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## Measurement of Activity and Stability of Physical Vapor Deposition (PVD) Coated Crystal Surfaces

Anuradha Krishnan  
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MEASUREMENT OF ACTIVITY AND STABILITY OF PHYSICAL VAPOR  
DEPOSITION (PVD) COATED CRYSTAL SURFACES

by

Anuradha Krishnan

A thesis submitted to the Graduate College  
in partial fulfillment of the requirements  
for the degree of Master of Science in Engineering  
Chemical and Paper Engineering  
Western Michigan University  
December 2016

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## MEASUREMENT OF ACTIVITY AND STABILITY OF PHYSICAL VAPOR DEPOSITION (PVD) COATED CRYSTAL SURFACES

Anuradha Krishnan, M.S.E.

Western Michigan University, 2016

The overall objective of this research work is to investigate the hydrophilic and hydrophobic forces acting on organic crystal planes and understanding the interaction of functional groups with additive molecular nanolayers. Crystals of acetaminophen and lactose were made and the surface forces were tested on Atomic Force Microscope (AFM). Uniformity of crystal surface was confirmed from the AFM study in the *first stage*. The growth factors of crystal planes as a function of time were studied. Adhesion forces on plain crystals were measured on both two different organic crystals made of acetaminophen and lactose. In the *second stage*, the crystals were coated with 10, 20 and 50 nm thick SiO<sub>2</sub> nanolayers using Physical Vapor Deposition (PVD). The adhesion forces were compared to that of the plain crystals measured in the first stage. In the *third stage*, two different AFM probes were functionalized with hydrophobic and hydrophilic functional groups of decane thiol and octanoic acid. In the *fourth stage*, the AFM forces were measured on the SiO<sub>2</sub> coated crystals with the functionalized AFM probes. Surface topography was found to change as a function of time. Uniformity and thickness of PVD coating was found to increase the adhesion forces on both crystals. Hydrophilic functionalization increased the adhesion force of SiO<sub>2</sub> coating. Hydrophilic functionalized groups were found to possess higher adhesion force than hydrophobic functional groups on SiO<sub>2</sub> nanocoated crystals. Chemical nature of crystal molecule was found to dramatically affect the adhesion force of functionalized group.

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Anuradha Krishnan

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## ABBREVIATIONS AND SYMBOLS

AFM	Atomic Force Microscopy
PVD	Physical Vapor Deposition
TEM	Transmission Electron Microscopy
SEM	Scanning Electron Microscopy
XRD	X- Ray Diffraction
SXRD	Silicon X-Ray Diffraction
API	Active Pharmaceutical Ingredient
APAP	Acetyl-Para Aminophenol
XPS	X-ray photoelectron spectroscopy
EDX	Energy-Dispersive X-ray
MgSt	Magnesium Stearate
CS	Colloidal Silica
ADNC	Automated Direct Nucleation Control

# 1. INTRODUCTION

Nanotechnology in pharmaceutical industry is an interesting area of scientific exploration due to its wide applications in particle interaction, powder flow, formulation and drug delivery platforms. Recently, several studies have been reported on how nanoparticles improve powder flow [1-4]. However, the particle interaction between micro particles and nanoparticles is not well understood. Although several studies were reported on powder flow including micro particles [5-10], the role of nanoparticles in altering the microparticle interaction continues to intrigue the scientists. In order to understand the particle interaction on micro scale and macro manufacturing, it is essential to have a fundamental understanding on a molecular scale on how these drug molecules behave when in contact with foreign particles. In this context, hydrophobic and hydrophilic forces are the most important which have a direct impact on drug release and dissolution of the active pharmaceutical ingredients (API). Therefore, it is essential to study the fundamental behavior of the drug molecules and their principal interactive behavior with hydrophobic and hydrophilic forces. Once this phenomenon is understood, it will be relevant to investigate the nanoadditive interactions that are caused due to the presence of lubricants or glidants (nanoscopic additive particles). Interestingly, previous studies indicate that a thin nanosmeared layer of lubricant or glidant can alter the overall drug release behavior [11, 12]. Hence, it is essential to study the interaction of lubricant (hydrophobic) and glidant (hydrophilic) with API (drug). The fundamental molecular understanding of these interactions at molecular scale can be better understood when crystals of drug materials are used. Hence, in this study, we investigated the hydrophobic and hydrophilic molecular interaction behavior with crystal planes of API (acetaminophen or acetyl-para aminophenol (APAP)). We made crystals of APAP, coated them with nanolayers of lubricant and glidant, and subsequently measured the AFM forces of

hydrophobic and hydrophilic functional groups with nanocoated layers on crystal planes. Adhesion forces of the functional groups with the crystal planes were studied by bringing the functional groups on AFM tips closer to the crystal surfaces.

## **1.1 PROJECT DEFINITION**

Crystals have been used for understanding surface modification; tablet making and film coating for making tablet. Initially crystals were made of required size where they were coated with hydrophilic and hydrophobic groups to check the surface modification and the force of attraction in crystal planes. The hydrophobic structure of ibuprofen drug showed bad dissolution and tableting behavior [13]. High cohesive nature of ibuprofen resulted in low flowability [13]. Ibuprofen had bad compaction behavior hence it had to be granulated usually before tableting [13]. A drug crystal which had characters and properties of ibuprofen was prepared [13]. In the past studies crystallization was carried out using the solvent change technique in the presence of different additives [13]. These additives were only present during the crystallization process and were removed by washing them off [13]. The shape of the crystal was like a plate which had high powder dissolution, increased flowability and good tableting behavior [13]. Past studies showed that ibuprofen molecules can form hydrogen bonds, additives interacted with these hydrogen bonds during the crystallization process modified the properties of the resulting crystals [13].

The presence of impurities and external disturbance can affect the chemical properties, nucleation and morphology of crystals [14]. In the past study, atomic force microscopy (AFM) and scanning electron microscopy (SEM) were utilized to investigate the influence of additives of acetaminophen (API) on its crystal surface [15]. To increase the flowability of materials and

improve tablet making, dimenhydrinate crystals as a model drug were used for crystal coating [16]. The influence of coating material and coating time affected several parameters such as the surface free energy parameters, the wetting of the samples, shape of the particles, the flow properties and surface free energy parameters, compressibility and compatibility [16]. The role of surface properties that were influenced by particle processing, in particle–particle interaction was investigated [17]. One of the most important drug delivery platforms was nano sizing approaches for the development of poorly soluble drug molecules [18]. Continuous coating process of a single API crystals in a tubular reactor to coat crystals was found [18]. Coating API crystals had various advantages where crystals could be prepared with coating to change in surface energies or to modify the drug release rate of the API i.e., the hydrophilic and hydrophobic nature of the drug, properties such as flowability and agglomeration [19]. To understand the underlying effects of the material science involved, atomic force microscopy (AFM) was used on an active pharmaceutical ingredient, acetaminophen (APAP) [21]. Atomic force microscopy (AFM) is an advanced and the most influential surface analysis technique used for micro structured and nanostructured samples [21]. The AFM is a flexible technique that can be used to obtain high-resolution nano scale images and study local sites in air or liquid surroundings which is also called the conventional AFM and the electrochemical AFM respectively [20, 21]. Hence AFM is an instrument where it helps us find the surface topographies of the sample surface, the forces of attraction and mapping of these attractions are recorded [21]. Previous studies showed surface adhesion and morphology as a function of relative humidity (RH) for acetaminophen, using both plain AFM tips and functionalized AFM tips with hydrophobic or hydrophilic [21].

The method Atomic Force Microscope Nano indentation measured the hardness of individual pharmaceutical solid particles that include sucrose, lactose, ascorbic acid, and ibuprofen

[22]. Past study showed that, thin coating was a method used for improving flow properties of cohesive drug powder [23]. This technique was based on some kind of deposition method where thin hydrophilic coating was done on individual Active Pharmaceutical ingredient [23]. The technique was a simple and effective method that could be used as a continuous process to modify API particle surface properties, which in turn improve the flow properties of powder [23]. Physical and chemical analysis of the surface was done by scanning electron microscope (SEM) with energy-dispersive X-ray analyzer (EDX) and analysis of crystal structure using X-ray diffraction (XRD) [24, 25].

## **2. PROJECT OBJECTIVE AND SCOPE**

Although several methods of crystals exist in practice, their interaction with hydrophilic and hydrophobic forces is least known in literature. Moreover, the effect of such forces in the presence of controlled humidity is relatively unknown. Hence, in this project, we addressed this area by investigating the hydrophobic and hydrophilic functional groups with API and excipient crystal surfaces. The project work is sequentially organized as follows. In the *first stage*, crystals of APAP and Lactose were made from saturated solutions of APAP and lactose. *Secondly*, thin layers of glidant (colloidal silica - CS) were coated on crystal planes using Physical Vapor Deposition technique (PVD). The crystal planes were tested for their growth behavior as a function of time and measured for their AFM forces to understand the effect of biomolecular chemical variations. This gave the topography of stability of the crystal plane. In the *third stage*, crystal planes were nanocoated using PVD technique with varying thicknesses of 10 nm, 20 nm and 50 nm. AFM forces were measured on nanocoated crystal planes and the effect of coating thickness

was studied. In the *fourth stage*, AFM tips were functionalized with hydrophobic and hydrophilic groups. In the *fifth stage*, mapping of adhesion forces on crystal planes was performed on both APAP and lactose crystals. These mappings were performed both before and after PVD coatings and the adhesion forces were compared on both crystal planes (coated and uncoated). In order to understand the interaction of these groups with excipient material in a pharmaceutical formulation during mixing, it is essential to relate the studies to excipient materials. We then finally replaced APAP (Active Pharmaceutical Ingredient) crystals with cellulose crystals and repeated the study all over again to compare the effects of chemical nature of crystal planes on nanocoating and functional interactions of both hydrophobic and hydrophilic groups.

### **3. BACKGROUND**

Saturated and supersaturated solutions are two successful methods of crystallization [26]. One among such methods, the most commonly used method was the solvent evaporation technique to achieve super saturation [26]. Another method to achieve super saturation the vapor diffusion method, where super saturation solution was obtained from solvent evaporation [26]. The crystallization process was influenced by the actual concentration distribution and to control super saturation, it was important to the crystalline formation [26, 27]. The constant composition method was suitable for the study of crystallization processes in previous studies [27]. Round and dark spots on crystal surface were examined [28]. X-ray diffraction analysis was used to investigate the crystallization process of indium tin oxide, which was in amorphous form to study the peak intensity, obtained through high-temperature [29]. Other techniques were used to estimate the growth parameters, nucleation, agglomeration and deactivation in crystallization [30]. Previous

studies showed experiments were carried out in several plastic bottles as crystallizers [30]. To obtain clear crystal with progressive nucleation to take place them crystallization solution was kept under a steady cooling temperature where it was found that new crystallites were continuously nucleated among the already growing ones [31]. Crystallization in pharmaceutical industry, was a major technological process for particle formation and played an important role in defining the stability, drug release rate [32]. Industrial aspects and regulatory aspects of crystallization were briefly reviewed with reference to its properties [32]. The process of crystallization in pharmaceutical industry incorporated many properties such as precipitation, solid-state transition, direct compression, separation of drugs, production of materials for inhalation drug delivery and injections [32]. Pharmaceutical regulatory bodies require minimal presence of solvent in an active pharmaceutical ingredient (API) after crystallization [33]. Crystals that were big in size, which had uniform size, and less agglomeration property were preferred [33]. Automated direct nucleation control (ADNC) was an approach that resolved problems like agglomeration and solvent inclusion [33]. Previous studies showed that this approach was able to produce larger and uniform crystals and removed the residual solvent trapped between the crystals compared to the typical crystallization operation [33]. In previous studies, Raman mapping was considered feasible and an attractive tool to understanding the crystallization mechanism of any drug with images [34]. Super saturation of a drug in a system where the drug is absorbed at a very slow rate was often consider a desirable therapeutic dose at a specific target [35]. Crystallization process of drug during storage was important for its longer life span than its usual life span [35]. One of the most important drug delivery platform approaches for the commercial development of poorly soluble drug molecules was nanosizing and all major pharmaceutical companies have understood the importance of drug nanocrystals and included this approach for manufacturing drugs [35, 36].

Preliminary studies of our group [37] have shown that the adhesion forces of APAP crystal on various crystal planes were different from each other. Although the same crystal was analyzed under AFM, adhesion forces on adjacent crystal planes were found to be different [37]. Interestingly, planes of similar family were found to exhibit similar forces compared to the planes of different families. However, humidity was found to have a significant impact on these forces. These measurements were done on uncoated crystal planes. In addition, the functional group interacting with the crystal surface was not confirmed. Moreover, the interaction with the excipient was also not studied. As an extension to the previous study, in this current work we propose to investigate the effect of thin PVD coating on crystal planes on the stability and adhesion forces interacting with the functional groups. In this thesis, we also propose to identify the effect of both hydrophobic and hydrophilic functional groups thereby gaining a thorough understanding on the interacting behavior of nanolayers with active drugs and excipients. The results of the previous work of our group explained the influence of humidity on uncoated crystal planes with respect to AFM study on APAP crystals. The proposed work for this thesis is a direct extension of previous work of the faculty advisor's research team [37].

## **4. EXPERIMENTAL METHODOLOGY**

### **4.1 MATERIALS REQUIRED**

Petri dishes and beakers were used during the experimental stage-1 (making of crystals). Crystals of acetaminophen were made from the saturated solution of active pharmaceutical ingredient (API). Demineralized water was used to make a solution. Active pharmaceutical ingredient and water was mixed in a mixer. This instrument had a small magnetic stirrer which

rotated automatically when the instrument was switched on. It had a heating system where stirring and heating could be done simultaneously. Heating effect and the speed of the stirrer was controlled with a regulator at the bottom of the instrument. As it was important to maintain a certain temperature when a super saturated solution was made, a thermometer was used to maintain the temperature between 72 °C and 80 °C. Optical microscope was required to check the size of the crystal and how clear the crystal was. The size of the crystal was important to analyze the surface using surface analysis mode in AFM and to measure the force curves using tapping mode in AFM on the crystal plane.

Atomic force microscopy (AFM) is a kind of microscope which is also called as scanning probe microscopy. This was needed to scan and map different surfaces of crystal before and after coating crystals. Any hydrophilic and a hydrophobic solution was used in order to dip the tip of AFM into the solutions to check the force of attraction between the tip of the AFM and the crystal surface. To coat on the surface of a crystal with silica-di- oxide ( $\text{SiO}_2$ ) was needed and coating on crystals was done with the help of Physical Vapor Deposition.

## **5. EXPERIMENTAL TECHNIQUES**

### **5.1 CRYSTALLIZATION**

The initial step to start with the research project was to grow a single, large and a clear crystal. Crystals are very sensitive to the atmosphere and external disturbance; it was important to grow crystals very carefully. Crystals from saturated and a super saturated solution were made. To avoid external disturbance a setup was constructed where the box was insulated so that the vessels used to grow crystals were placed inside. Petri dishes were used to keep the saturated solution and

the super saturated solution. A saturated solution was prepared by mixing acetaminophen powder which is an active pharmaceutical ingredient with demineralized water in a stirrer and stirred until the powder was dissolved completely in water. The solution was filtered and left undisturbed for weeks in a vessel. This vessel was covered with perforated sheet in order to reduce the speed of evaporation. Another method where crystals can be made was with a super saturated solution. Acetaminophen powder was mixed with demineralized water and stirred until the powder was mixed. The solution was heated up from 72 °C to 80 °C so that the powder was entirely dissolved; solution was filtered and left for slow evaporation. The crystals were not exposed to atmosphere as they may get oxidized and form dark spots on them. Different kinds of crystals were obtained using both the processes. The crystals were either like flakes or solid crystals.

## **5.2 OPTICAL MICROSCOPE**

The next step was to analyze the size and shape of the crystals obtained from crystallization with the help of an optical microscope. Stereotype optical microscope is a kind of microscope that was used during the process. The optical microscope had lens and a camera; the camera was attached to the computer and light. Individual crystals were taken and kept under the microscope by adjusting the focus and the lens, where an image was shown on the computer screen. Pictures were captured on the computer screen.

### **5.3 ATOMIC FORCE MICROSCOPE**

Atomic Force Microscope (AFM) was the next method that was required to check the force on the crystal surface. AFM is one kind of a scanning probe microscope. AFM used was a simple setup where it consisted of a scanner, a microscope with a camera, a probe holder. The AFM was connected to the software for the analysis of the samples. The AFM's software had all the mode that could help us obtain our results such as a 'scan syst' mode, contact mode, tapping mode and electromagnetic and mechanical mode. These modes operated differently for different samples. The probe holder as the name implies holds the probe that scans the surface of any sample. The laser beam had to be adjusted so that it falls on the tip of the probe so that it can identify the surface and stop engaging with the surface when it comes into contact. The tip of an AFM was tapped on the surface of the crystal at various points so that mapping could be done. AFM tip was dipped in that is a hydrophilic solution, 8-mercaptooctanoic acid ( $\text{HS}-(\text{CH}_2)_7\text{COOH}$ ) and 10-decane thiol which is a hydrophobic chemical solution. By this procedure, a monolayer of functionalized groups is expected to attach to the AFM tips. This helped us find the force of attraction in the surface when the crystals were coated. The forces curve for the functionalized tip with different chemicals on the crystal facets was determined. A comparison of the adhesion forces using hydrophobic, hydrophilic and plain AFM tip was performed on all the crystal facets.

### **5.4 PHYSICAL VAPOR DEPOSITION**

The crystals were coated with Physical Vapor Deposition (PVD), which further continued for the proposed work, and with the help of PVD a thin layer of silica-di-oxide was coated on the crystal. The main advantage of using PVD was that it could coat the samples at the room

temperature, thickness of the coating could vary up to 10 nm to 100 nm and finally it took less than 30 minutes for coating to take place. Entire PVD system was computerized and changes could be made if necessary. The crystals were mounted on a metallic disc and loaded into the system. Coating the crystal and with the functionalization of the AFM tip the forces between the hydrophilic and hydrophobic groups were checked.

## **5.5 SPUTTERING SYSTEM**

This is another kind of PVD. The sputtering system was used to gold coat the AFM probe for functionalization of the probe with the hydrophilic and hydrophobic solution. The sputtering system had its own software where the temperature, coating thickness, total time for the coating to be done was displayed. The probe was mounted on a plate and then loaded into the system. The entire process took place at room temperature. Total time took to gold coat the probe was about seven to eight minutes. Finally, the tips were functionalized with hydrophobic and hydrophilic groups.

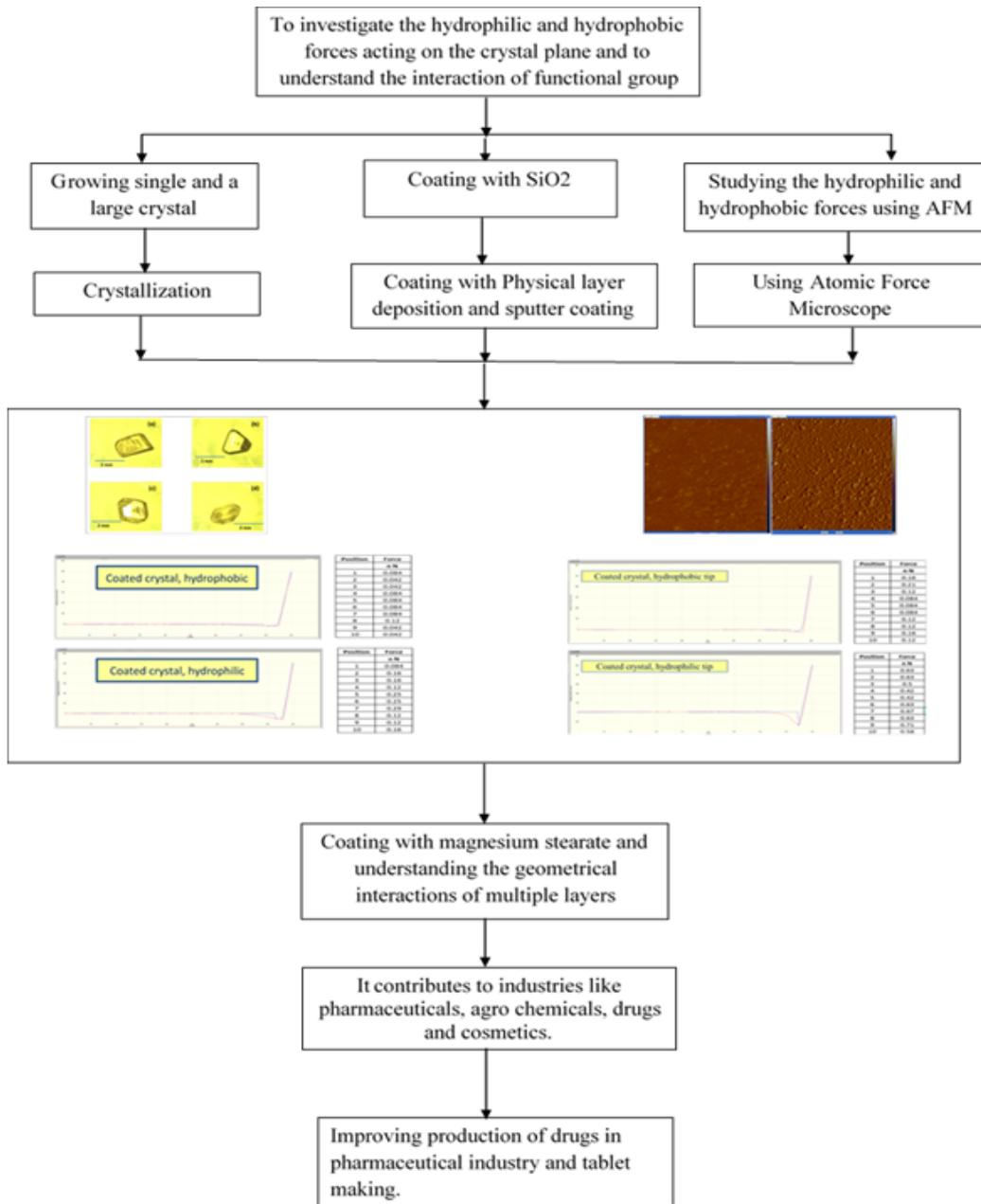
## **5.6 FUNCTIONALIZATION OF AFM TIPS**

The gold coated tip was dipped in the hydrophobic solution (10- decane thiol) for 24 hours. After the time span of 24 hours it was taken off the solution and allowed to dry for three hours. The above procedure was repeated for hydrophilic solution (8-mercaptooctanoic acid). Self-assembled monolayers were expected to form on the gold coated tip as gold is widely known to thiolate. Hence the thiol group is expected to attach to the gold coating on the AFM tip and the

carbon chain is available as an interacting chain with crystal planes. The thiolated AFM tips were later tested under RAMAN spectroscopy to confirm the functionalized groups. RAMAN imaging mapping was performed at various locations on the AFM probe to confirm the presence of thiol.

## **6. OVERVIEW OF THE WORK**

The overview of the project is shown in Figure 1 in the form of a flow chart. The objective of the research work is to investigate the hydrophilic and hydrophobic forces acting on the crystal plane and to understand the interaction of functional group. Organic drug crystals (APAP) were made from saturated solution. Single, large and a clear crystal was selected to check the size and clarity under the optical microscope. Force curves were measured using Atomic Force Microscope. Firstly, forces on plain APAP crystal and plain AFM probe were measured. These crystals were then coated with SiO<sub>2</sub> with the help of Physical Vapor Deposition. The coating thickness were 10 nm, 20 nm and 50 nm. Uniform coating was achieved by 50 nm thickness. AFM tips were gold coated with sputtering system and then functionalized with decane thiol and octanic acid, which were hydrophobic and hydrophilic functional group. Forces on coated crystal surface and the functionalized AFM tips were studied. Forces on ten different points on the crystal surface were measured. In the future work, these crystals will be coated with magnesium stearate and the geometrical interaction of these multiple layers will be studied. This research work contributes to pharmaceutical industries. Finally, improving the drug release rate and the dissolution rate can be improved.



**Figure 1: An over view for this thesis showing the objective, specific aims, methods used, results, future work, scientific contribution and conclusion.**

## 7. RESULTS AND DISCUSSION

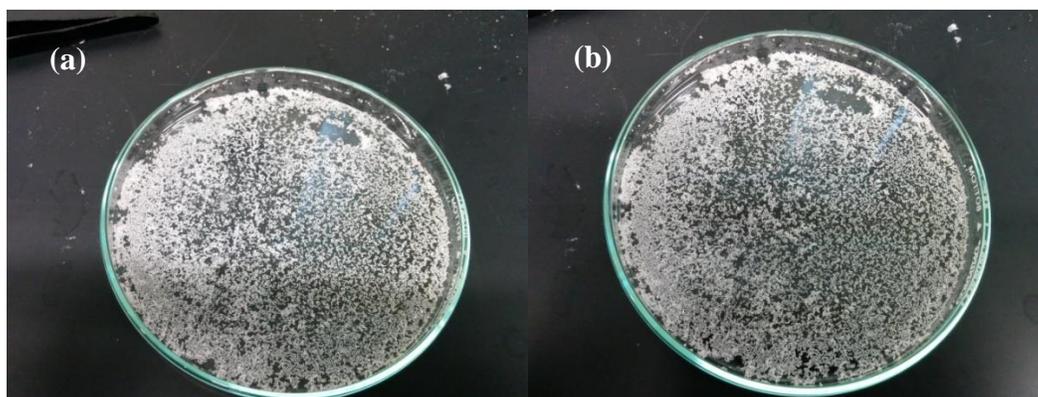
Several geometries of crystals were obtained from the saturated solutions of APAP. Multiple batches of solutions were prepared to obtain several sets of geometrical variations of organic crystals. After the drying of these crystals for few days, it was observed that some crystals were brittle and some were stable and independent. Several such independent crystals were separated from the batch and collected as sample materials for further analysis as shown in Figure 2.



**Figure 2: Crystals obtained from saturated solution.**

These crystals were taken out and kept separately for further use. The geometries of crystals were checked further under an optical microscope to confirm the size and clarity. Some crystals were found to be brittle in nature and were discarded. Only the transparent and stable crystals, which were free of dark spots, were collected and used for further testing. We also found that the crystals were of different dimension and shape. The sizes of most of the crystals were 2 mm to 3 mm. Some crystals were clear where as some were a little hazy. Most of the crystals were found to be triangular in shape, some rectangular and some had another crystal attached to each other. These independent crystals which were confirmed for geometrical uniformity and clear surfaces free of dark spots were further be used for AFM studies and PVD coating.

Another set of crystals that were made from super-saturated solution shown in Figure 3 were obtained as flakes and not solid crystals. As a result, these crystals could not be used as they had no clear size, shape or geometry. Handling of these crystals would have been difficult since nanocoating of these crystals requires that the crystal surface be a clean and smooth surface to study the surface topography and force curves.



**Figure 3: Flaky crystals obtained from super saturated solution.**

Looking at the crystal samples, we could clearly differentiate the types of crystals obtained from saturated and super saturated solution. The flaky crystals were obtained when the super saturated solution was used and the solution was subjected to faster evaporation. The samples of this batch were kept open without protective cover. This facilitated faster rate of evaporation of solution. We attribute this phenomenon to insufficient time for crystal growth process due to faster rate of evaporation of aqueous phase from the solution. During the preparation of saturated solution, we also observed that some of the acetaminophen did not dissolve completely. However, this was observed beyond a saturation stage in the solution. Such insoluble suspended APAP particles were filtered off and the pure saturated solution was collected.

It is worth mentioning here that all solutions used in the preliminary stage of experiments were subjected to various rates of evaporation. Some samples were collected in a glass vial, and some were collected in a flat glass plate. All samples were covered with a sheet to protect outside contamination. Perforations were made on the sheet in order to facilitate a slow rate of evaporation. These samples were left for natural evaporation in which the process was forced to slow down by incorporating perforations on the covered sheet on the top of the sample holder. We obtained crystals of desired size and shape due to this process.

Some of the samples were also kept in a refrigerator and also in freezer in order to facilitate freeze drying. The faster solidification of solution resulted again in flaky substances in the form of sheets. Although certain crystals were obtained, they were found to be quite brittle. The hardness and stability of such crystals is uncertain and hence we could not use these crystals further in the experimental analysis.

Pure, bright and solid crystals were obtained from saturated solution (not supersaturated solution). These crystals were found to be clean and bright on the first appearance. It had taken 5 to 7 days per batch for these crystals to develop to this size, shape and structure. However, not all crystals were clean as some crystals were found to have some black spots. This could be attributed to the over exposure to open atmosphere beyond evaporation of aqueous medium. There were black spots on many crystals because of the possible reaction with atmosphere. As we know that the crystallization process from saturated solution is very sensitive to any physical vibration or mechanical disturbance, any slightest vibration induced in the solution during the development phase is expected to cause irregular growth of crystal. Figure 4 show the crystals obtained with black spots. Saturated solution was refrigerated and irregular crystals were obtained due to external disturbance as shown in Figure 5. Figure 6 shows the solution that didn't evaporate which was

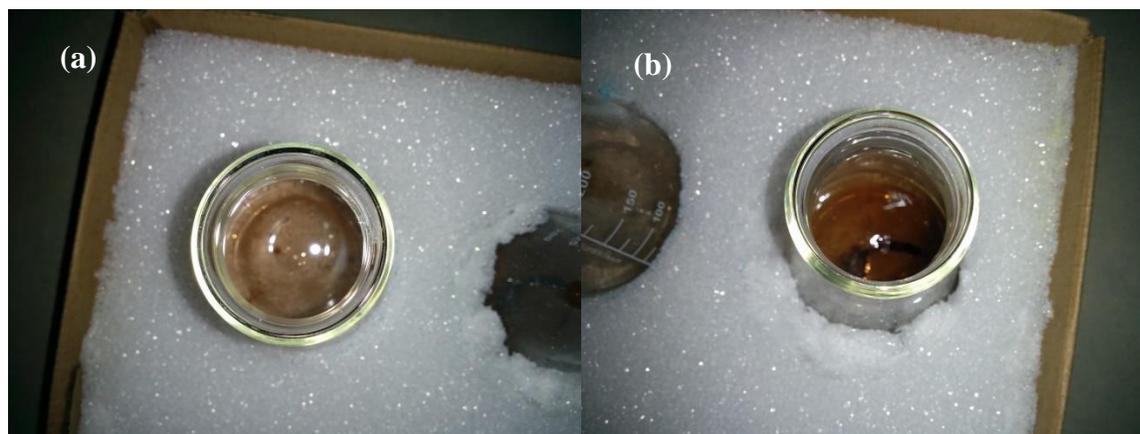
left out as black or dark liquid in the beaker. In such a situation, we observed that the crystallization process did not initiate and the process of crystal making was not successful. Eventually, several conditions and parameters were learnt from these initial steps. Finally, we were able to get good crystals whose description is provided in the next section. These were obtained where the evaporation rate was slow and the conditions where the crystals are obtained as per desired parameters were recorded.



**Figure 4: Crystals oxidized due to exposure to atmosphere.**



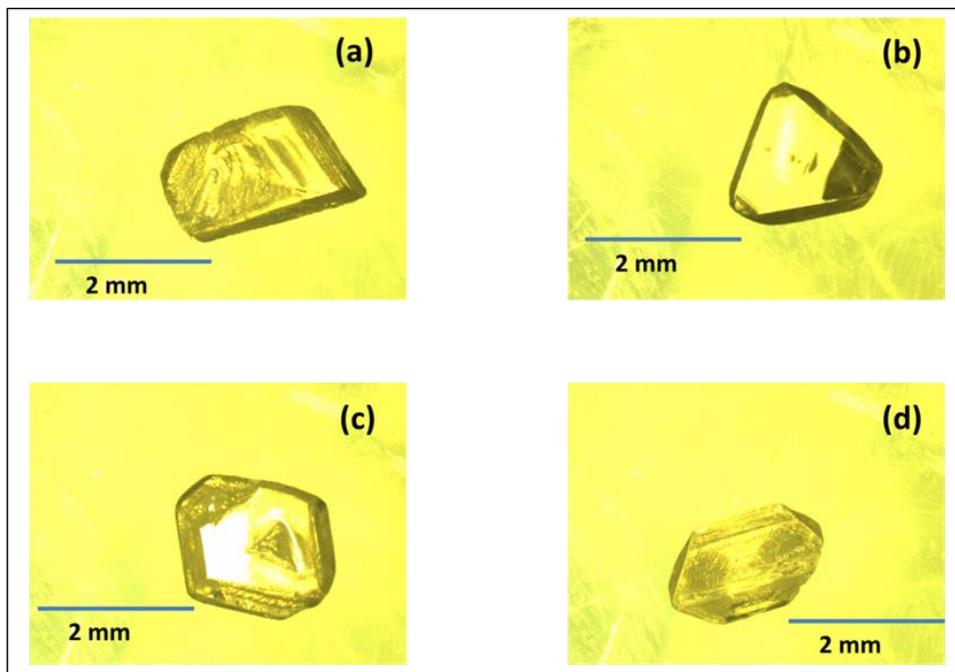
**Figure 5: Irregular growth due to disturbance.**



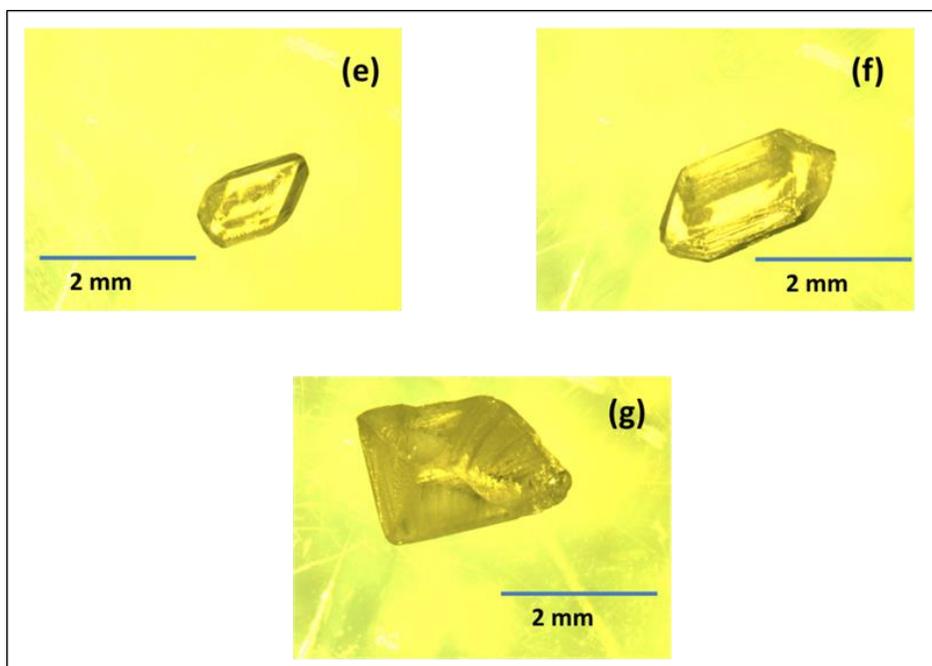
**Figure 6: Saturated solution of Acetaminophen which, was oxidized due to atmospheric conditions.**

Finally, clean and solid crystals were obtained from saturated solution prepared at room temperature. The solution obtained was a clear solution after mixing acetaminophen and water. The solution was filtered for clearer solution directly into the petri dish for evaporation. The petri dish was left undisturbed from the time when the solution was transferred. Petri dishes were kept

insulated with compact foam to protect the solution from external disturbance. The top of the box was covered with a perforated sheet to reduce the rate of evaporation as clear crystals were obtained from slow evaporation. We made sure that the solution has not contacted with the sheet that was used as a cover. It is due to the fact that the sheet would react with the solution which could increase the formation of black spots on the crystals. The solution was left without any disturbance for one to three weeks. After a week we observed that there were crystals forming partially and followed by clear, large and strong crystals formed after three weeks. Figures 7 and 8 show the crystals obtained from saturation solution. These crystals were observed under the optical microscope to check the size of these crystals and the clarity of these crystals. It was important to check the size of the crystal for proper uniform coating and to perform AFM to study the force curves. Clarity of the crystal is important to know if the crystals are not oxidized or there are no dark spots on the crystal surface.



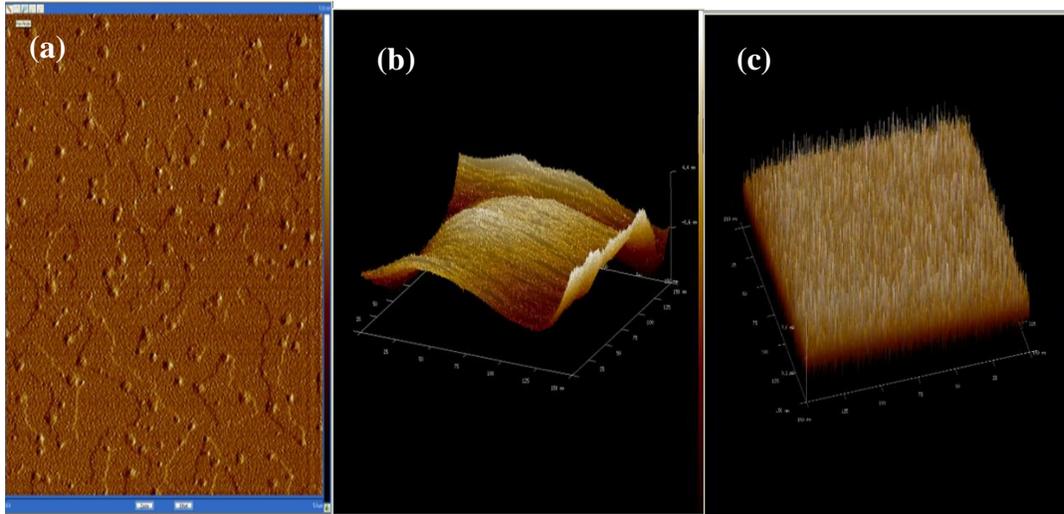
**Figure 7: Images of crystals obtained from saturated solution. (a), (b), (c), (d) were the shape and size of the crystal under the optical microscope.**



**Figure 8: Images of crystals obtained from saturated solution. (e), (f), (g) were crystals of different size and shape under the optical microscope.**

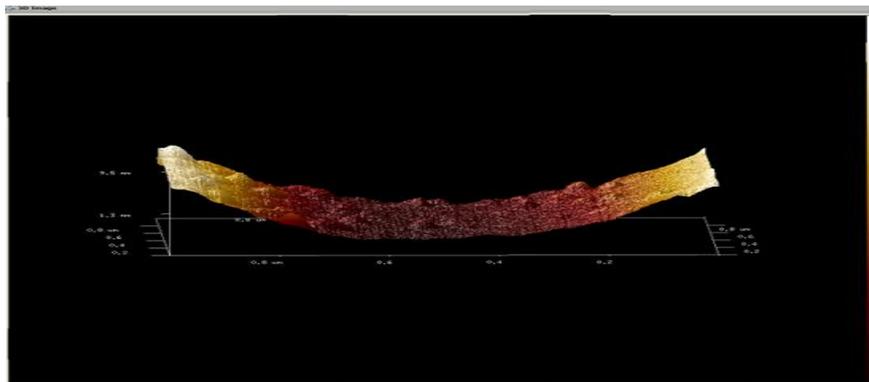
## **7.1 SURFACE TOPOGRAPHY OF APAP CRYSTAL**

After analyzing the clarity and size of crystals under the optical microscope from Figures 7 and 8, the surface topography and imaging were done under an atomic force microscope. The crystal was placed on a 15 mm size metal disc and was mounted on the magnetic sample holder that was on top of the scanner tube of the Scanning Probe Microscope (SPM) system. The head of the AFM has a probe holder, which are screwed tightly, and holds the probe that scans through the sample. Once the head was fixed to the AFM the laser had to be aligned and made sure that the laser fell on the tip of the AFM. This was a necessary step as the laser beam had to fall on the tip of the probe so that it can identify the surface and stop engaging with when it came in contact with the surface of the sample. Topography of acetaminophen crystal was first scanned.



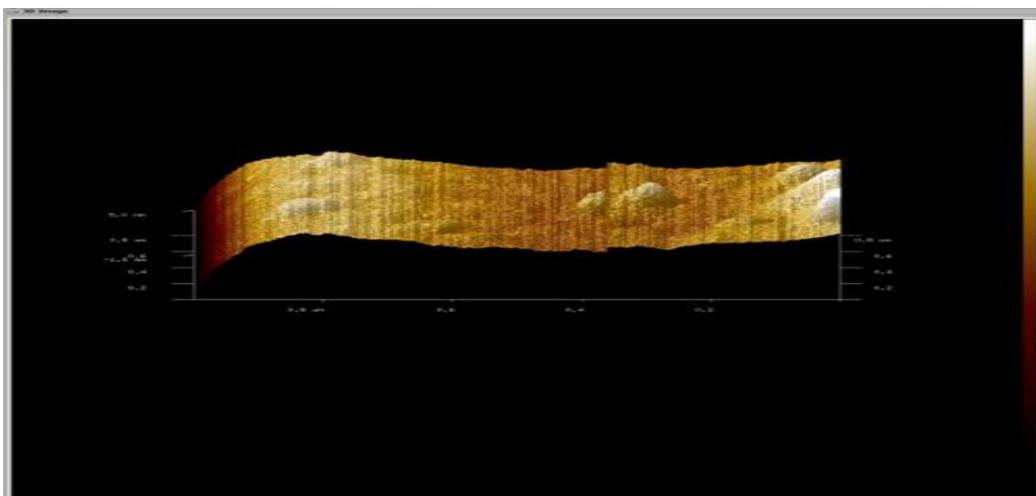
**Figure 9: Surface topography of acetaminophen crystal under AFM.**

Figure 9(a) is a two dimensional image showing the smooth surface of an APAP crystal. Figure 9(b) is a three dimensional image of the previous one and Figure 9(c) is a peak force of the crystal surface. The peaks in the image show the uniformity of the crystal surface. Surface topography from the results depicted that the crystals had a rough surface or a smooth surface. It was important to know the surface as it would be easier to do the force curves and needed to get in contact with the surface. AFM imaging showed variable surface topography overall with different points on the surface showing different surface geometries.



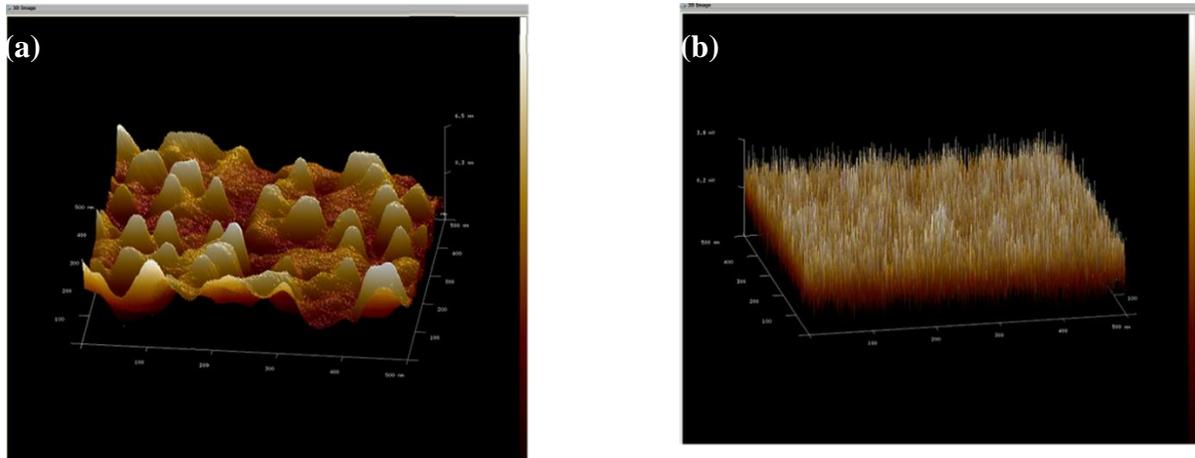
**Figure 10: A 3D image of an uneven surface on the crystal plane.**

Figure 10 illustrates an uneven surface at one of the points on the crystal plane. It showed that there was a deep dip on the crystal plane. Figure 11 shows an even and smooth plane, which had not many ups and downs on the plane. This uneven planar development can be attributed to the improper development of crystal planes during the formation stage. However, we found interesting that some planes were deep and some were smooth and high. In order to test the effect of surrounding environment, we left the crystals to stay in the sample holder for 12 months. It was interesting to see that the crystal surface planes again recrystallized which can possibly happen due to the effect of humidity, time and exposure to surroundings. In this work, recrystallization of surface planes occurred with additional nucleation and variations in the growth rates, which made the surface rugged over a period. The results clearly showed the impact of time on crystal growth, which also indicated that the stability of crystal is a key factor that cannot be ignored for chemical or physical testing. However, retaining the stability of organic crystals is indeed a challenging aspect when the biomolecular interaction is considered.

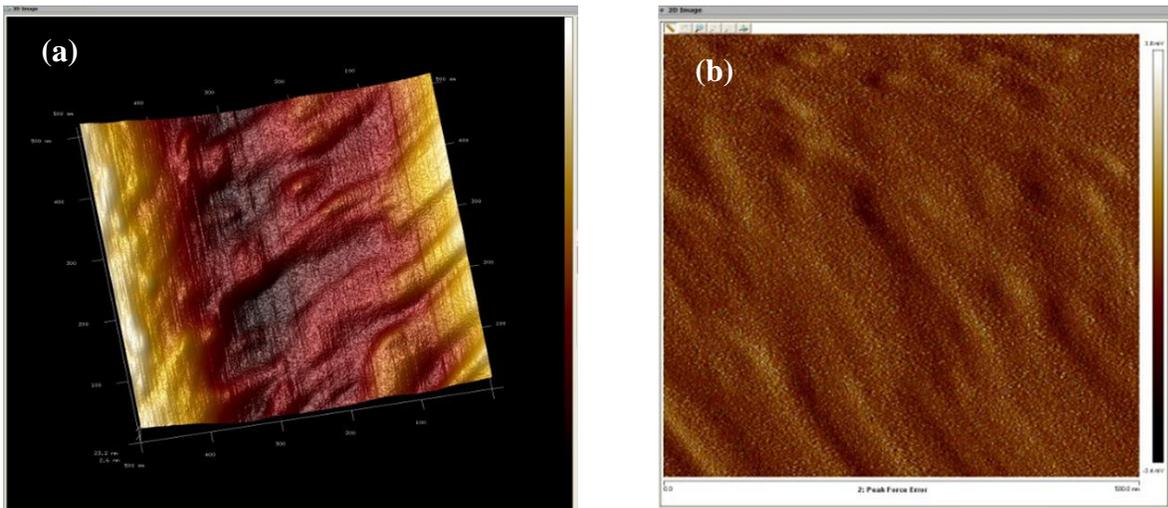


**Figure 11: A 3D image of an even surface on the crystal plane.**

Figure 12 is a three-dimensional image showing how recrystallization took place within the period of one year. Small white blocks are crystals that are growing which were not visible to our eyes.



**Figure 12: Effect of humidity over a period.**



**Figure 13: New batch of crystal with smooth surface.**

Figure 13(a) is a 3D image of APAP crystal, which had a smooth surface and Figure 13(b) is a 2D image of the peak force showing a uniform surface of the crystal. It did not show any signs

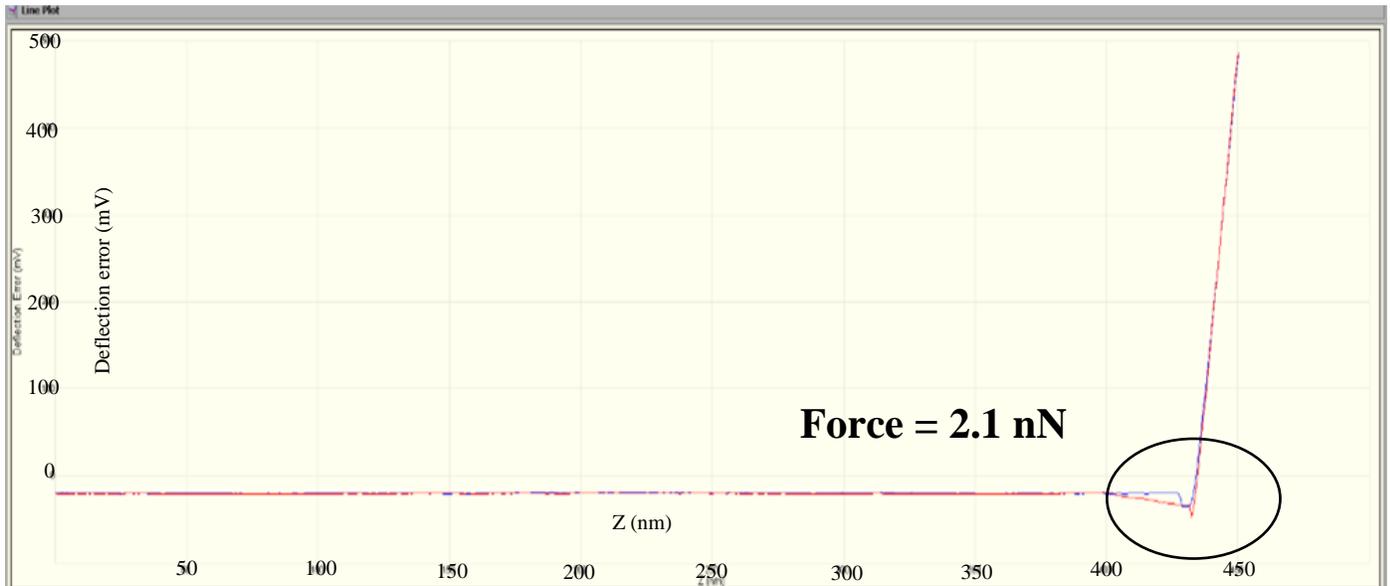
of recrystallization. There was no effect of humidity as the crystals were made recently. A detailed understanding of interpretation of the above results can be seen from the graphical representation showing the smoothness and the roughness of the surface. Figure 14(a) is a graph of a smooth crystal. The blue line represents “the trace” and the red line represents “the retrace”. When the AFM probe touches the surface of the crystal it moves back and forth while scanning the surface. The trace represents the probe moving in one direction and retrace is the probe moving back in the same direction. It is important to have the “trace” and “retrace” overlapping so that there is an accuracy in the results hence by adjusting the scale, we could overlap the trace and retrace in the graph.

Image 14(b) show the bumps on the crystal surface which confirms that the crystals had an uneven surface.



**Figure 14: Graphical representation of crystal surface.**

After the analysis of the crystal surface, the next step was to obtain the force curves on plain APAP crystal. As mentioned earlier, the AFM was changed to the contact mode where it touches the surface of crystal and moves away. By this method, the forces on the crystal surface were obtained in the form of a graph.



**Figure 15: Force curves on plain APAP crystal and plain AFM tip.**

Figure 15 shows the forces of plain APAP crystal. The force found in this case was low as represented in Figure 15. The red line in Figure 15 that is being pulled downwards indicates the strength or the maximum pull between the crystal surface and AFM probe. The force on plain crystal was low because there is no kind of interaction happening between the surface of the crystal and the AFM probe. Ten different points were chosen to take ten different force curves to analyze any kind of variation in the force. The forces on ten different points on the crystal surface show that there is no interaction happening on the surface of the crystal. These points were tabulated as shown in Table 1. The force obtained from Figure 15 and taking the position 1 from Table 1, we can calculate force as follows:

$S = 70 \text{ nm/V}$  (constant)

$K = 0.6 \text{ N/m}$

$F = K \cdot d$

Where,  $d = s \cdot \text{divisions} \cdot \text{scale}$

$= 0.6 \text{ N/m} \cdot 70 \text{ nm/V} \cdot 0.5 \text{ div} \cdot 0.1 \text{ V/div}$

**$F = 2.1 \text{ nN}$**

Here,

S- Spring constant = 0.6 N/m

K- Young's modulus

d- Divisions

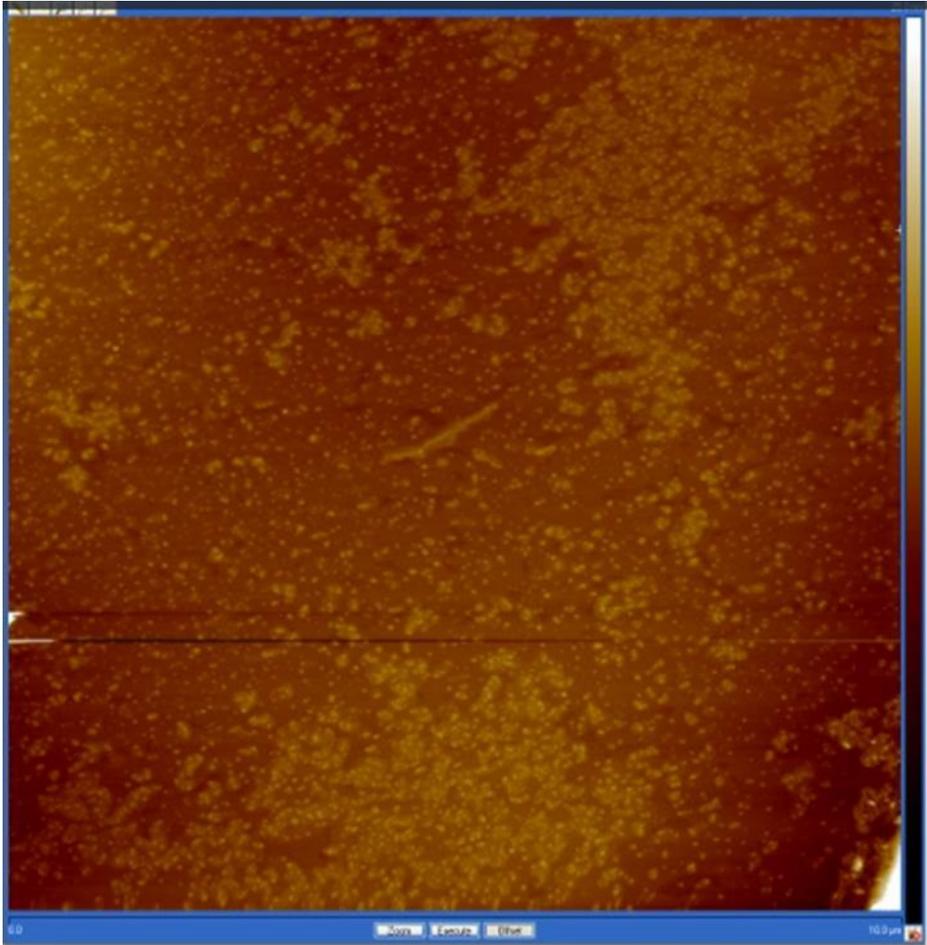
The above calculation is repeated for all the force values obtained.

**Table 1: Different positions on the crystal and their force curves.**

<b>Position</b>	<b>Force</b>
	<b>n N</b>
1	2.1
2	2.5
3	1.26
4	2.1
5	1.26
6	2.94
7	2.94
8	1.26
9	2.1
10	0.84

After obtaining the force curves on the plane crystal, the crystals were coated with  $\text{SiO}_2$  with the help of Physical Vapor Deposition (PVD). The PVD used for coating was E- beam evaporation system. The crystals were mounted on a metallic disc and loaded into the system through load- lock assembly, without breaking vacuum and finally exposing the crystal to air. The

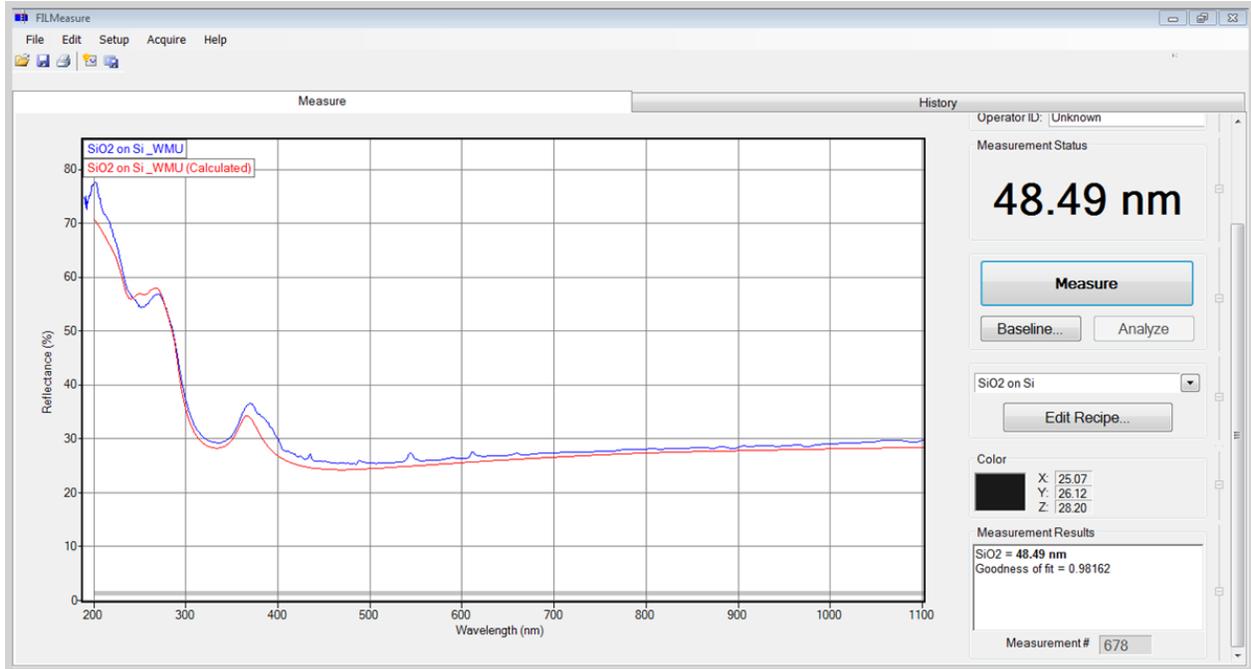
temperature inside the system was maintained at room temperature. Once the system was on the metallic disc rotates at 20 rpm which helped us yield better uniformity on the crystal. This PVD system could coat from 10 nm to 100 nm thickness. Initially coating of 10 nm was done. When they were analyzed under the AFM, it was found that there was an uneven coating of SiO<sub>2</sub> on the surface on the crystal (Figure 16).



**Figure 16: Non-uniformity of SiO<sub>2</sub> coating on the surface of crystal.**

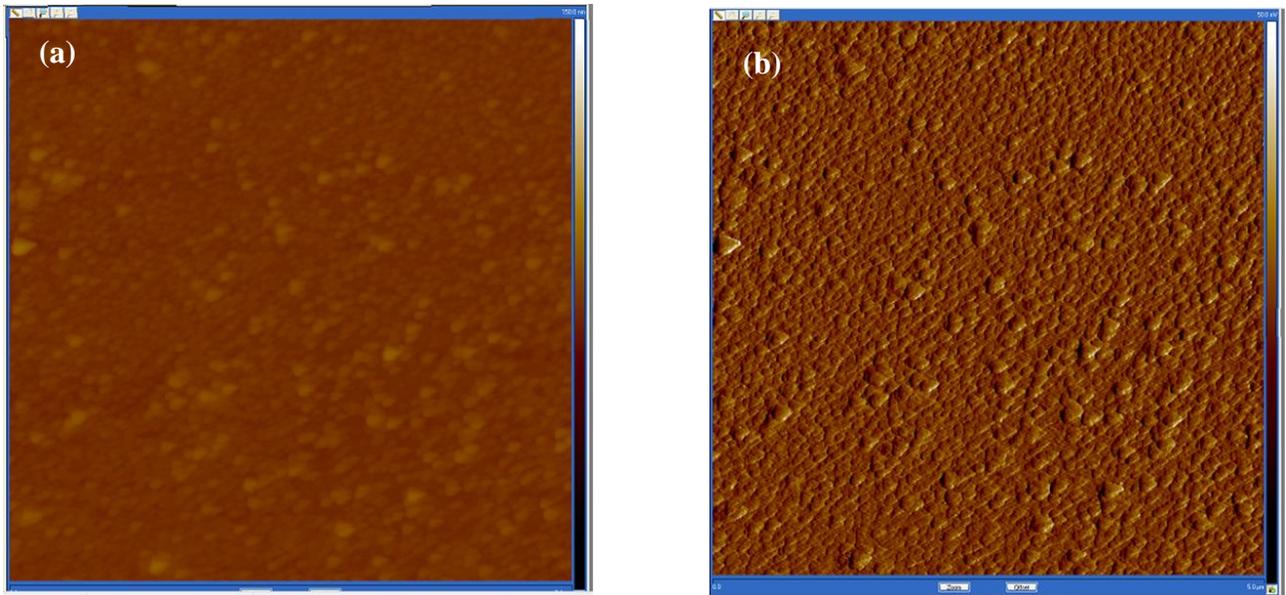
From Figure 16, it is seen that 10 nm or 20 nm coating resulted in non-uniformity on the surface of the crystal. The white patches of coating can be clearly seen from Figure 16, arising from the non-uniform coatings on crystal surfaces. Since the coating was non uniform with 20 nm, another coating 50 nm thickness was done on the crystal. The same procedure

used for 20 nm was also repeated for 50 nm coating. However, the time of exposure was held longer compared to that of the coating of 20 nm.



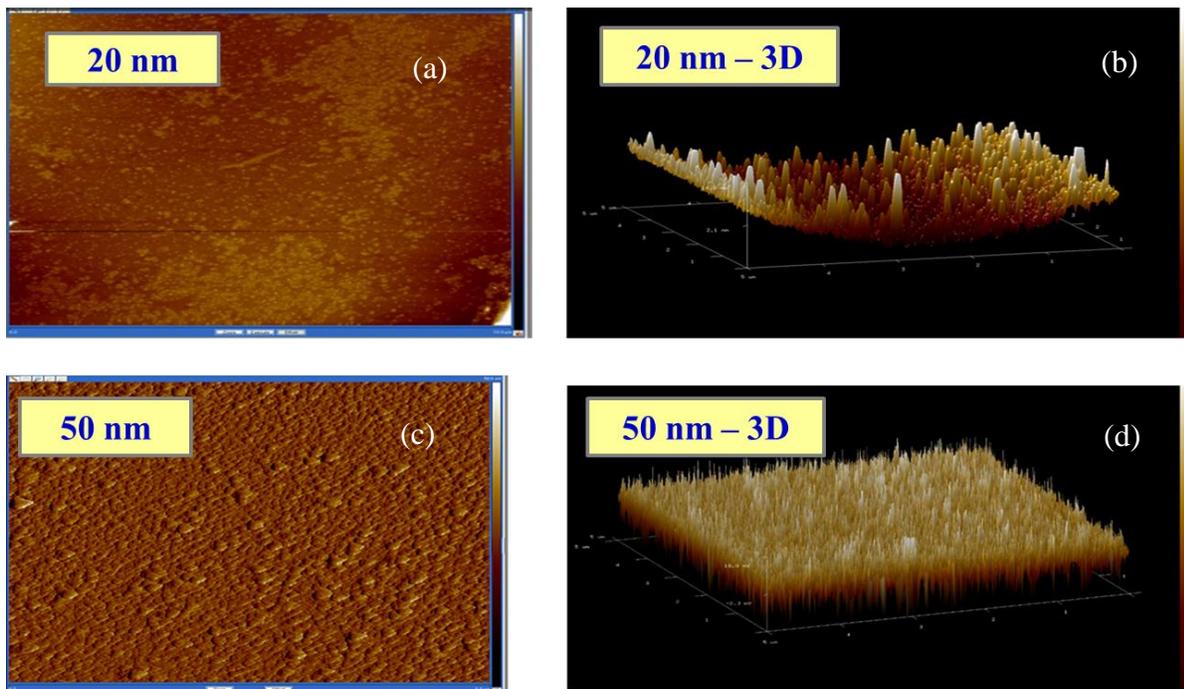
**Figure 17: Conformation of PVD coating on crystal surface with approximately 50 nm thickness.**

After coating was done, the thickness of coating was confirmed using a software called *film measure*. The crystal was kept under a beam of light where a laser hit the surface of crystal and the software calculated and showed the results on the computer screen. This confirmed the coating thickness was close to 50 nm as shown in Figure 17. For further understanding, we placed the crystals coated with 50 nm thickness under an AFM to check the uniformity of coating.



**Figure 18: 50 nm coating thickness of SiO<sub>2</sub> on APAP crystal.**

Figure 18(a) shows the uniformity of SiO<sub>2</sub> coating on APAP crystal and Figure 18(b) is an image of the peak force that shows uniform coating on the surface of crystal. The crystal surface was scanned under the AFM.

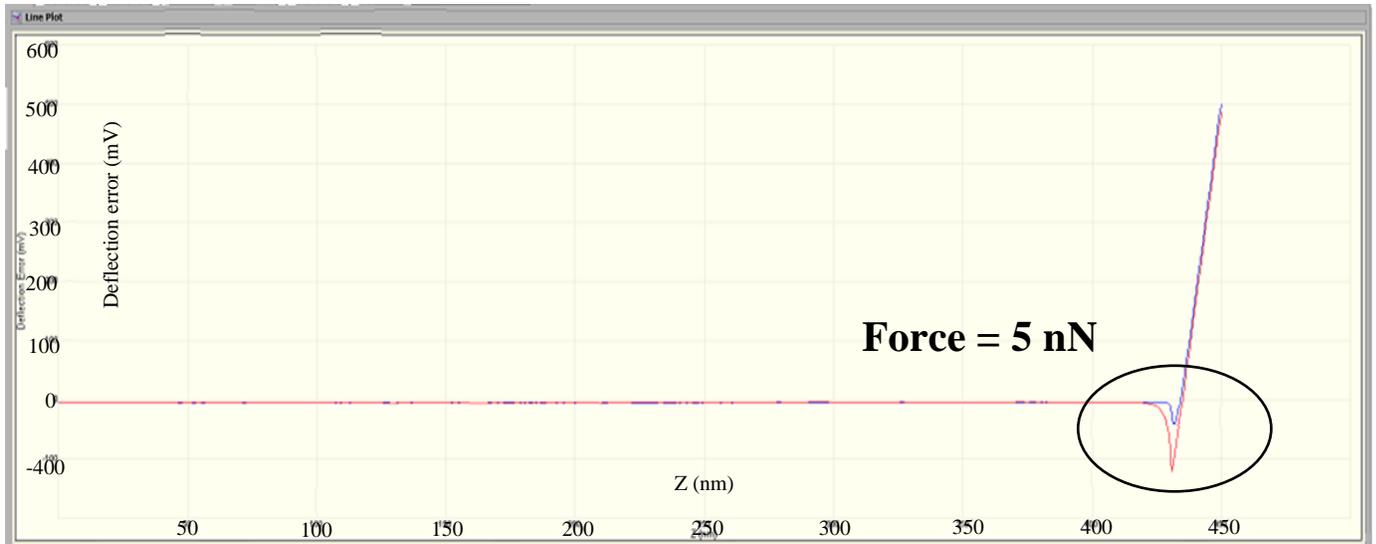


**Figure 19: Comparison of 20 nm and 50 nm coated surface.**

A comparison was made for better understanding when the crystal was coated with 20 nm and 50 nm. Figure 19(a) shows a three dimensional image of crystal surface coated with SiO<sub>2</sub> with the thickness of 20 nm. The white patches on the surface show that the coating was not uniform. Figure 19(b) shows the peaks forces were uneven as the coating was not uniform on the surface of the crystal. Figure 19(c) shows crystal surface with 50 nm coating thickness and Figure 19(d) shows the even peak force due to uniform coating thickness. This showed that the entire crystal plane was successfully covered with the coating when the nanocoating thickness was increased to 50 nm.

The next step was to take the force curves on this 50 nm nanocoated crystal. APAP crystal coated with 50 nm was kept under the AFM and the mode was changed to contact mode. The probe came in contact with the crystal and the force curves were captured on the computer screen. After coating with SiO<sub>2</sub>, from Figure 20 it was found that the force (5 nN) curves were higher on Silica coated crystal when compared with the force (2.1 nN) curve in uncoated APAP crystal (Figure 15). The force was high on coated crystal is because of some kind of interaction taking place between SiO<sub>2</sub> and the APAP crystal surface. Ten different points were chosen to take ten force curves and were tabulated.

The X- axis of the graph is Z (nm) and Y- axis is the deflection error. The red line called “The retrace” is taken into consideration and shows how strong the forces were. Using the previous calculations where the force was found is repeated again which gives us the value for the force to be 5 nN. Table 2 shows ten points taken on the crystal surface and the force curves at those points.



**Figure 20: Force curve on coated APAP crystal.**

**Table 2: Ten different positions on coated APAP crystal.**

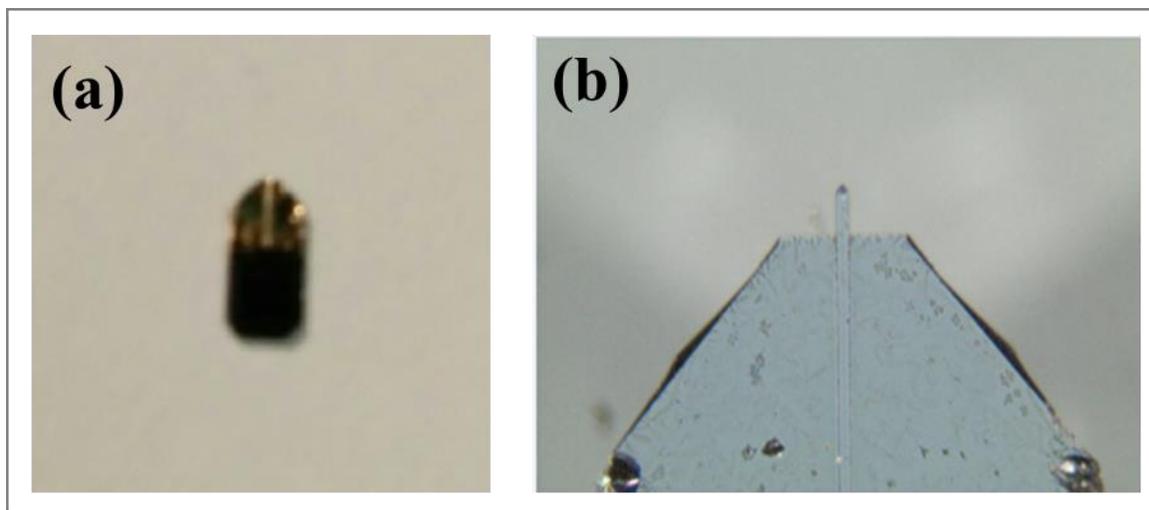
Position	Force
	n N
1	5
2	5
3	4.2
4	5
5	5
6	5
7	5.4
8	5
9	5.4
10	5.8

The force curves were found to be almost the same in different points taken on the surface of the crystal. The force on APAP crystal coated with SiO<sub>2</sub> was higher (Table 2) compare to plain APAP crystal (Table 1) because there is some kind of interaction taking place between the surface of the crystal and the layer of SiO<sub>2</sub> coating.

## 7.2 FUNCTIONALIZATION OF AFM PROBE (HYDROPHOBIC SOLUTION)

This section deals with the functionalization of AFM probe. Functionalization was done by first gold coating the AFM tip. The gold-coated tip was then dipped into the 10- decane thiol, which is a hydrophobic solution, for 24 hours. After 24 hours, the tip was removed and dried for three hours. Due to the thiolating nature of gold, the gold-coated part of the AFM tip is expected to be functionalized with a self-assembled monolayer of hydrophobic thiol, with thiol group (S-H) attached to the gold side of the tip and alkane group available for SiO<sub>2</sub> interaction.

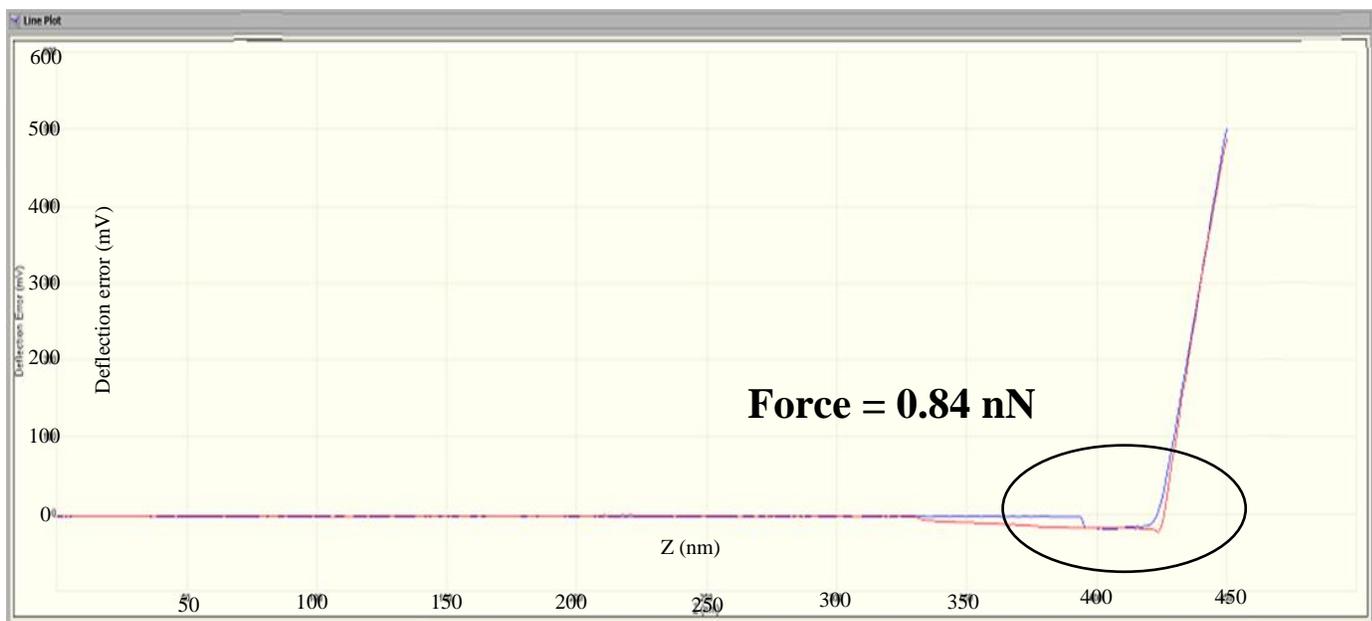
Gold coating of the tip was done by the sputtering system. The sputtering system works like PVD. The tip to be coated was first mounted on a plate and then loaded into the sputtering system. The temperature was maintained at the room temperature. The part of the AFM tip that was gold coated and this part of the cantilever was used to measure the adhesion force on AFM.



**Figure 21: Gold coated AFM tip.**

Figure 21(a) is the image of AFM probe that is gold coated. The cantilever probe can be seen from Figure 21(b).

In the next stage, the functionalized hydrophobic AFM tip was used to capture force curves on SiO<sub>2</sub> coated crystal surfaces (Figure 22). It was found that the adhesion forces were lower (Force = 2.1 nN) compared to that of previous experiments (Figure 15) on plain crystal without any coating. This proved that the interaction between hydrophobic alkane thiol and SiO<sub>2</sub> molecules on crystal plane was weak. The study showed that the molecular interaction affinity of SiO<sub>2</sub> with alkane group was ineffective over all the positions of the SiO<sub>2</sub> coating. Ten different force curves were taken at various points on crystal surface and the results are tabulated and shown in Table 3. Using the previous calculations the force for Figure 22 was found to be 0.84 nN.



**Figure 22: Force curves on coated APAP crystal, functionalized AFM tip with hydrophobic solution.**

A steady trend throughout the surface of the crystal confirmed the affinity of alkane group, which is hydrophobic in nature. Hence, we can expect a similar behavior on micro particles, which is evident from the results at molecular scale from this study. The results from Figure 22 points

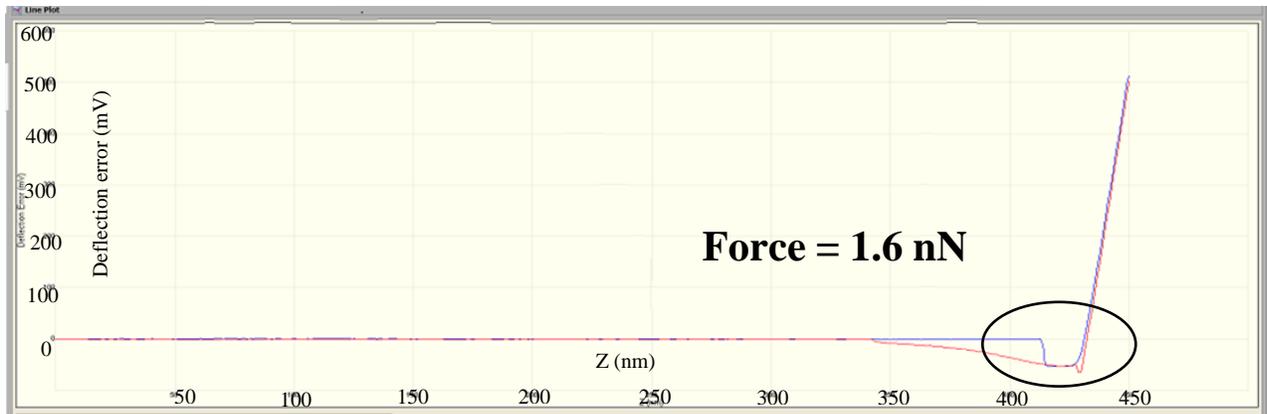
towards an important direction to follow in terms of hydrophobic and hydrophilic interaction both at nano and micro scales, connecting the phenomena on multi-scales.

**Table 3: Force curves at ten different points on coated crystal.**

Position	Force
	n N
1	0.84
2	0.42
3	0.42
4	0.84
5	0.84
6	0.84
7	0.84
8	1.2
9	0.42
10	0.42

### **7.3 FUNCTIONALIZATION OF AFM PROBE (HYDROPHILIC SOLUTION)**

The tip was then functionalized with a hydrophilic solution 7- carboxy thiol. The above procedure mentioned in previous section functionalization of AFM probe (hydrophobic solution) was also repeated in this stage for hydrophilic functionalization of AFM tip. The tip was gold coated, dipped in hydrophilic solution for 24 hours and then dried for three hours. The force curves found on coated APAP crystal are shown in Figure 23. The red line called “the retrace” shows a strong pull which indicates that the force is 1.6 nN which is higher compare to the force in Figure 22 which was 0.84 nN.



**Figure 23: Force curve of coated APAP crystal and tip functionalized with hydrophilic solution.**

**Table 4: Force curves at ten different points.**

Position	Force n N
1	0.84
2	1.6
3	1.6
4	1.2
5	2.5
6	2.5
7	2.9
8	1.2
9	1.2
10	1.6

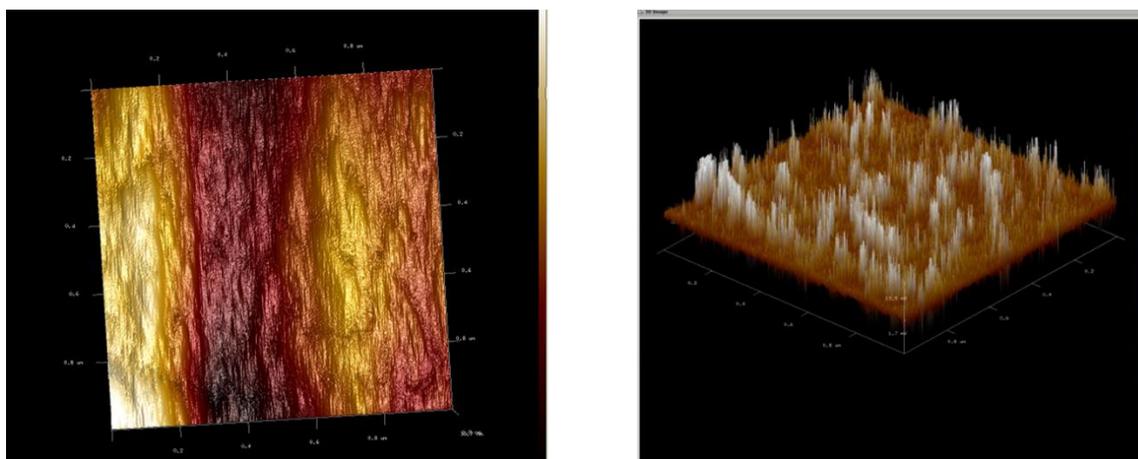
The results showed that the hydrophilic adhesion forces from Figure 23 were stronger compared to that of the hydrophobic functionalized tip from Figure 22. The results indicated a stronger affinity of hydrophilic carboxyl group with SiO<sub>2</sub> nanocoating layer, which is also a hydrophilic group. The readings were similar at multiple points taken for adhesion force throughout the crystal planes and were tabulated as shown in Table 4. The plane of the crystal was not only smooth with the nanocoating, but also showed higher affinity towards the carboxyl groups, indicating that the hydrophilic forces bind stronger than the hydrophobic forces. The

results may point an interesting trend, which is reflective in case of hydrophobic lubricants, and hydrophilic glidants that are commonly added on to the particle surfaces in bulk pharmaceutical manufacturing.

The entire procedure was repeated with an excipient, lactose crystal. Lactose crystals were made of saturation solution where lactose was mixed with distilled water in a mixer and kept in a container which was insulated to avoid external disturbance. The container was covered with perforated cover so that the evaporation is slow. After five to seven days' crystals were formed. Crystals formed were very tiny and brittle. A clear and smooth lactose crystal was picked and kept under the AFM to check the surface topography and perform SiO<sub>2</sub> nanocoating.

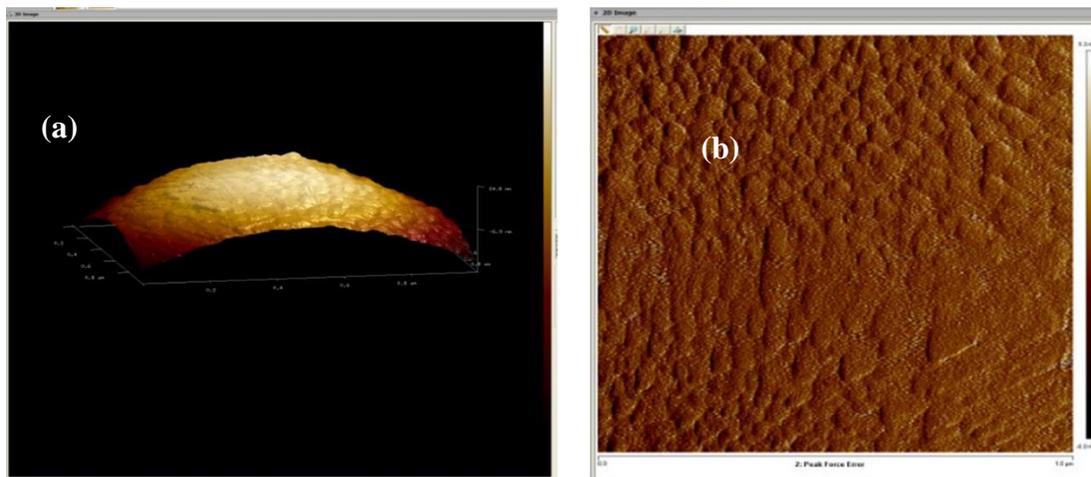
#### **7.4 SURFACE TOPOGRAPHY OF LACTOSE CRYSTAL**

Figure 24(a) shows a 3D image of lactose crystal where the surface is smooth and clean. The clear and smooth area was first identified by putting the AFM probe in the scanning mode and check for the uniformity of scanned surface. The uniform peaks seen from the 3D image in Figure 24 (b) showed the smoothness and uniformity of the crystal surface.



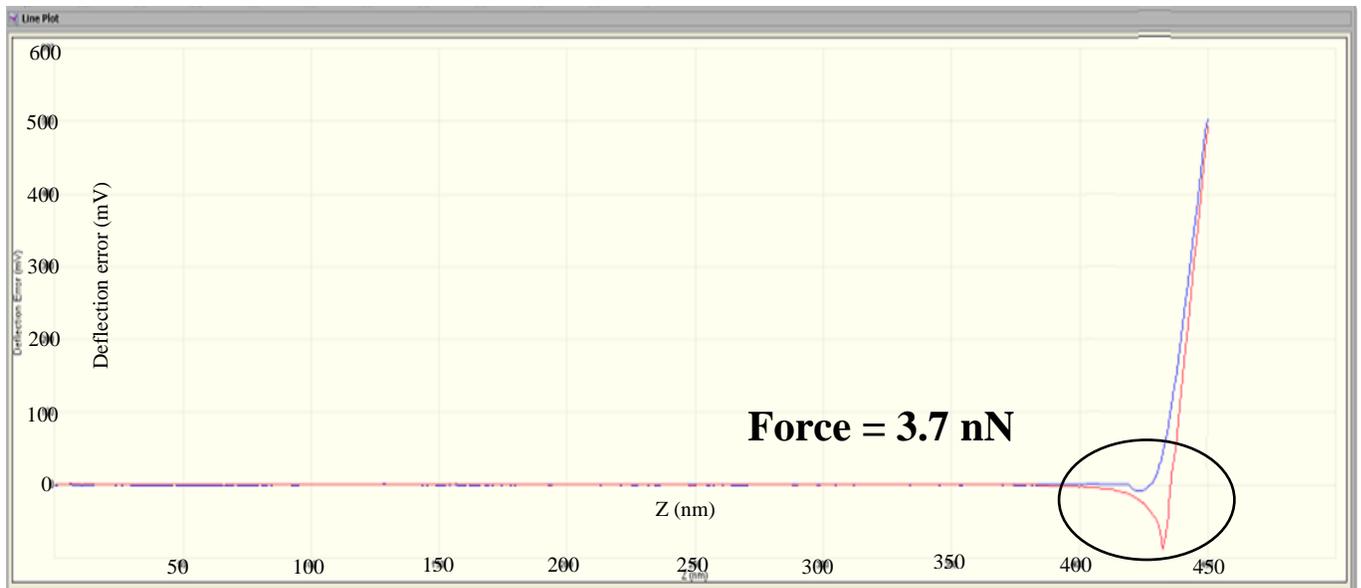
**Figure 24: Surface topography of lactose crystal under AFM.**

The lactose crystal was then coated with 50 nm SiO<sub>2</sub> nanocoating with PVD. The same procedure that was used in previous sections for coating APAP crystals was also repeated for coating lactose crystal. The crystals were coated in an E- beam evaporation system at a room temperature.



**Figure 25: Surface topography of lactose coated with SiO<sub>2</sub> with thickness of 50 nm.**

Scanning mode peak force images were taken after the nanocoating on lactose crystal. The above Figure 25 shows the surface of lactose crystal after coating with silica having a thickness of 50 nm. Figure 25 (a) is a 3D image of the nanocoated lactose crystal surface and Figure 25 (b) shows the uniformity in coating on the surface. In the next step, force curves were analyzed on both coated and uncoated lactose crystal.

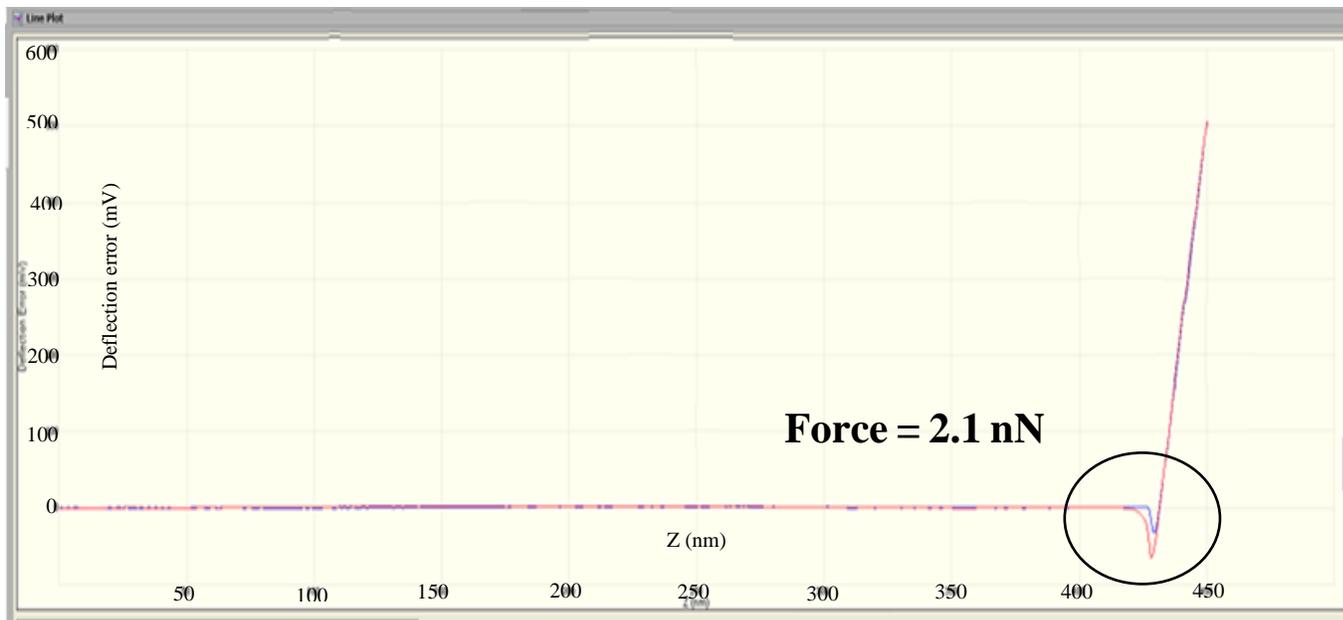


**Figure 26: Force curve of plain lactose crystal.**

**Table 5: Different points of force curve on lactose crystal surface.**

Position	Force
	<b>n N</b>
1	3.7
2	3.7
3	3.7
4	3.7
5	3.7
6	4.2
7	2.5
8	2.5
9	2.9
10	3.3

Figure 26 shows the force on plain lactose crystal. The adhesion force on plain lactose crystal was high due to its uneven surface. The unevenness of the surface, high surface energy and attrition was the cause of high force. Using the calculations mentioned in the previous section the force was found to be 3.7 nN for the Figure 26. Table 5 shows ten different point on the lactose crystal were chosen and the forces were measured.



**Figure 27: Force curve of coated lactose crystal.**

**Table 6: Different points of force curve on coated lactose crystal.**

<b>Position</b>	<b>Force</b>
	<b>n N</b>
1	2.1
2	2.1
3	2.1
4	2.1
5	2.1
6	2.1
7	1.2
8	0.84
9	1.6
10	1.6

Figure 27 shows that the force curves varied when the crystal was coated. Since there was a uniform coating on the lactose crystal, the surface after coating was a smooth surface that in turn lowered the surface energy and attrition hence the force (2.1 nN) was low on SiO<sub>2</sub> coated lactose when compared to uncoated (force = 3.7 nN). Force curves at ten different positions were tabulated

for the uncoated lactose crystal as seen in Table 6. Since the surface of the crystal had a uniform coating the forces were constant.

In the next stage, functionalized hydrophobic AFM tip was used to check the force curves on coated crystal planes as shown in Figure 28. The force curves showed a decrease in adhesion force as seen in previous section (from Figure 22). Overall, hydrophobic forces were weaker than the hydrophilic forces, irrespective of the chemical motif of the excipient or an API. Table 7 shows ten different points chosen on the lactose crystal and the forces were measured. Due to the weak affinity of functionalized hydrophobic and coated layer hydrophilic the forces (Table 7) were low.

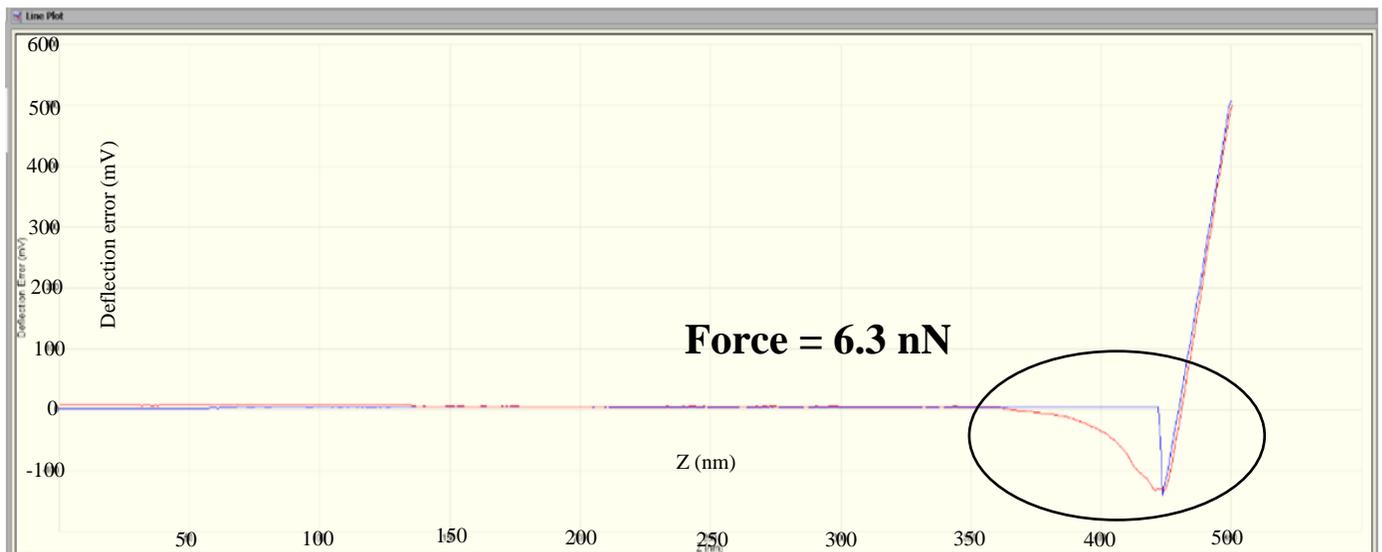


**Figure 28: Force curve of coated lactose crystal, functionalized AFM tip with hydrophobic solution.**

**Table 7: Force curves at different points on the lactose crystal.**

Position	Force n N
1	1.6
2	2.1
3	1.2
4	0.84
5	0.84
6	0.84
7	1.2
8	1.2
9	1.6
10	1.2

The hydrophilic functionalized tip was then used to check the forces on lactose crystal planes. Figure 29 shows that the hydrophilic forces (6.3 nN) were stronger for the hydrophilic interaction with SiO<sub>2</sub>. The force curve of hydrophilic tip and coated lactose crystal was found to be stronger when compared with force curve of hydrophobic (1.6 nN) tip. This is because the strong interaction between the hydrophilic tip and the hydrophilic nature of SiO<sub>2</sub>.



**Figure 29: Force curve of coated lactose crystal, functionalized tip with hydrophilic solution.**

Overall, the forces with the functionalized tip of 8-mercaptooctanoic acid solution and coated lactose crystal was found to be the highest (Figure 29). Table 8 shows ten different points on the lactose crystal surface. This indicates that the adhesion was stronger for (Table 8) lactose crystal (excipient materials) coated with SiO<sub>2</sub> shown in Figure 29 than that of APAP crystal (active drug material) coated with SiO<sub>2</sub> shown in Figure 23 (Table 4). The results also indicate that the adhesion of nanolubricants, additives and glidants can be higher on excipients. This finding is significant as the popular belief is that the drug carries most of the additive coatings and the scientific community mostly focusses on the interaction of API with the nano-lubricant. However, our results in Figure 29 showed that the excipient materials, which is lactose, were significantly effective in attracting the lubricants and glidants. Going by the findings of this research work, the focus of pharmaceutical material interactions can be put on both API and excipient rather than on just API alone.

**Table 8: Force curves at different points on lactose crystal.**

Position	Force
	n N
1	6.3
2	6.3
3	5
4	4.2
5	4.2
6	6.3
7	6.7
8	6.3
9	7.1
10	5.8

## 8. CONCLUSIONS

- ✓ Surface topography of APAP crystals changed with exposure to humidity over a period. The surface of crystal started to re-crystallize over a period. The growth of crystal was found to be non-uniform and was dependent on several external factors such as moisture, humidity, contamination, solubility, and solution purity. Dynamic surface topographies were observed on both APAP and lactose crystals that depended upon the condition during the crystal growth. Uniform crystal planes were observed on all crystals with some areas having non-uniform surfaces. Lactose crystals possessed more uneven surfaces than APAP crystals.
- ✓ Adhesion forces on plain crystals showed that APAP crystal had higher force than cellulose crystal because of the smoothness of the crystal surface varied on APAP and lactose. Adhesion forces taken at multiple locations were consistent showing the development pattern of the crystal plane.
- ✓ Uniformity and extent of nanocoating was found to increase the surface adhesion force.
- ✓ Nanocoating thickness of SiO<sub>2</sub> was found to influence the adhesion forces on crystal planes. Crystal planes that were coated with 10 and 20 nm showed variable adhesion forces. This was also confirmed from the topographical images taken in the scanning mode.
- ✓ A uniform nanocoating of SiO<sub>2</sub> was found at a coating thickness of 50 nm. At this coating thickness, it was found that the entire crystal plane was covered with nano SiO<sub>2</sub> coating.
- ✓ Adhesion force on coated crystal was found to be higher than the uncoated crystal surface indicating a stronger force of hydrophilic-coated nanolayer.
- ✓ Hydrophobic (alkane thiol) and hydrophilic (carboxy-alkane thiol) functionalization of AFM tip facilitated a functional interaction group with nanocoated crystal surfaces.

- ✓ Hydrophilic functional group had a stronger force of adhesion compared to that of the hydrophobic functional group.
- ✓ Chemical nature of both API (APAP) and excipient (lactose) was found to influence the magnitude of adhesion force with both hydrophobic and hydrophilic functional groups.
- ✓ Excipients were found to have stronger forces than active drug substances.

## **9. FUTURE RESEARCH WORK**

In the current work, only SiO<sub>2</sub> coated crystal surface interactions were tested and measured. However, in future measure magnesium stearate because of the technical complexity of the material and its chemical nature of the lubricant that will be difficult to clean after the coating is done. Hence, in the future research work, we intend to extend our work to first explore several different ways to PVD coat MgSt (Magnesium stearate) as the technical complexity of the material and its chemical nature of the lubricant that will be difficult to clean after the coating is done.. Once this is figured out, we will focus on layer-by-layer coatings of both MgSt and SiO<sub>2</sub> in various mixing orders. SiO<sub>2</sub> will be coated first followed by MgSt coating on top of it. This order will be exchanged in the next stage, by first coating with MgSt, followed by SiO<sub>2</sub>. Mixing order chemistry as a function of chemical nature of API and other excipients will be studied. Finally, a multi-scale molecular interaction parameter will be developed systematically, and various mechanistic approaches will be explored. The formation mechanism of several nanolayer interactions will be investigated to discover the principle causes of multi-scale phenomenon. Once this experimental model is developed, the results will be validated with theoretical models both at molecular and

particle scales. Overall, chemical interaction phenomena at nano, micro, meso and macro levels will be developed to connect the scales using the multi-scale theoretical and experimental models.

The studies will then be related to particle interactions on bulk scale during macroscopic bulk manufacturing and industrial pharmaceutical manufacturing. Results of this study will be related and validated for a bulk scale manufacturing in order to understand the interaction phenomena of multi-scale particles, typically used in pharmaceutical formulation, powder flow and mixing. Overall, forces contributing to the problems of over-lubrication and under-lubrication will be studied both at molecular and particle level interactions.

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