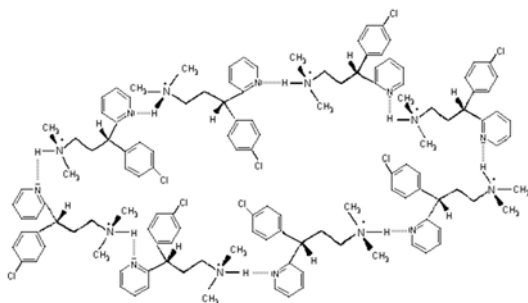


*Figure 2: Section analysis of DCPM from viscoelastic data (phase images).*

The measurement of the internal diameter of DCPM rings, which was found to be about 3.5 nm clarified the limitation of topographic imaging: the AFM probe could not detect such a cavity because it was much smaller than the tip diameter, which is about 20 nm.

The first analysis derived from these results was to consider if the observed rings were single molecules or aggregates of them. To this end, from the measured ring diameter, the perimeter of the observed thoroids was calculated, which was about 15 nm. On the other hand, the length of a single DCPM molecule was estimated from the interatomic distances in the molecular formula, and was about 15 Å. Therefore, we assume the presence of up to 10 DCPM molecules per ring.

In aqueous medium, and in the presence of the maleate anion, the ammonium group of the dexchlorpheniramine is positively charged, and therefore leads to the formation of hydrogen bonds with the pyridinic nitrogen of another dexchlorpheniramine molecule. The proposed model for the chemical structure of the observed DCPM rings is presented in Fig. 3. However, the planar representation of the molecules does not exactly match their 3D conformation: the interaction would be stabilized by the so-called  $\pi$ -stacking phenomenon, consisting of the piling of aromatic rings, which depends on the environment, since it would be affected by the presence or lack of other rings.

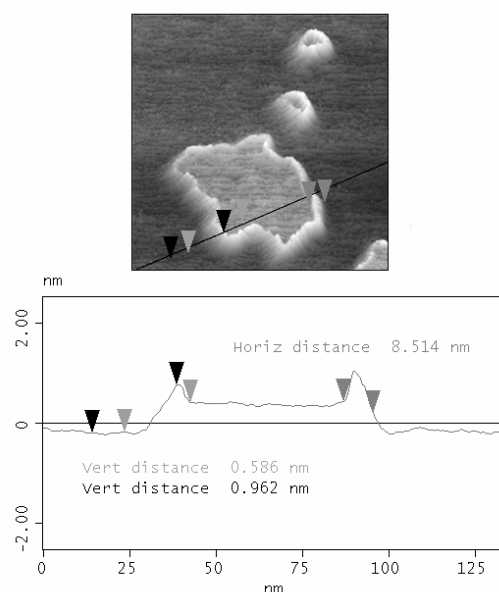


**Figure 3.** Proposed chemical structure for DCPM rings observed on HOPG terraces.

Since HOPG is an inert, highly hydrophobic substrate and thus no capable of interacting with hydrophilic molecules, intermolecular interactions among sample molecules take more relevance

on HOPG than molecule-substrate interactions. Conversely, freshly cleaved mica is a negatively charged surface, and therefore highlights the interactions between the substrate and the sample. DCPM amphiphilicity allowed to characterize it also on mica and, consequently, to evaluate its behavior in charged media, obtaining more information about DCPM structure and self-assembling capabilities.

In that respect, although ring-like structures were still observed, these exhibited irregular shape and much bigger diameters, suggesting a different interaction mechanism that involved from several tens of molecules to hundreds of them. Further, the presence of a layer of ~6 Å high (Fig. 4) of unknown nature inside DCPM rings, pointed out the need for acquiring also phase images, in order to identify the nature of the structures being imaged.



**Figure 4.** Section analysis of DCPM on mica from topographic data.

Figure 5 shows a comparison between topographic (5a) and phase (5b) images, the latter exhibiting the presence of three different chemical structures: It is plain that the darker area corresponds to the mica substrate. Further, the diameter of the strings is in the same order of magnitude than that measured for DCPM chains on HOPG, confirming that these were indeed DCPM chains. But the chemical nature of the

white areas included within DCPM margins was still unresolved.

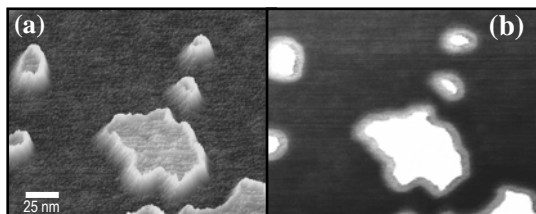


Figure 5. Topographic (a) vs. phase images of DCPM on mica substrate.

Data from the literature demonstrate that in ambient conditions, water condenses on freshly cleaved muscovite mica forming flat, two-dimensional films with an ice-like structure. The reported average thickness of these monolayers was about 2 Å at ~50% RH [12]. Furthermore, molecularly thin films of water are very stable because of the strong binding of water on mica due to its polar nature.

On the other hand, the quaternary ammonium group of DCPM is positively charged in aqueous medium at acidic-neutral pH values ( $pK_a(-NH_4^+) = 9$ ), and will therefore interact ionically with the freshly cleaved mica surface, which appears to be negatively charged [10,12]. Then, it is coherent to assume that up to three water films can be confined inside DCPM chains, stabilizing the interaction. The strong phase signal also agrees with these assumptions.

Finally, the different particle sizes exhibited by DCPM on HOPG vs. mica surfaces reflect its capability to undergo conformational changes depending on the interaction mechanism, which is an indicator of the self-assembling potential of DCPM molecules (Fig. 6).

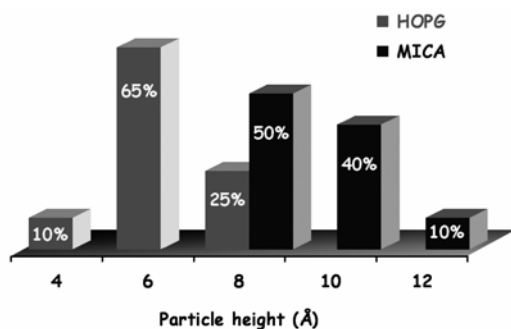


Figure 6. Particle size distribution of DCPM chains on HOPG and mica substrates.

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