EXAMINING MECHANICAL PROPERTIES OF SINGLE ACETAMINOPHEN CRYSTAL USING NANOINDENTATION METHODS

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ABSTRACT OF EXAMINING MECHANICAL PROPERTIES OF SINGLE ACETAMINOPHEN CRYSTAL USING NANOINDENTATION METHODS

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The pharmaceutical industry incurs substantial loss in revenue and consumer confidence with inefficient manufacturing practices. Large scale processing of organic compounds is challenging due to its sensitivity to environmental conditions and the unpredictable breakage behavior of tablets under applied stress. Tablet compaction and particle size reduction through milling induces variability in the end product. Variability in powder flow, stress induced transformation in polymorphic compounds, re-crystallization after compaction, and lack of content uniformity are some factors that translate into poor product quality. These challenges can be partially resolved by a better understanding of mechanical properties of crystalline pharmaceutical materials at single particle level. The endeavor of this study was to understand the breakage behavior of various planes of a single Acetaminophen crystal using nanoindentation instrumentation.

The results of the study indicated that the Acetaminophen crystal is anisotropic with respect to hardness and Young's modulus values. Analysis of the load-depth curve, discontinuities on the loading and

unloading cycle were observed, as well as pop-in events during constant load intervals. Furthermore, the frequency of pop-in events on the loading depth curve was found to correlate with the elasticity of the planes in question. It was also apparent that the organic compound was sensitive to environmental conditions. Varying strain rates effects different planes of the same crystal and also in adhesion reflected sensitivity to environmental conditions. The exact mechanism by which the crystal deforms is still unknown. However it is theorized that it could be through partial dislocations and crack propagations.

ACKNOWLEDGEMENT AND/OR DEDICATION

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1. INTRODUCTION

It has been suggested that improvement upon current manufacturing processes in the pharmaceutical industry may result in an annual saving of $90 billion [1]. With rising cost of health care, increasing cost of commercialization, abbreviated exclusivity periods for intellectual property, stringent government quality criteria, and diminishing returns on research and development endeavors, this would be a welcomed relief.

Much of the cost associated with pharmaceutical industry is assumed to be derivative expense from research and development of novel drug products. Manufacturing costs and practices of existing and prospective drug products have been largely ignored. It has been estimated that cost of manufacturing a brand name drug can range between 27-30% of sales; trumping research and development costs for the brand [2]. Proposed manufacturing cost for generic drugs are a staggering 50% of total revenue sales [2]. These statistics convey an austere reality for the pharmaceutical industry; it's pending resolution can ultimately lead to cost optimization that would be mutually celebrated by consumers and industry.

Manufacturing cost entail: raw materials, machinery and operating procedures. The increase in cost is most likely a consequence of batch-to-batch production. Batch-to-batch production can lead to variability in

solid-state properties of a yield and results in considerable waste of pharmaceutical product due to inadequate compliance with quality standards. Continuous processing, which can regulate operations though sophisticated computer systems is not yet established for the pharmaceutical industry or a viable option for all processing steps. Other product flaws of particular interest arise from tablet compression and milling. Poor tablet manufacturing can lead to chipping, delamination, capping, and friability. These characteristics can lead to poor shelf life and variations in bioavailability. Bioavailability is of particular concern, since content uniformity is not a guarantee in solid dosage form. The uncertainty in quality from a compromised tablet renders it ineffective. Milling unit operation, which is considered to an essential and versatile tool in pharmaceutical manufacturing, is problematic. The milling process has been accused of causing stress induced polymorphic changes, unwanted Oswald ripening, stimulating crystal and surface energetics, and changing powder flow properties [1,3]. These effects, of course have multitudes of down stream processing implications.

Understanding the micro and atomic behavior of the material being processed can isolate macro production challenges. Excipients and Active pharmaceutical ingredients, which are two necessities in tablet formulation, are synthesized in crystalline form. Therefore, a comprehensive understanding of mechanical properties and their dependence on crystal structure is a critical first step to alleviating

manufacturing challenges associated with tablet compression and milling operations. This study seeks to investigate the mechanical properties of multiple planes of a single Acetaminophen crystal using nanoindentation methods.

2. LITERATURE REVIEW

2.1 Background

Presently, there is a lack of foresight, with respect to manufacturing capabilities, when evaluating a prospective drug candidate by research and development team. A probable drug candidate is shuffled through various stages of development, which include: discovery, formulation, chemical development and human testing, by meeting limited criteria [1]. This “throw off the wall approach” advances drug candidates without collecting adequate information pertaining to crystal form, physical properties and manufacturing viability [1]. The delayed scrutiny slows process development, which hinders product launches, product quality and leads to decreased efficiency. These deficiencies are more or less resolved by manipulating drug and excipients ratios, applying granulation techniques, utilizing novel technologies and constructing complex drug delivery systems [1,4,5]. However, a better strategy would be one that is less reactive and more predictive. In order to forecast the behavior of a bulk active ingredient, there should be a clear understanding of its crystalline structure. More than 90% of small drug molecules, in fact are delivered in crystalline form [5]. The bioperformance of these active pharmaceutical ingredients’ [API] are contingent upon crystalline attributes like: size, shape and form. Therefore, it is beneficial to understand single crystal behavior and

how it can be related to bulk properties that translate into manufacturing success.

Isolating a drug compound in a crystalline form is advantageous for several reasons. Foremost, it allows for a rigorous isolation process that authenticates the purity of the molecule. Being able to confidently isolate the desired molecule in the case of a polymorph is important because of stability and toxicity concerns. Crystallization is a reliable and scalable method that offers high degrees of impurity rejection and selectivity, often achieved through solvent mediation [1,4,5]. Crystalline compounds are also favorable because they provide for easier processing and longer storage periods. Thermodynamic stability ensures crystalline substances are physically and chemically stable, which extends their shelf life.

Acetaminophen is the active pharmaceutical ingredient that is the subject of our study. It is also known as Paracetamol and is abbreviated as APAP. Acetaminophen was first marketed in 1956; sales in 2008 tallied 24.6 billion APAP tablets sold, owing to its popularity as an over the counter analgesic and antipyretic [6]. It is a white crystalline powder that is chemically know as N-acetyl-*p*-aminophenol, and bears the following molecular structure as shown in Figure 1: $C_8H_9NO_2$.

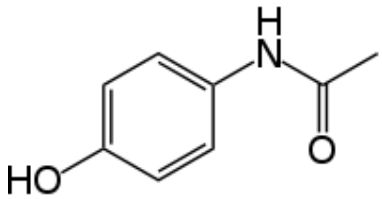


Figure 1: Molecular structure of APAP molecule

2.2 Theory of Nucleation and Growth

Solution crystallization is an integral, and sensitive operation within the pharmaceutical industry and is implemented during the intermediate and final stages of purification and separation. The crystallization unit operation is critical to manufacturing of crystalline API and excipients because it dictates important physical attributes like: size, habit, structure and concentration of defects, as well as discerning chemical purity. Crystallization is typically a non-continuous operation, and elicits batch-to-batch variability. This inconsistency in quality of the crystals presents real challenges for down stream processing: drying, filtration and milling. In order to resolve some of the external and internal factors that influence crystal growth, so as to obtain a greater control over reproducibility of stable form and physiochemical attributes; one must understand nucleation theory. Crystallization occurs in several complex stages: supersaturation, nucleation and crystal growth.

2.2.1 Supersaturation

Supersaturation is the fundamental driving force behind crystallization, and is equated to the difference between the chemical

potential of molecules in a solution and the chemical potential of the bulk crystal phase [7]. A chemical potential difference that is greater than zero indicates a supersaturated solution and implies that nucleation and crystal growth are plausible. Conversely, when the change in chemical potential is below zero it suggests that the solution is under-saturated and therefore dissolution is more probable. Quantity of crystals and the size of the crystals are depended on degree of supersaturation. For instance, crystals with high friability, decreased stability, low purity, high surface area, and small particulate size are all characteristic of crystallization from supersaturation conditions close to the metastable limit; a consequence of high nucleation and poor crystal structure [1,7]. Conversely, large crystals with high purity and stable crystal structure are resultant from operating close to the solubility curve [1].

Influence of degree of supersaturation has been observed in APAP crystals. Supersaturation effects the growth rate and crystal habit of the Paracetamol crystal by accentuating one crystal face over another. It has been remarked that with low supersaturation the (110) face is dominant along with the columnar crystal habit [8]. Increasing the supersaturation levels results in the dominance of the plate like feature and (001) plane [8]. Furthermore, it has been studied that the initial surge in supersaturation results in a higher water content in the crystal, instead of the expected change in crystal habit. Along with the increased water

content, there is an associated decrease in enthalpy of fusion and dissolution rate of the Acetaminophen crystal [9].

2.2.2 Nucleation

Nucleation results in the genesis of a new crystalline product from a solution. Molecules move in random motion in a solution, and through constant collisions result in aggregation of clusters. These clusters can form a nucleus depending on their critical size either irreversibly evolving into a macrocrystal or reversibly, disintegrating back into the solution. The jostling between the volume and surface dimensions is the prime arbitrator of whether the modest aggregates will get an opportunity to grow further. Specifically, volume term favors growth of the clusters because it reduces the Gibbs free energy via exothermic process [7]. Meanwhile, the surface term favors dissolution. Therefore the clusters that exhibit a high surface to volume ratio will disassociate, and the clusters that have larger volume to surface ratio will propagate. For that reason, critical radius is a standard for classifying a cluster as a nucleus. Below, Figure 2 represents the nucleation and crystal growth cycle.

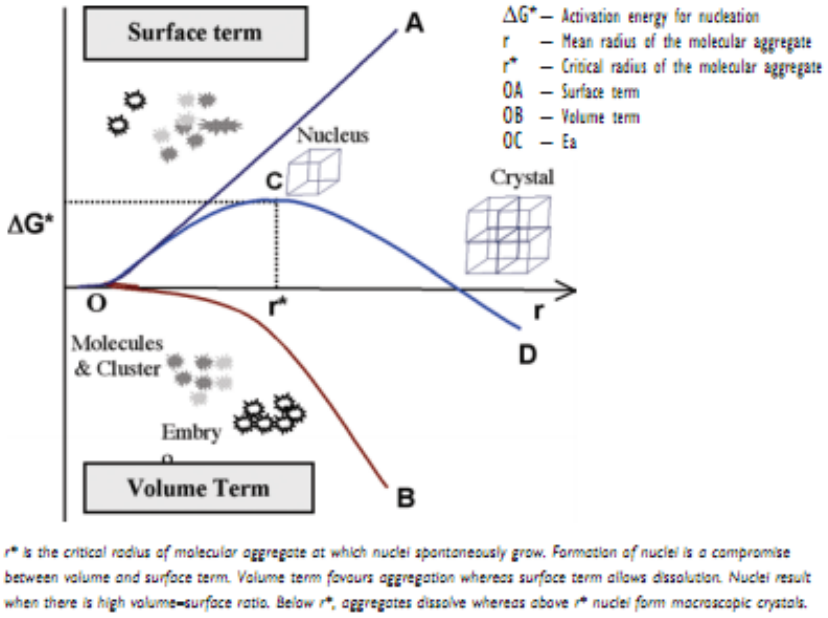


Figure 2: Thermodynamic Relationship Between Activation Energy and Mean Radius of Molecular Aggregate

Banga, Sheere, Garima Chawla, and Arvind K. Bansal. "New Trends in the Crystallisation of Active Pharmaceutical Ingredients." *Business Briefing:Pharmagenerics* (2004): 1-6. Print.

Nucleation is classified as either primary or secondary, and the former classification is further subdivided into homogenous and heterogeneous sect. Homogenous nucleation is an idealized process that is devoid of all impurities. In contrast, heterogeneous nucleation is a dynamic process that encompasses random impurities and additives. Secondary nucleation is a process that utilizes seed crystals of the desired composition to induce nucleation. The favorability of a nucleation process is contingent upon the surface free energy. In fact, the activation energy and critical radius of the total free energy function are emphatically influenced by surface free energy. Surface free energy at lower supersaturated concentration is reduced considerably by either

incorporating impurities or minimizing the discrepancy between the substrate material and the crystallizing product. As a result the heterogeneous nucleation process is more feasible than the homogenous nucleation process. However, reduction in surface free energy is greatest in secondary nucleation process because the substrate and the crystallization product are the same.

2.2.3 Crystal Growth

Crystal growth is the final phase in the particulate formation cycle characterized by augmentation of the nuclei into a large crystalline particulate. From Figure 2, it can be seen that after the activation energy barrier has been crossed, the increase in volume term facilities the growth of the nuclei. Even in the final stage, there is a competition between nucleation and crystal growth over available solute molecules. The rate at which molecular resource is consumed determines the crystal size. Large particles are a result of early nucleation, where as later the system nucleates the more rich in fines the product will be [5].

External and internal factors can manipulate crystal growth. External factors include: temperature, degree of supersaturation, solvents, pH, stirring rate, presence of impurities and rate of evaporation or cooling of the solution. Internal factors include: crystalline defects and crystalline structure. These intrinsic features decide the behavior and intensity of intermolecular interaction between the crystal surface and solution.

As mentioned above, temperature and pH can be used to induce crystal growth change. Acetaminophen crystals can be altered with modulations in temperature. At 3°C the dominant crystal plane is (001) [9]. However, when the temperature is increased to 47°C, the longest crystal plane is (110) [9]. The manner in which pH influences crystal growth rate and crystal habit is still somewhat enigmatic. One study theorizes that pH of crystallizing medium influences crystal growth by the adsorption of OH^- and H_30^+ ions by the surface [9]. This is thought to impact the crystal active growth site.

It should be noted that for pharmaceutical crystals, the crystals are comparatively more delicate than inorganic based crystalline particulates. They are susceptible to erosion, clumping and sensitive to fragmentation [1]. Organic crystals generally exhibit higher degree of amorphism and solidify in no certain structure. This phenomenon is referred to as: “oiling out” [1].

2.3 Crystal Structure

2.3.1 Crystal Habit

Crystal habits are important consideration in tablet compaction and down stream processing of pharmaceutical product. Through crystal shape, and the structure, the product’s physical and mechanical properties can be modified. The crystal morphology can contribute to the ease or difficulty of processing by affecting mixing and demixing, flow and milling. With respect to tablet stability, it can cause lamination and

capping issues derived from contributing factors like: particle slippage, particle bonding, preferred orientation and pressure transmission [1]. It can also affect dissolution and particle uptake, thus making it very sensitive to bioavailability concerns.

Crystal habit is a product of its crystallization conditions. The Law of Rational Indices clarifies criteria of a stable crystal face. The stability of a crystal face is associated with low energy, which is associated with slow crystallization. This is prevalent in nature. Also, stable crystal faces tend to have the high density of atoms and are found perpendicular to large interplanner spacing [1].

Crystal habits are often manipulated by using different solvents, concentration of impurity or additives, temperature and degree of supersaturation. By using these factors, crystal habits can be engineered to give desired results.

Pharmaceutical products are generally organic anisotropic materials, and their morphology is usually tweaked in order to optimize bioavailability, or processing characteristics. Isotropic spherical and cubic habits are considered ideal for manufacturing.

Solvent mediated crystal transformations are widely used to alter the crystal habit. Solvent-solute interactions play a key role in which crystal habit is created [9]. Figure 3 shows the change in morphology of Acetaminophen using different solvents. Crystal morphologies between polymorphs also vary greatly, which is logical because polymorphs can

display different physiochemical properties. In the case of Acetaminophen, its crystal habit can be converted by using benzyl alcohol from orthorhombic needle shape to monoclinic plates shape [5].

The shapes that are featured in the micrographs were similar to the habits obtained experimentally in our lab using ethanol, methanol, acetone and water.

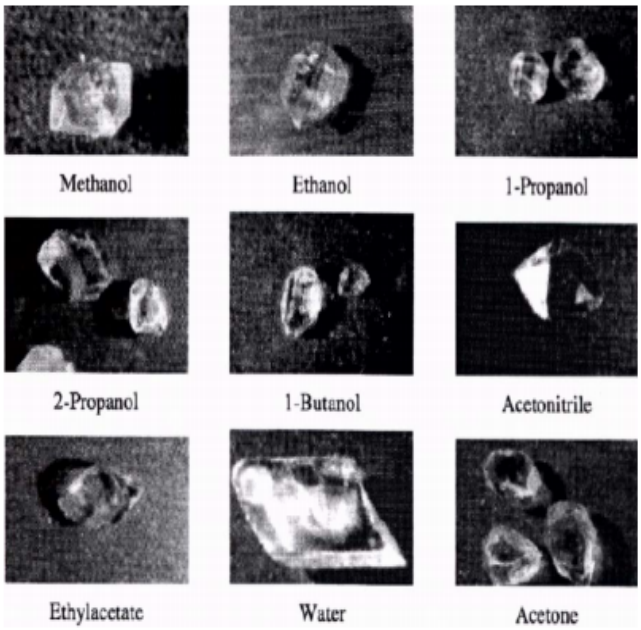


Fig. 6 Paracetamol crystals obtained from various solvents [18].

Figure 3: Acetaminophen crystal habits from various solvents [8]

Impurities and additives can also be used as growth inhibitors or accelerators to dictate which direction growth can continue, and thereby facilitating creation of a designer crystalline product. Additives change the crystal structure in three ways: by restricting the adsorption of solute molecules, become incorporated into the crystal lattice by planting to the surface, and inhibit the nucleation process [8,10,11]. The restriction with

using additives at times is because of the decrease in purity of the final material, due to the by inclusion of the solvent and an increase in crystalline defects [12].

Though there are options for deliberate adjustment of crystal habit, most pharmaceutical products are not adjusted intentionally. Mostly, it is a “by-product” of downstream processing, as was the case in the transformation in Acetaminophen. Metacetamol, which was a reaction by product during a manufacturing process, altered the spherical habit of APAP crystal into needle morphology [5]. Needle morphology is extremely difficult to process in terms of drying, filtering, handling in powder form or using in a formulation [5].

2.3.2 Crystal Packing

Crystal packing determines the physiochemical properties of the single crystal and eventually the interactions of bulk products. And is therefore responsible for product performance under applied stress. Organic crystals are a result of collection of responses to the intermolecular force system. These forces are responsible for binding atoms together to form molecules and are relatively weak interactions.

These intermolecular forces can be broken down as being either attractive or repulsive. The attractive forces can be further sectioned into three categories: non-bounded, ionic and electrostatic interactions. Of these three, the non-bounded interactions are of particular interest in pharmaceutical product analysis.

The non bounded interactions are also referred to as non-covalent interactions and can be discussed in terms of Van der Waals forces and Hydrogen bonding. First, Van der Waals forces are highly depended upon polarizability, dipole moment and electronic distribution of molecules. Hydrogen bonds necessitate acceptor and donor functional groups. The proton donors are NH and OH groups and acceptors are C=O and OH [13]. HABIT, a computer program quantized the OH—H hydrogen bonds to be -24.12 KJ/mol and the NH—O bond to be -10.68 KJ/mol [13]. Hydrogen bonds are also directional and anisotropic. Acetaminophen crystal is primarily constructed by hydrogen bonding and Van de Waals forces. In fact the molecules are packed as sheets in the crystal lattice, and their only means of interaction is through Van der Waals forces [13]. In a monoclinic APAP crystal, used by Boldyreva et al.'s group, found the hydrogen bond network along with the strong interactions between the benzene ring to account for 60% of the lattice energy [13].

2.3.3 Polymorphism

Polymorphism defines a materials ability to have multiple crystalline forms that have different molecular packing configurations. These differences in structural ambiguity arise by the behavior of the intermolecular forces described earlier. These variances by molecular interaction force determine periodicity of molecules, which in turn promote energy differences that translate in to changes in physical properties.

The pharmaceutical industry requires the use of the most stable form of a polymorph, and this is a common concern, since most organic compounds are dimorphic. Novir™ an HIV drug is a cautionary tale of how a marketed drug failed after a more stable form of the active ingredient: ritonavir appeared during production [5]. Its appearance was triggered by a by-product reaction during manufacturing that corrupted the entire supply of Novir™. It should be noted that pharmaceutical products, like Paracetamol, also experience stress-induced transformations, from milling, drying and compaction, though this was not the case in this particular instance [14]. The new thermodynamically stable form exhibited much lower dissolution rate, which is inline with the most stable phase. As a consequence, Abbott, the producer of Novir™ suffered significant economic loss and consumers had to wait for a life saving drug.

Polymorphs are dictated by thermodynamic stability, and crystallize above or below their transition temperature. Polymorphs can be discriminated based on the same criteria mentioned earlier: use of solvents, seed, additives or degree of supersaturation. In the case of Acetaminophen, excipients have been used widely to curb the appearance of the unstable form II from aqueous solution [13]. In the same effort, high degree of supersaturation has been used to selectively create the more stable form I of APAP crystal.

Acetaminophen has two definite polymorphs and a third unstable polymorph. The commercially used form of Paracetamol, which is stable at room temperature, is monoclinic form I. It also has poor compaction properties. Form II is orthorhombic and is metastable at room temperature. It has poor shelf life at ambient conditions; one study reported a conversion from form II to form I after three to four months [15]. The same study also observed a transformation with increased temperature: 90°C for one hour [15].

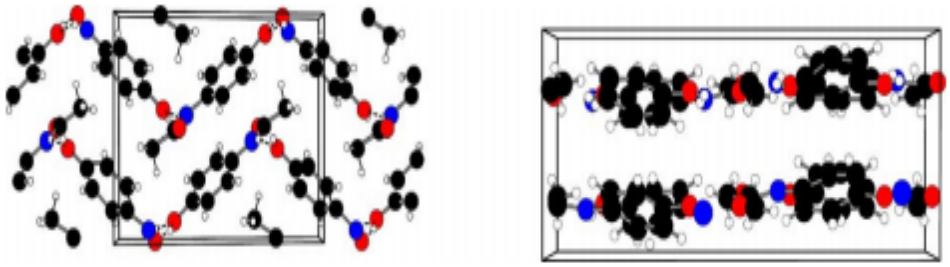


Fig. 1. The fragments of the crystal structure of the monoclinic (left) and orthorhombic (right) polymorphs of paracetamol. (For colour in this figure, the reader is referred to the web version of this article.)

Figure 4: Acetaminophen Polymorphs. Monoclinic form I (left) and orthorhombic form II (right) [4]

As discussed earlier, hydrogen bonding and Van der Waals forces are the primary interaction forces in APAP crystal lattice. The structure of the lattice varies with form I and form II. Form II has planer molecular sheets meanwhile form I, has sheets that are in a zigzag pattern [16]. The packed molecules in the sheets also follow the respective packing patterns of the associated form. As previously mentioned these molecules interact mainly through hydrogen bonding. Despite having similar structures

these polymorphs behave very distinctively when considering compaction behavior.

Studies that have compared the Young's modulus between different polymorphs have concluded that the most stable polymorph usually has a higher Young's modulus, yield stress, tensile strength and true density. Thus indicating that stable polymorphs will be resistant to deformation and this implies weaker compaction behavior than their less stable counter parts. A sufficient dwell time is needed for plastic flow to initiate as well as allowing the formation of bonds [17]. Stable polymorphs generally are considered to have greater lattice energy, this confers the increase in tensile strength [17].

2.4 Anisotropy

Organic compounds have directional bias when reacting or expressing mechanical. This intrinsic bias adds to the complexity of the breakage behavior of pharmaceutical products. However, anisotropic behavior could be used in the future to design drug substances that give desired features to ease manufacturing or customize bioavailability.

Acetaminophen, an aromatic polyfunctional molecule operates through hydrogen bonding forces that induce highly anisotropic behavior. Structural deformations are the means by which hydrogen bonds adjust to temperature and pressure fluctuations. Pressure changes encourage greater molecular distortion, and were used in the study conducted by Boldyreva et al. [13]. The aim of the study was to gain a deeper insight as

to how the intermolecular forces that dominate the organic molecule affect it under high pressure.

The concluded by stating that ethanol-water derived APAP crystals experienced expansion in several directions with the increase in pressure. However, there was also contraction in the volume of the crystal, which was attributed to the hydrogen network. Also, charge distribution within the molecule is contingent upon the maintenance of a hydrogen network. Interactions are of a highly cooperative nature and benzene rings enable the molecule to be more flexible [13].

The specificity of the structural changes is discussed in terms of intramolecular and intermolecular bonding. With the increase in pressure, C=O bond length was found to have been stretched [13]. And a shortening of the OH—O was noted [13]. The study did find considerable change in the torsion angle between the planes of the crystal, making it flatter.

The intermolecular forces experience a noticeable change with increasing pressure. The OH—O, NH—O, and the molecules between different planes were observed to have shortened [13]. Furthermore, the NH—O bond was to found to be more compressible than the stronger, shorter OH—O bond [13].

The flattened Acetaminophen molecule allows for denser packing, and in result is more stable crystal. This flattened molecule is attributed to the shortening of the hydrogen bonds discussed above. These

shortened bonds, that are an outcome of increase pressure, decrease the dihedral angle. The dihedral angle, which is the angle between the amide group and benzene ring is sensitive to the protonation and deprotonation of the OH and NH groups [13]. For instance, in a stable conformation the dihedral angle is estimated to be 0.3°, but when OH group is deprotonated the dihedral angle changes to 90.0° [13].

Contraction of the molecule was also a side effect of the increased pressure. This contraction was preferential, and occurred where NH—O and OH—O bonds were linked in alternating chains [13]. In contrast the reported expansion occurred in the NH bonded chains. The rotation of the benzene ring is also responsible for the observed compression. The rotation of the benzene ring contributes to the decrease in steric hindrance between molecular fragments and leads to increase in packing density at higher pressures [13].

The hydrogen-bonded cycle, which forms a two dimensional network in structures was observed to give structure changes in Paracetamol molecule with increasing pressure with respect to: deviation from the plane, distance from the cycle centroid, and the changes in sum and individual angles. The discernable change observed in the molecular structure did not modulate the shape and size of the hydrogen-bonded cycle.

Anisotropy dictates physical and chemical properties observed in an organic crystal. Hydrogen bonding determines the extent and nature of

molecular interaction within the APAP crystal. It is responsible for the observed imbalance in mechanical properties from plane to plane. Introduction of pressure allows for these intermolecular forces to be observed. The minute changes in bond length, angle and degree of rotation of intermolecular forces have wide spread implications that can compromise the physical stability of an organic product. And so it is imperative to scrutinize these interactions, as was done by Boldyreva et al. [13].

2.5 Adhesion

Inspection of a crystalline surface can reveal interesting information about the impact of environment and the general history of the material. Surface inspection can reveal surface deformation like cracks and fracture, as well as surface interactions like adhesion. Adhesion is the manifestation of an attractive force when two materials with different surfaces are brought together. Adhesion interactions are suspected to be magnified by environmental conditions. Because most pharmaceutical ingredients are hygroscopic, sensitive to humidity, this poses a great concern for bioavailability, processing and shelf life. Pingali et al. evaluated crystalline surfaces of Acetaminophen crystals using AFM to understand the dependence of adhesion on relative humidity with respect to exposed hydrophobic and hydrophilic groups [18]. The study observed that with hydrophilic AFM tips the adhesion reached a maximum peak and then tapered off with increase in relative humidity [18]. Over all it

was found that hydrophilic AFM tips garnered greater adhesion than the hydrophobic [18]. Heng et al.'s group also investigated the hydrophilic and hydrophobic nature of Acetaminophen crystal in both its polymorphic forms [19]. Their studied sought to understand the varying wetting behavior between different facets of the crystals. It was found that the, advancing contact angle, θ_a, was the largest when the associative attachment energy was the weakest. In the study the facet (010) of Paracetamol I had the weakest attachment energy, and the $\theta_a = 67.7° \pm 2.5$ [19]. The large contact angle implies that the plane is hydrophobic. Meanwhile, the most hydrophilic plane was (001) with a contact angle of $\theta_a = 15.9° \pm 3.1$ [19]. The study suggests that the hydrophilic and hydrophobic state of a plane is dependent on the OH group at the surface of the plane to forming bonds with external molecules [19]. The study also noted that the XPS predicted surface polarity rankings of the plane further validated the results they observed. Finally, Pingali et al.'s study also observed topographic transformations at high relative humidity that suggests re-crystallization of the surface [18]. The results of this study further speak to the surface sensitivity of organic crystals to environmental and external conditions, which is derived from Van der Waals forces.

Nanoindentation is an analysis tool that evaluates the mechanical properties of a material by probing its surface. However, in its contact analysis it neglects adhesion interactions. It assumes that when the load

is zero that the contact area is also zero [20]. This method of analysis is incomplete when investigating surfaces that illicit an adhesion interaction.

Adhesion manifests when a two different surfaces come into close contact. Landman et al.'s group suggests an atomistic interpretation of adhesion interaction by experimenting with a nickel tip probing a gold surface [21]. The explanation starts with the nickel tip inducing plastic deformation when indenting the gold surface. As the tip makes contact with the gold surface it tears the surface. At this point a part of the surface adheres to the nickel tip, as it penetrates deeper into the sample. The tip penetrates further into the sample causing adhesion induced flow that is supported by slip planes that ultimately cause point defects in the material. When the tip is finally retracted from the sample there is ductile mode I fracture. There is a crystalline neck that tries to hold the two surfaces together, and it stretches until there is a clear separation between the surfaces. The neck retains a crystalline surface and decreases in cross-sectional area as its being stretched. Elastic and plastic yielding that is conjunction with particle rearrangement and stretching of the bonds explain this event. Adhesion is depicted by unloading curve dipping past the x-axis and then recovering back to the x-axis in an asymptotic manner.

2.6 Mechanical Properties

A crystalline product is defined by regularly repeating unit cells that impose long-range order. This unit cell has a distinct orientation and shape defined by translational vectors: a, b and c. Crystal can be classified by 7 crystal systems, 14 Bravais lattice classes, and 230 space groups. Though, it may seem that permutations of these classification systems would be overwhelming, Cambridge Structural Database claims that 90% of all compounds can be grouped into 17 popular space groups [4].

Crystals are highly ordered structures, but are not devoid of imperfections. In fact, real crystals lattices are abundantly filled with these defects, which manifest in likes of: point defects, line defects and planner defects [22]. Vacancies, impurities and interstitial occupancies are common point defects. Planner defects include grain boundaries, stacking faults and external surfaces. Edge and screw dislocations are grouped as line defects and are of particular interest in the discussion of breakage behavior of pharmaceutical materials [23].

Defects are mostly intrinsic, and develop during crystal growth, however some are caused by external factors like pretreatment or processing. With added pressure, the crystal lattice experiences various stresses and strains that contribute to the concentration of flaws and have far reaching consequences in fracture mechanics of the substance [23].

Mechanical properties significantly influence milling processes. Milling is an inevitable processing step in pharmaceutical industry, and often the only mechanism to modulate solid-state attributes of API and excipients. Crystallization affords the industry to abide by stringent purity standards, however most drugs are not delivered through crystalline means. Solid dosage form however is the most favorable method of drug delivery. Effectiveness of the active ingredient rests with the drug engaging with the correct target in the body at a molecular scale. Size reduction of larger particles facilitates the particles to penetrate restricted areas in the body such is the case with pulmonary delivery, which requires particle size to be in the range of 1-6 μm [5]. Particle size distribution allows the industry to maintain high quality standards because they can regulate size distribution, thus affecting content uniformity in blends, and optimize compaction behavior over all.

When considering size reduction of organic particles through milling process, it is helpful to understand effect of intrinsic defects of the crystalline material and its behavior under applied stress. A previous study investigated the relationship between crystal flaws and their influence on particle fracture in jet mill. The study specifically sought to understand why there is a variance between crystals that were identical in size, but reflected different fracture strengths. The size of the crystal, flaw size, and number of flaws were considered when experimenting with fracture mechanics. It was concluded that flaw density was more

significant contributor in fracture strength than size of the flaw. This further proves the significance of understanding intrinsic qualities of a crystalline matter.

When powder is compressed or reduced by impact, particles rearrange as a result. In the same manner, when a crystal is introduced to stress the crystal molecular arrangement adjusts in order to sustain the shock. A crystal can react to the force in either an elastic, plastic or viscoelastic manner.

Elastic response is reversible and time independent. Prior to application of a force that is under the yield pressure of the substance, the molecules are comfortably vibrating at their equilibrium positions. After the force is applied, the molecules are perturbed and disperse to accommodate the external stress. As the external pressure is lifted the molecules spontaneously return to their equilibrium positions. The stored energy is dissipated when the applied stress is lifted and increases when the force is applied.

Elastic response is based on spontaneous recovery after the load is removed. The strain that is associated with impact is studied in terms of final applied stress. Hooke's law is represented by an ideal spring to understand the stress and strain relationship at low levels of strain. This relationship is encapsulated in the following equation:

$$\sigma=E\varepsilon \quad (1)$$

Stress (σ) over strain (ε) equates to the Young's modulus (E) as shown by the manipulation of the above expression. Young's modulus quantifies the propensity of a material to deform under stress; measures the stiffness of a material. Hardness is a similar material property that is often discussed in conjunction with Young's modulus. Hardness is the resistance to applied stress. Hardness measures plasticity, while Young's modulus measures elasticity. Both these material properties are influenced by the anisotropic nature of an organic pharmaceutical crystal.

In contrast, plastic deformation is irreversible, and its analysis is path depended. For plastic deformation to ensue, yield stress must be exceeded. Yield strength of a material is stress that must be exceeded in order to deform the material and cause irreversible damage. This is illustrated in the stress and strain curve in Figure 6. The point of inflection where yield occurs can be ambiguous for many materials. A yield strength was defined that would necessitate a 2% strain upon removal of the applied stress.

While both elastic and plastic deformations are independent of time, creep is time-dependent permanent deformation. Creep suggests that plasticity can progress slowly and over time after stress has been applied. At a molecular level it speaks to mobility of the molecules. In an elastic deformation, the molecules are rigid and can only functionally respond in a spring motion. Meanwhile, in a viscoelastic event, the

mobility of the molecules is such that they can interact by moving past each other. This allows for a gradual deformation process to ensue.

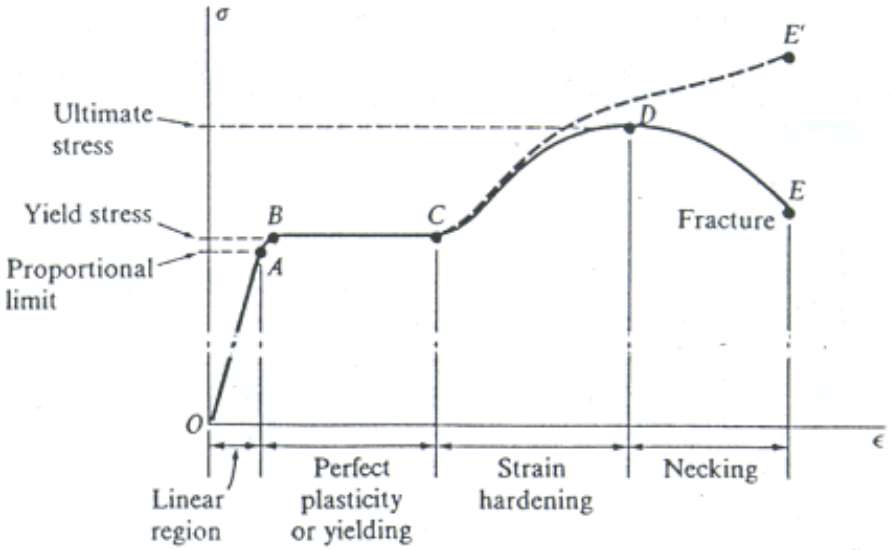


Figure 5: Stress and strain curve

Yield Strength and Heat Treatment. TPP Information Centre. *Steels, Heat Treatment, Case Study - Technology, Product & Processes*. 2006. Web. 03 Oct. 2011. <http://www.tppinfo.com/>.

Fracture is characterized by failure of the product, which culminates in breakage of the specimen. For plasticity to manifest, appreciable amounts of force need to be applied to the material so as to surpass the energetic barrier and enable flow in the most favorable slip system [24]. Fracture can be brittle or ductile, however at an atomic scale bonds are broken. A brittle fracture is reflective of minute changes in shape where as in a ductile material there is a noticeable change. With in a crystal, brittle fractures can be noted by fracture along the cleavage planes. Cleavage planes are planes that are the weakest and therefore fracture with ease. A criterion for a brittle fracture is the existence of a

crack flaw or a stress large enough that can induce crack growth [23]. Ductile fractures are thought to occur through slip planes via shear stresses [24].

Duncan-Hewitt's study on anisotropy of APAP crystal suggested Acetaminophen to be a semi-brittle material that operates on a lone slip system: (010)<011> [24]. This creates high stress concentration because for generalized flow to occur, at least five slip systems should be in play. This increase stress level leads to breakage. In terms of tablet compression, this validates Paracetamol's supposed poor compact behavior. Because it operates on single slip system, this creates realignment in the <010> during punching, which coincides with the (010) cleavage plane. This would be cause for capping during decompression and ejection.

Capping (top) and lamination (right) tablet failure modes

Figure 6: Capping failure (top) and lamination failure (right) [26]

Tablet defects translate into lost productivity, loss in time, repair costs, maintenance costs, and increased downtime, and these are

reflected in an overall loss in revenue [25]. The most commonly observed defects in tablets are: capping and lamination, hardness variability and sticking and picking [26]. Upstream operations, and in particular milling, quality of raw material, and properties of drug formulations are contributing factors of this defect. Capping is described in the figure above, where the tablet top of the tablet has detached from the whole tablet. Lamination is the detachment of any area of the tablet from the body of the tablet. Both are caused by poor compressibility arising from poor powder flow and excessive fines, which lead to failure of the particles to interlock and bind. Thus producing tablets that are prone to defects.

Sucrose is a brittle substance that has capping and delaminating concerns during tablet manufacturing. K. J. Ramos using nanoindentation instrumentation investigated the single crystalline properties of this substance by indenting on multiple planes of the crystal [27]. Table 1 below summarizes the values for hardness and elastic modulus for the various planes he inspected. Sucrose crystals were evaluated in two conditions: as received state and after growth of large transparent crystals. In both crystal planes (001) and (100) were analyzed. These crystals were stored in calcium sulfate desiccant and tested under relative humidity range of 16%-35%.

Table 1: Summary of the indentation results on various sucrose planes [27]

Indentation Surface	Reduced elastic modulus (GPa)	Hardness (Gpa)
(001) habit plane as grown	38.2±0.4	1.57±0.07
(100) habit plane as grown	33.0±1.1	1.44±0.07
(100) cleavage plane as grown	33.7±0.7	1.68±0.15
(100) habit plane as received	33.3±0.5	1.55±0.12
(100) cleavage plane received	34.0±0.4	1.82±0.04

From this investigation, reduced modulus behaved in an anisotropic manner, however hardness was more isotropic. The (100) plane showed elastic modulus value of 33GPa and 38 GPa for (001) plane. This orientation dependent bonding strength is characteristic of complex structures that have minimal slip plane systems [27]. The hardness discrepancy in the value of the cleavage plane being higher than the habit plane was explained by surface roughness. Plastic flow generated pile up around the indentation surface, dislocation mechanism was said to be responsible.

The results that were generated from this study were similar to the microindentation results produced in prior studies of sucrose. Microindentation study had stated an elastic modulus value of 32.3 GPa and reported isotropic hardness value of 645 MPa for (001), (100), (110), and (010) planes [27]. The inconsistency in the values of the microindentation study and the nanoindentation study were thought to

arise from the surface roughness, scale of investigation and pre-existing cracks or crack propagation during examination.

A studies lead by Sherwood examined APAP single crystals involving microindentation [28,29]. Results of these studies are show in the figure below. They reported that the hardness value was a function of tip orientation. And further stated that the hardness values within the same plane were isotropic, however, compared to different planes of the same crystal were comparatively anisotropic.

Table 2: Paracetamol microindentation study [28]

Finnie, Prasad, Sheen, and Sherwood

Table II. The Geometry of Dislocation Slip Systems in Several Organic Solids

Material	Crystal system	Dislocation geometry	Number of distinct slip systems
Paracetamol	Monoclinic	(010) [001]	
		(010) [100]	2
RDX (24)	Monoclinic	(010) [001]	
		{021} [100]	3
PETN (24)	Orthorhombic	{110} ⟨111⟩	4
Adamantane (28)	FCC	{111} ⟨101⟩	12
dl Camphor (29)	FCC	{111} ⟨101⟩	12

Table 3: Microindentation data for Paracetamol [28]

Table I. Comparison of the Mechanical Properties of Paracetamol with Those of Other Organic Solids

Material	Face	Range of H_V (MPa)	Range of H_K (MPa)	Extent of deformation μm (load, g)
Paracetamol	{001}	352–392	235–412	50 μm (50 g)
	{20$\bar{1}$}	382–442	304–456	45 μm (50 g)
	{110}	352–392	353–402	55 μm (50 g)
	{011}	362–402		40 μg (50 g)
RDX (24)	{210}	372–382	314–431	91 μm (50 g)
PETN (24)	{110}	147–167	127–245	160 μg (20 g)
	{101}	157–167	137–176	160 μg (20 g)
Adamantane (28)	{222}	40–50	40–60	100 μg (1 g)

Table 4: Fracture toughness values for Paracetamol crystal [29]

Fracture of Paracetamol Crystals

Table I. Fracture Toughness Values for Paracetamol Crystals

Fracture plane	Indentation plane	Crack length, c (μm) and crack type	Fracture toughness K_c, (MPa $m^{1/2}$)	Attachment energy, E_{at} (k cal.mol^{-1})	Slice energy, E_{sl} (k cal.mol^{-1})
(010)	(001)	39 ± 2; Radial	0.043 ± 0.007	−5.30	−18.05
	(110)	36 ± 2; Radial	0.048 ± 0.008		
	(201)	41 ± 2; Radial	0.041 ± 0.005		
	(011)	38 ± 2; Radial	0.043 ± 0.005		
	(010)	25 ± 2; Lateral	0.050 ± 0.008		
(210)	(001)	26 ± 2; Radial	0.105 ± 0.008	−15.30	−9.75
(110)	(001)	35 ± 2; Radial	0.052 ± 0.006	−10.00	−13.36
	(011)	30 ± 3; Radial	0.085 ± 0.0010		
(011)	(110)	30 ± 3; Radial	0.060 ± 0.010	−11.09	−12.27
(100)	(010)	25 ± 2; Radial	0.076 ± 0.010	−6.56	−16.79

The Sucrose study also discusses pop-in events during loading, creep behavior after imposing a dwell time and slight elastic recovery upon elastic unloading. Ramos makes a distinction between kinks, which he refers to as yield points, and pop-in events. Yield points are considered low load inflection points and they indicate a transition from elastic

deformation to plastic deformation. Several mechanisms that are related to materials that possess similar slip systems are suggested to explain the observed plastic events on the loading curve. These include: nucleation, multiplication and cross slip systems [27]. Meanwhile pop-in events, which are expressed on the loading curve as large discontinuities or sudden increase in depth on a loading cycle, were attributed to nucleation of dislocations in a perfect crystal or proliferation of dislocation from a limited population [27]. Furthermore, time dependent plasticity was also observed on all planes in the Sucrose study. Creep behavior was correlated to temperature and environmental conditions, operating through dislocation mechanism. Sensitivity to processing conditions is a prominent concern for organic compounds. The study observed circular pits that were analogous to observation from a previous study of dark prismatic zone representative of a hydrated crystal. It is believed that the Sucrose crystal retains water and incorporates it into its crystal structure via screw dislocations. The observation of hydrated Sucrose crystals implies that the retention of water has implication in deformation mechanism by affecting creep and hardness values. Water imbedded in the molecular structure could become the driving force for plasticity when placed under appropriate environmental conditions.

Olusanmi et al. recently conducted nanoindentation study of Aspirin breakage behavior that analyzed various planes of single Aspirin crystal [30]. The summary of the data for hardness and elastic modulus

are reported in Table 6. The study reflected the inconsistency observed in the aforementioned Sucrose investigation in terms of anisotropy between hardness and Young's modulus. The elastic modulus varied between the two planes: (100) and (001). Through the evaluation of the pop-in events of the loading depth curve it was resolved that the cleavage plane was (100). This plane expressed more plasticity than the (001) pane. The study also stated that crack propagation through preferential cleavage planes, and the existence of a weaker plane was integral to the discussion of fracture mechanism of Aspirin. Cleavage planes were said to be derivative of the molecular packing patterns that are intrinsic of the crystal lattice.

Table 5: Variation of hardness and Young's modulus with indentation depth on Aspirin planes [30]

ASPIRIN (001) at 5mN/s				
Indentation depth range (μm)	Average hardness (Gpa)	CV%	Average Young's Modulus (Gpa)	CV%
3-4	0.14	13.4	3.11	7.8
4-5	0.12	13.1	2.93	10.0
5-6	0.12	12.6	2.71	5.0
6-7	0.11	11.6	2.81	8.6
7-8	0.11	7.84	2.81	9.3
ASPIRIN (100) at 5mN/s				
Indentation depth range (μm)	Average hardness (Gpa)	CV%	Average Young's Modulus (Gpa)	CV%
3-4	0.28	17.7	7.16	12.3
4-5	0.27	16.9	6.97	8.8
5-6	0.23	15.9	6.59	9.5
6-7	0.22	7.25	6.85	17.3
7-8	0.11	15.5	4.73	7.9

A portion of Milca Aponte-Roman's Ph.D. thesis also investigated mechanical properties of Acetaminophen single crystal [31]. Her evaluation of the Young's modulus of the single APAP crystals using the same nanoindentation instrumentation, under the load of 1000μN, was 12.6 ± 1.4GPa.

2.7 Nanoindentation Review

Nanoindentation is a simple, nondestructive method used to elicit a mechanical response from a solid under stress. The mechanical reaction is then translated in form of loading depth curve that quantifies values of reduced elastic modulus and hardness. Reduced elastic modulus and hardness are traditionally used to define a material's mechanical capabilities. However, it is the dissection of the loading and unloading curves that offer real insight into the anatomy of the deformation. A material can deform via two pathways, either elastically or plastically. Elastic deformation is reversible, instantaneous recovery, when the stress is lifted the material will regain its form. Elastic behavior implies that at a molecular level, the bonds are being stretched. Meanwhile, plastic deformation is a permanent, irreversible change, which suggests breaking of minimal atomic bonds. Loading depth curve analysis is predicated on the assumption that the loading curve is prone to elastic-plastic deformation behavior, where as the unloading curve is exclusively elastic behavior. The following analysis aims to: identify the

observed events from different crystal planes of two individual monoclinic single acetaminophen crystals, and compares the associated reduced elastic modulus and hardness of those crystals planes.

Nanoindentation is rapidly gaining popularity in the pharmaceutical industry as means to measure mechanical properties of pharmaceutical product. Generally, it is assumed that major challenges posed in drug development arise from synthesizing the proper active ingredient. Even drug companies have mistakenly overlooked the complexities of manufacturing, and its importance to drug development until the product is at a fairly progressed stage of development. This lack of foresight, leads to loss of substantial funds, market compatibility, loss of resources and most importantly time. Therefore, any instrumentation that allows pharmaceutical companies to gain insight into the prospective material's deformation behavior, characterized by their associative: hardness, elastic modulus and creep strain rates, will be considered especially beneficial. Specifically, the popularity of nanoindentation in the pharmaceutical industry is attributed to two key reasons. Foremost, it reduces cost of research and development by minimizing the amount of product needed to adequately probe mechanical properties of a new substance. Existing practices rest on empirical evaluation of pharmaceutical substances that require pilot scale experiments to decipher the adequate conditions of commercially competitive process. This requires time and large amount of product. Nanoindentation allows

pharmaceutical companies to gauge the feasibility of the manufacturing process and therefore the feasibility of the new drug substance to reach the masses, with substantially less product requirement. The data gathered from the studies, also enables researchers and manufacturers to estimate how the substance will react in the future. And secondly, it mitigates the complications arising from bulk inspection of mechanical properties, by facilitating particle investigation at a nano length scale. Performance of a compact presently is scrutinized in bulk, powder system. Usually, relying on powder system to communicate the compaction performance provokes further complications because powder flow can behave in a dualistic manner, exhibiting both liquid and solid properties. Furthermore, variability in powder features like porosity, size and shape also add to the complicated analysis. Interestingly, compaction behavior is referenced by intrinsic and extrinsic qualities of crystal structure and molecular configuration. Thereby further bolstering the utility of nanoindentation in the pharmaceutical industry.

Furthering the case for implementing nanoindentation instrumentation to examine pharmaceutical material is a study conducted by Cao et al., which sought to correlate the bulk powder compaction behavior to particle hardness [32]. The premise of the study was to investigate if material hardness and elastic modulus had any significant predicative value with relation to tableting performance. Duncan-Hewitt and Weatherly claim that it is possible to gauge the

compressibility of a powder by knowing its single crystal mechanical properties, however their suggestion has been polarizing [32]. They proposed that a correlation could be made via a strain index, which is equated to hardness over reduced elastic modulus. Hiestand anticipated powder compression performance of compacts by using a ratio of compression stress over dynamic indentation hardness, where poor tableting behavior was equated to a ratio greater to or equal to one [29]. The results of this study, which analyzed twelve compacts and sixteen pharmaceutical particles including Acetaminophen, corroborate Duncan-Hewitt and Weatherly theory. Cao et al. results show that hardness of a single crystal can be predictive of powder compaction behavior [32]. Particles with low or high values correspond to poor compaction behavior, meanwhile particles with medium hardness exhibited optimal compaction behavior. In the case of Acetaminophen, nine particles were tested and average hardness and reduced elastic modulus and elastic modulus were found to be: 1.0±0.2 GPa, 1.9±0.2GPa and 1.7±0.1GPa respectively. The strain index was 0.53. In terms of Acetaminophen compact, compression stress was reported to be 104MPa and 43MPa dynamic indentation hardness. The associated ratio of compression stress over dynamic indentation hardness was noted as 2.42. The study rated the overall compaction performance as poor. The hardness values reported for single crystal Acetaminophen matched the results obtained in our study, however the reduced elastic modulus was comparatively much lower. This

variability could be attributed to surface roughness of our samples, conditions under the samples were examined and how the crystals were grown. The study also reported noticing cracks, material pile up, sink-ins in AFM examination of post indentation events.

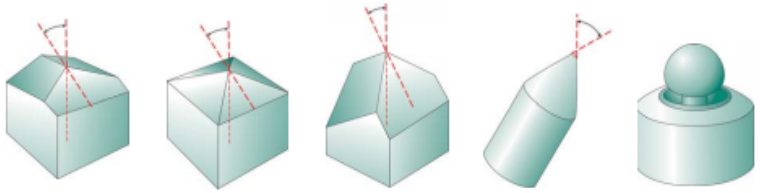
Another novelty of nanoindentation, besides its length scale implications and its flexibility in amount of product needed for investigation, is its ability to investigate variable surfaces. It's has been exercised on unique and contrasting surfaces ranging from human dentine, thin films, surface coatings, to organic and inorganic crystals [20,33,34]. Nanoindentation method offers modifications of the loading unloading cycles that make it possible to carry out different types of analysis. There are options that enhance plasticity analysis and allow for observation of time dependent deformation. Furthermore, variations on the loading unloading cycles can include but are not limited to: partial unloading in segments, superimposing oscillatory movement on loading, and placing a hold at maximum load to observe change in depth [20]. Overall, nanoindentation test can calculate typical hardness and elastic modulus values, but also convey phase transformations, crack propagations, energy absorption, time dependent deformation and strain hardening from depth and loading curves. Nanoindentation also calls for restraints to be set with respect to either load control or depth control. In case of load control, a maximum value will be set for peak load for a given

cycle. Similarly, with depth control a maximum value of depth can be placed, which will signal conclusion of an indentation cycle.

Indentation analysis has been routinely applied on the micron and millimeter length scales. Major indentation techniques include: Vickers, Knoop, Brinell, and Rockwell. The distinction between aforementioned established approaches and the nanoindentation method is not only resigned to length scale, but also in the indirect manner in which quantity of hardness is extracted. With regard to microindention, the hardness is equated to the maximum load divided by the projected area. This projected area is deciphered through physical measurement facilitated by optical instrumentation. Because nanoindentation performs on a nano length scale, optically measuring the residual plastic indent to determine the contact area as a function of load is challenging. This difficulty in evaluating contact area is mitigated by using measured depth penetration and known indenter geometry [20]. The depth of penetration is continuously recorded as the indentation tip breaches the surface of the specimen up to the designated maximum load. The associated indenter geometry can be variable depending on which indentation tip is applied: Vickers, Berkovich, conical, cube corner and spherical. The majority of the indentation tips for nanoindentation test are either pyramidal or spherical [20]. Table 6 summarizes the qualities of the various indenter tips in terms of their respective projected areas, geometries, and angles.

Table 6: Nanoindentation tip specifications and application recommendations [35]

Indenting Tips Summary



	Berkovich	Vickers	Cube-Corner	Cone (angle ψ)	Sphere (radius R)
Features					
Shape	3-sided pyramid	4-sided pyramid	3-sided pyramid w/ perpendicular faces	Conical	Spherical
Applications	Bulk Materials, Thin Films, Polymers, Scratch Testing, Wear Testing, MEMS, Imaging	Bulk Materials, Films and Foils, Scratch Testing, Wear Testing	Thin Films, Scratch Testing, Fracture Toughness, Wear Testing, MEMS, Imaging	Modeling, Scratch Testing, Wear Testing, Imaging, MEMS	MEMS
Available as Traceable Standard	Yes	Yes	Yes	No	No
Parameter					
Centerline-to-face angle, α	65.3°	68°	35.2644°	—	—
Area (projected), A(d)	$24.56d^2$	$24.504d^2$	$2.5981d^2$	πa^2	πa^2
Volume-depth relation, V(d)	$8.1873d^3$	$8.1681d^3$	$0.8657d^3$	—	—
Projected area/face area, A/A_f	0.908	0.927	0.5774	—	—
Equivalent cone angle, ψ	70.32°	70.2996°	42.28°	ψ	—
Contact radius, a	—	—	—	d tan ψ	

In this study, Berkovich tip was exclusively applied for several reasons. Foremost, Berkovich tip enables a more decisive navigation of the surface, primarily due to its sharper point. And that makes it a much more attractive candidate for small-scale analysis.

The projected area for this tip is given by the following equation:

$$A=3\sqrt{h_c^2}\tan^2\theta \quad (2)$$

$\theta=65.27°$ and h_c = depth of penetration

After substituting value of theta into the projected area equation we are left with the following expression for Berkovich tip:

$$A=24.5\ h_c^2 \quad (3)$$

Finally, this expression is translated into hardness value via following equation:

$$H=\frac{P\ (\text{Maximum Load})}{A=24.5\ h_c^2} \quad (4)$$

Value of depth of penetration, h_c, is derived from the load displacement curve. The following, figure x, discusses the myriad of ways to analyze the depth of penetration. The permutations arise from the dynamic process of indenting a surface. For instance, h_r is the impression cast by plasticity of the indent. Meanwhile, h_e is the displacement from proposed recovery of elastic unloading. The plastic depth, h_c is the depth of the contact circle. The total surface at the maximum load is equated to h_{max}. At last, h_a is distance from surface at the maximum load to edge of the contact.

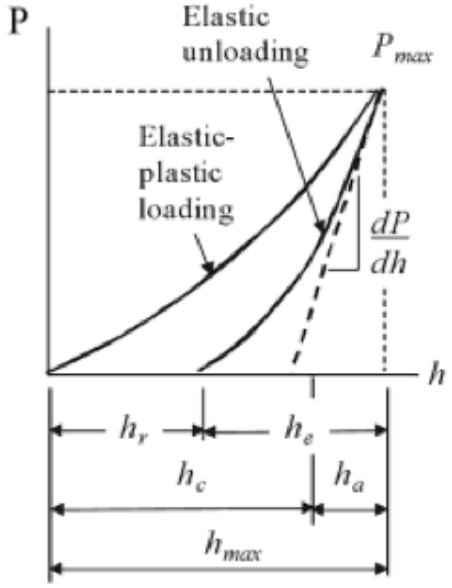


Figure 7: Loading depth curve for plastic-elastic loading and elasting unloading. hr is the depth of residual impression. he is the elastic displacement during unloading of actual cone. hc is depth of contact. hathe distance from the edge of the contact specimen surface at full load. hmax is the depth beneath the specimen free surface. [20]

Elastic modulus of a material can also be deduced from nanoindentation testing. The analysis leading to the value of elastic modulus commences by initially analyzing the load-displacement curve. The slope of the line tangent to the unloading curve, which is the change in load over the change in depth, reflects the stiffness of the contact. This approach to the evaluation of elastic modulus is based on the method of Olive and Pharr [36]. This stiffness can be used to determine the indentation modulus, which in principle is the same as the elastic or Young's modulus of the specimen. The following equation defines the slope of the unloading curve to the reduced modulus.

$$E_r = \frac{1\sqrt{\pi}\, dP}{2\sqrt{A}dh} \quad (5)$$

The reduced elastic modulus takes into consideration the elastic response from both the indenter tip as well as the material. To ascertain elastic indentation modulus, which is assumed to be equal to the Young's modulus of the material, the following equation is used:

$$E = \frac{(1-\nu_s^2)}{(1/E_r)-(1-\nu_i^2)/E_i} \quad (6)$$

In the above expression, the term ν_s is the Poisson's ratio of the material under investigation and ν_i is the Poisson's ratio for the indenter. E is indentation modulus or the corresponding modulus of the material under inspection. E_r and E_i are the reduced modulus and the indenter modulus respectively.

Conventionally, a dwell time will be inserted into a load function at segmented intervals where load is changed and at maximum loads. This serves not only to stabilize the specimen and instrument; so that accurate readings of load and depth can be measured, but also helps to abbreviate time dependent deformation effects, or creep [20,37]. Creep is observed by change in depth at constant a load. Unlike plasticity, which is presumed to an instantaneous event, creep is a time depended response to applied stress. Creep is typically discerned by the elongation of the nose of the loading depth curve, as noted the figure below.

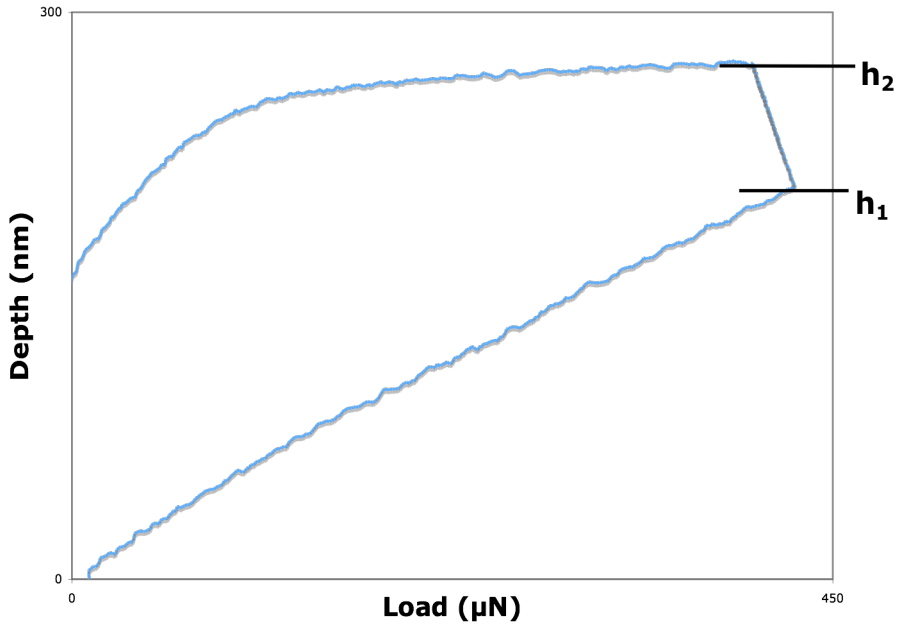


Figure 8: Calculation of the change in depth with constant load

Creep is expressed as a strain rate and is quantitatively evaluated by the change in indentation depth at constant load over dwell time.

$$C_{IT} = \frac{[(h_2 - h_1)/\ h_1]}{\text{time}} \quad (7)$$

Load displacement curves are not resigned to simply aiding quantitative analysis, but rather offer a detailed qualitative analysis on deformation mechanism of the substance under evaluation. Features of the loading and unloading curves, under proper scrutiny, can reveal yielding, phase transformations, crack propagations and adhesion. The following Figure 9 distinguishes the notable features that convey the aforementioned conclusions.

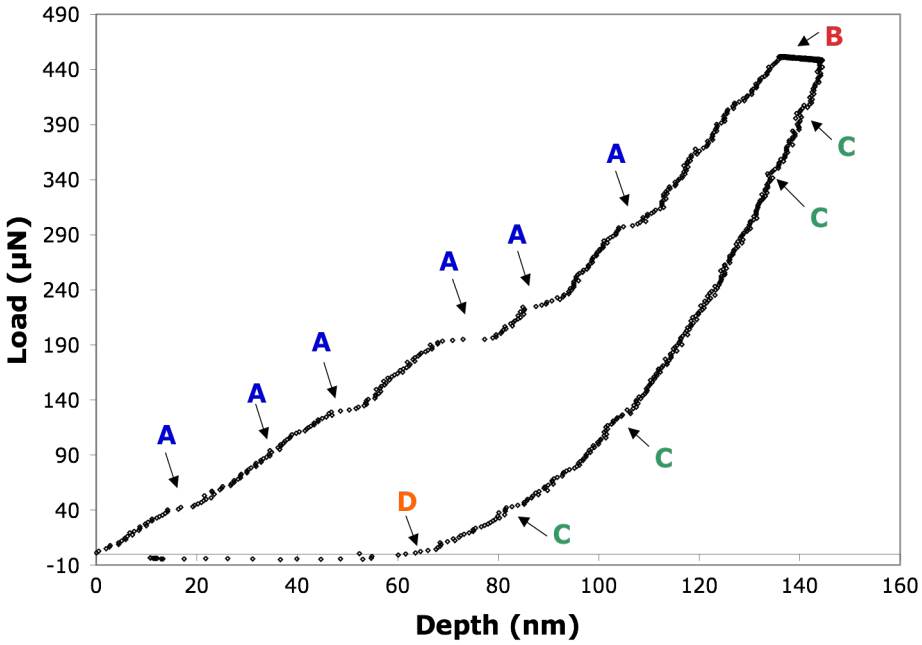


Figure 9: Loading depth curve with several effects. A is pop-in events. B shows creep. C is discontinuities in unloading curve. D represents the location where adhesion is featured on the curve

Fragmentation or discontinuities on the loading and unloading cycle are referenced as: pop-in and pop-out events, as shown in Figure 9 as A and B respectively. These events, which are accompanied by an abrupt increase in depth, are usually a sign of onset of plasticity, and reflect crack propagation [30]. However, fragmentation on the unloading cycle could be a sign of phase transformation [30]. An elbow, is characterized by a subtle change in slope of the unloading curve, and could be indicative of a phase transformation as observed in silicon. Alternatively, an elbow can also be residual affects of creep decaying, and thus modifying the shape of the unloading curve. Creep, time dependent

deformation, is identified as B in the above Figure 9. Finally, adhesion can be spotted as dipping of the unloading curve below the x-axis and then arching up to plateau close to the x-axis. Figure 9 marks the location of where adhesion is typically observed as D. The actual features of adhesion will be discussed later in the study.

Nanoindentation test is an incredibly sensitive instrument and has several sources of error that should be considered. In this study three sources of error were considered in particular: specimen surface roughness, sample tilt and calibration of indentation tip. Surface roughness of a sample can have substantial cumulative effect on a study because it can lead to ambiguity in documenting projected area. The uncertainty in projected area translates into distrust of hardness and elastic modulus measurements. Tilt of a sample can also lead to over estimation of projected area, though a recent study suggests that the use of a Berkovich indentation tip alleviates much of the uncertainty [20]. Finally, calibrating the indentation tip, if other samples of variable hardness are being tested with a large discrepancy in applied load. With load fluctuation being applied, the tip can loose its sensitivity in the process; thus recording inconstant measurements.

3. EXPERIMENTAL

3.1 Crystal Growth

Acetaminophen powder (SigmaUltra, ≥99.0%) from Sigma-Aldrich was used in this study, along with distilled water, ethanol, methanol and acetone. Glass and plastic Petri dishes were used as vessels to grow crystals in. Great effort was made to follow stringent sterilization practices to limit contamination from impurities present in the environment. These practices included, washing the dish with soap and water and then rinsing it further in succession with ethanol, methanol and acetone.

Several methods, including vapor diffusion and solvent diffusion techniques were attempted to produce crystals that could withstand the vigorous examinations of x-ray diffraction and nanoindentation. However it was clear that simple slow evaporation technique was the best option for growth of Acetaminophen crystals.

Slow evaporation technique is a gradual process that takes upwards of two to three week's time frame at ambient conditions. Acetaminophen crystals were attempted with four different solvents: distilled water, ethanol, methanol and acetone. And each solvent was tried with two options of saturation and supersaturation.

A saturated solution was made up of 30 mL of solvent and then appropriate amount of Acetaminophen, so that there was a slight

accumulation of the product at the bottom of the beaker. This accumulation signaled that the solution was saturated. The solution was agitated via magnetic tablets that worked in conjunction with the hot plate. Though no heat was used in this process. The solution was not filtered through another medium, because of fear of adding dust particles and contaminating the sample. Instead, the solution was slightly tilted and meticulously poured into the Petri dishes so as to not include the excess powder. Finally the Petri dishes were covered with perforated Parafilm, so as to limit dust particles from settling into the solution. The Petri dishes were labeled and set aside in an area that would experience the least perturbation.

Supersaturation solution essentially followed the same protocol except addition of heat to increase acetaminophen concentration in the solution. The solution was heated until there was slight accumulation of powder at the bottom of the beaker. Same manner and rate of agitation used in saturation process was applied in this process. The solution was then set aside a partially covered with lid. It was observed that supersaturation most often resulted in small crystallites and was not a fruitful endeavor.

For this study, only crystals grown from a distilled water solution were used. Growing large batches of APAP crystals from a distilled water solution was made more difficult by possibility of oxidation. Oxidation in the growing crystals was exposed by discoloration of the standing

solution from clear to brownish or pinkish hue, and was incorporated into the crystals, which turned reflected a shade of pink or brown. This difficulty was observed in several papers that discussed growing large APAP crystals from an aqueous solution. One study reported oxidation, occurring within one to two weeks time frame at room temperature at 35°C, and with in one to two days at 65°C [8]. Oxidation is said to inhibit crystal growth and should be avoided to maintain crystalline purity. Oxidation effects were minimized by taking the following precautions: batches were restricted to 30 mL, lab experiments were conducted at lower temperatures, and extracted before oxidation could set in. The size of the crystal is in millimeters and were large enough to handle.

3.2 Nanoindentation Analysis

Hysitron TriboIndenter was used to carry out nanoindentation on a single Acetaminophen crystal using a Berkovich indentation tip. Each plane was carefully mounted on a metal stud using crystal wax. Great caution was taken when mounting and dismounting the crystal to maintain the integrity of crystal so that multiple planes could be indented. Great care was also taken when crystals were exposed to heat, because Acetaminophen is a brittle organic substance, it is venerable to thermal shock and cracking upon sudden introduction to high temperatures.

The load function was load-controlled mode, where the maximum applied load was 500 μN. There were a total of ten indents per face,

spaced between five to ten nanometers apart, depending on the size of the surface. The crystal planes were indented using the following load function: five seconds loading time, five second hold time and then five second unloading time. The crystal planes were meticulously sketched so that the plane under investigation can be identified after x-ray diffraction.

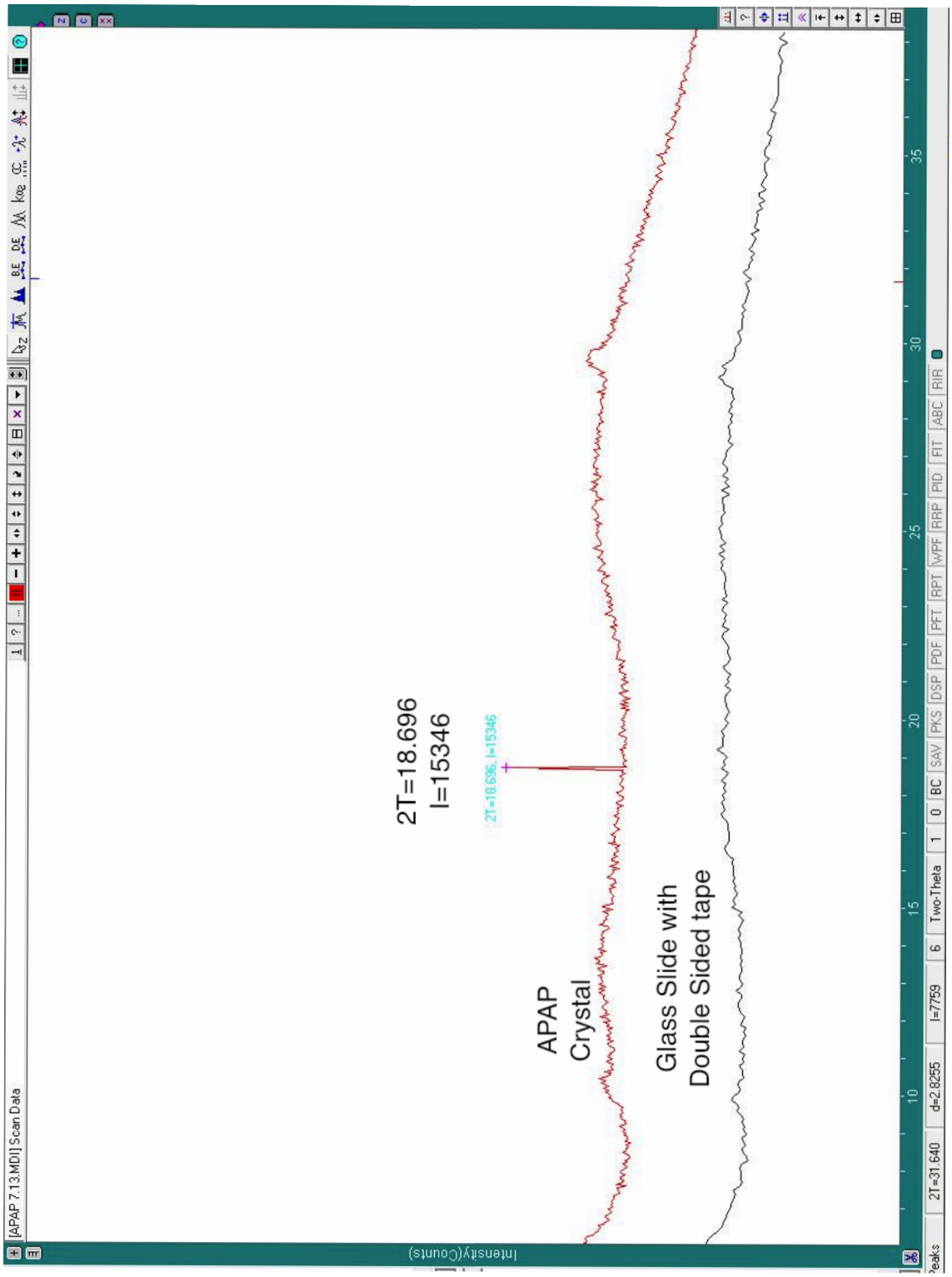
3.3 XRD Analysis

An attempt was made to use the Siemens D500 instrument. This unit uses Cu radiation with operating conditions of 40KV/30mA. The experiment was conducted with scan range for 2θ between 5°-40°. The step size was set to 0.05 and dwell time was 2 seconds. The crystal was mounted on a glass side with double stick tape. This platform was used so that amorphous nature of glass would not output strong peaks that would complicate the data. The peaks from the double stick tape between 5°-40 were minimal, and broad. Another reason this method of sample prep was used, was the ease of mounting and dismounting the sample; causing minimal impact. Furthermore, this allowed for easy rotation of the crystal so that it could be mounted to inspect various planes.

This method of inspection proved fruitless. Extracting a peak from the single APAP crystal was challenging, and reproducibility was nearly impossible. This could have been because the sample was not at the proper alignment of the x-ray or at the same level of depth. Regardless, one peak was ascertained from the June APAP crystal. Figure 10 displays

the full spectra acquired from the scan, and magnified feature of the peak is shown in Figure 11. A sharp peak was noticed at θ= 18.7 °. Using the literature x-ray diffraction data from single crystal diffraction of APAP crystal, Figure 13, the 2θ value was matched to (020) plane, assuming the unit had an offset of 0.2 in unit 2. This made θ= 18.7 ° either 18.5 ° or 18.9 °. However, my results did not match that of Dr. Thomas Egme, when he inspected the same crystal and identified the plane in question as being (0-11).

Figure 10: X-ray diffraction pattern of June APAP single crystal from Siemens D500 instrument



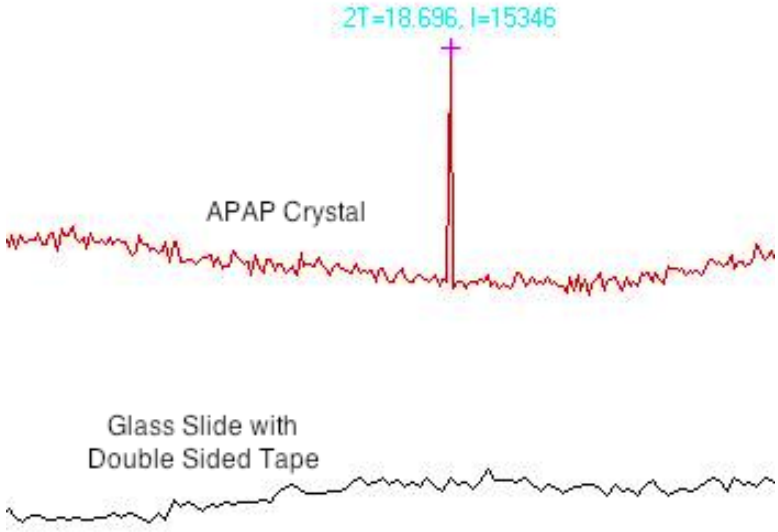


Figure 11: Magnification of the peak from Siemens D500 instrument analysis of APAP June crystal

Figure 12: APAP single crystal sample prep for XRD. The crystal is mounted on a glass slide with double stick tape.

Table 3—Experimental Powder X-ray Diffraction Data for Paracetamol Forms I and II

form I				form II			
hkl	*d* (Å)	°2Θ	I/I_{max} (%)	*hkl*	*d* (Å)	°2Θ	I/I_{max} (%)
011	7.301	12.112	26	200	8.562	10.323	4
10$\bar{1}$	6.398	13.830	18	210	6.935	12.755	5
002	5.809	15.240	3	020	5.907	14.986	14
101	5.709	15.508	72	211	5.061	17.508	26
110	5.640	15.700	4	220	4.862	18.232	22
11$\bar{1}$	5.294	16.732	11	021	4.618	19.203	49
111	4.877	18.175	68	121	4.463	19.878	3
020	4.690	18.905	13	400	4.284	20.716	9
021	4.355	20.376	39	221	4.066	21.844	22
11$\bar{2}$	4.276	20.756	7	002	3.699	24.038	100
112	3.849	23.087	9	102	3.616	24.597	14
12$\bar{1}$	3.785	23.483	62	230	3.579	24.855	9
022	3.650	24.367	100	420/112	3.464	25.700	13
10$\bar{3}$	3.596	24.741	5	131/202	3.401	26.177	5
12$\bar{2}$	3.355	26.545	62	231	3.222	27.663	12
21$\bar{1}$	3.280	27.165	11	022	3.139	28.407	3
20$\bar{2}$	3.201	27.847	4	122	3.084	28.931	21
122	3.140	28.403	2	312	3.006	29.700	5
211	3.075	29.012	7	222	2.945	30.325	27
113	3.048	29.273	6	600	2.857	31.280	5
023	2.987	29.887	3	240	2.792	32.026	7
12$\bar{3}$	2.858	31.273	5	322	2.747	32.572	6
22$\bar{1}$	2.805	31.877	3	141/431	2.706	33.081	4
032/131	2.747	32.575	17	132	2.662	33.639	2
13$\bar{2}$	2.622	34.173	2	611	2.600	34.462	5
132	2.513	35.706	3	232	2.573	34.840	7
114	2.480	36.193	10	422	2.530	35.454	3
033	2.434	36.904	18	512	2.461	36.486	3
20$\bar{4}$/22$\bar{3}$	2.398	37.466	7	440/621	2.431	36.955	17
213	2.372	37.896	4	630	2.314	38.891	7

Figure 13: XRD diffraction data for Paracetamol polymorphs I and II [38]

A Bruker SMART APEX single x-ray diffraction unit identified crystal planes of both the APAP single crystal. Both the single crystals

submitted for testing were identified as part of the monoclinic crystal system and belonging to the P21/n space group. The unit cells of both crystals were roughly equivalent: a=7.1, b=9.4 and c=11.7. Below are the identified crystal planes of the two APAP single crystals. This results were also confirmed by Naumov et al.'s group analysis of Acetaminophen structure at 150°K [39].

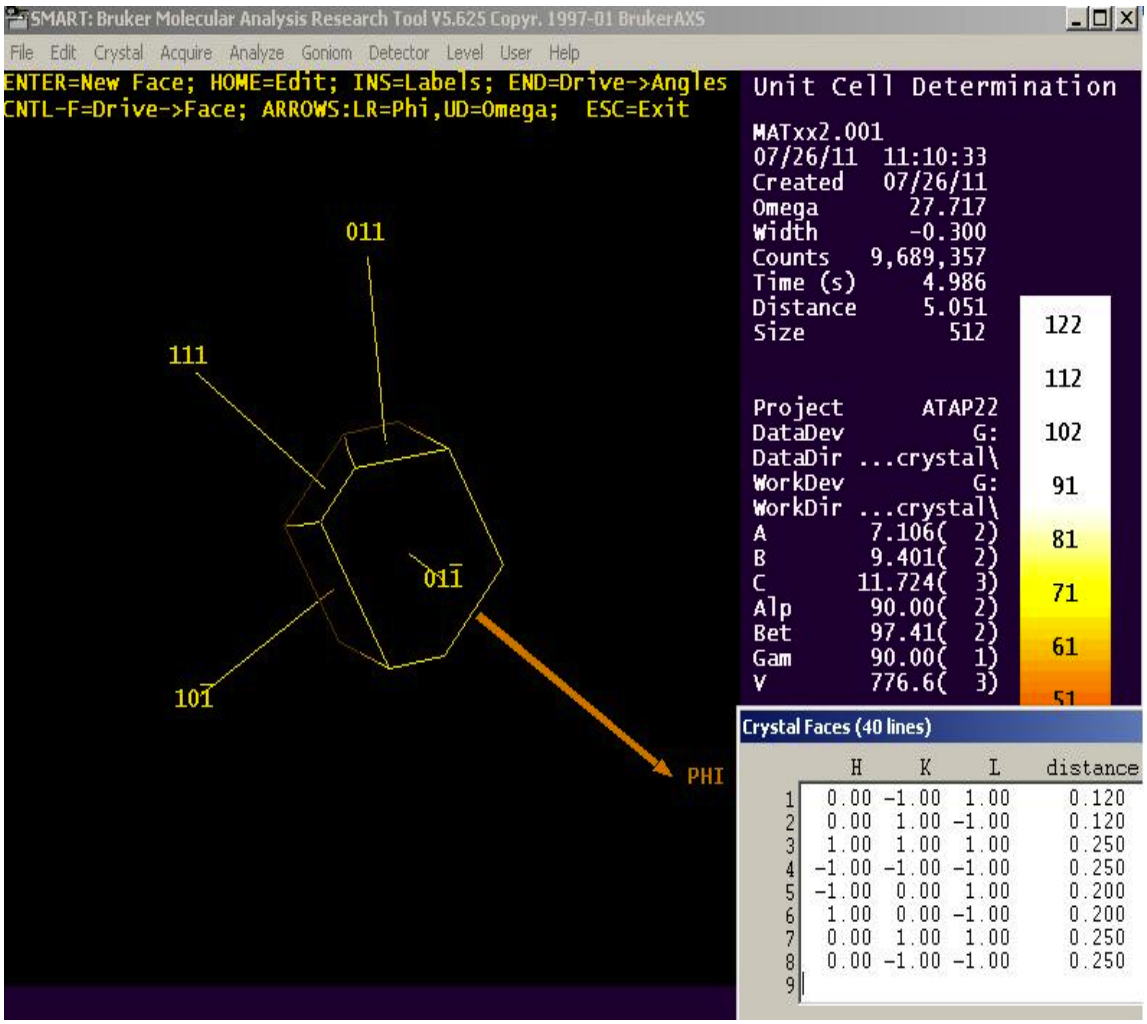


Figure 14: Identified JUNE Acetaminophen crystal planes from single crystal XRD

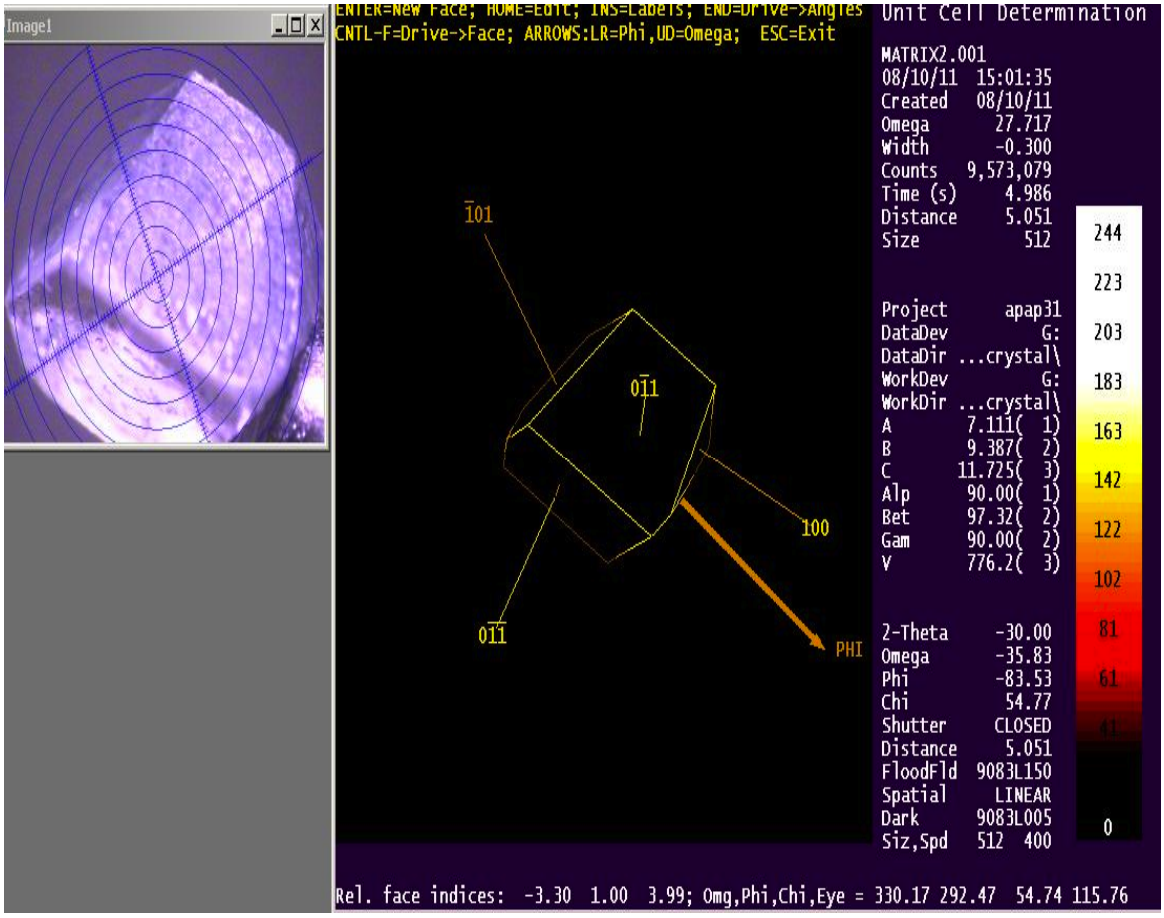


Figure 15: Identified JULY Acetaminophen crystal planes from single crystal XRD

4. RESULTS AND DISCUSSION

Acetaminophen is a brittle organic substance, and the anisotropy observed between the single crystal planes indicate that some faces are more vulnerable to plastic deformation than others. Examining the features of the loading depth curve, coupled with reduced modulus and hardness analysis will help to resolve the observed variability deformation mechanism.

Hardness is a material property that measures a specimen's ability to resistance plasticity. The anisotropic nature of the organic single crystal is communicated through the variability in hardness measurement of various planes. Plastic deformation of a single crystal is restricted to preferred slip systems. And therefore, each plane translates into a unique load displacement curve. The curves featured in Figure 16 and Figure 17 below are scrutinized by the following parameters: frequency and severity of fragmentation, time dependent plasticity, depth of penetration and the slope of the unloading curve.

An immediate observation is the linear behavior of the loading curve profiles of all three months: June, July and August. The loading curves are expected to be more parabolic in an ideal loading depth curve. This linearity is most likely an indication of the brittle nature of the organic crystal.

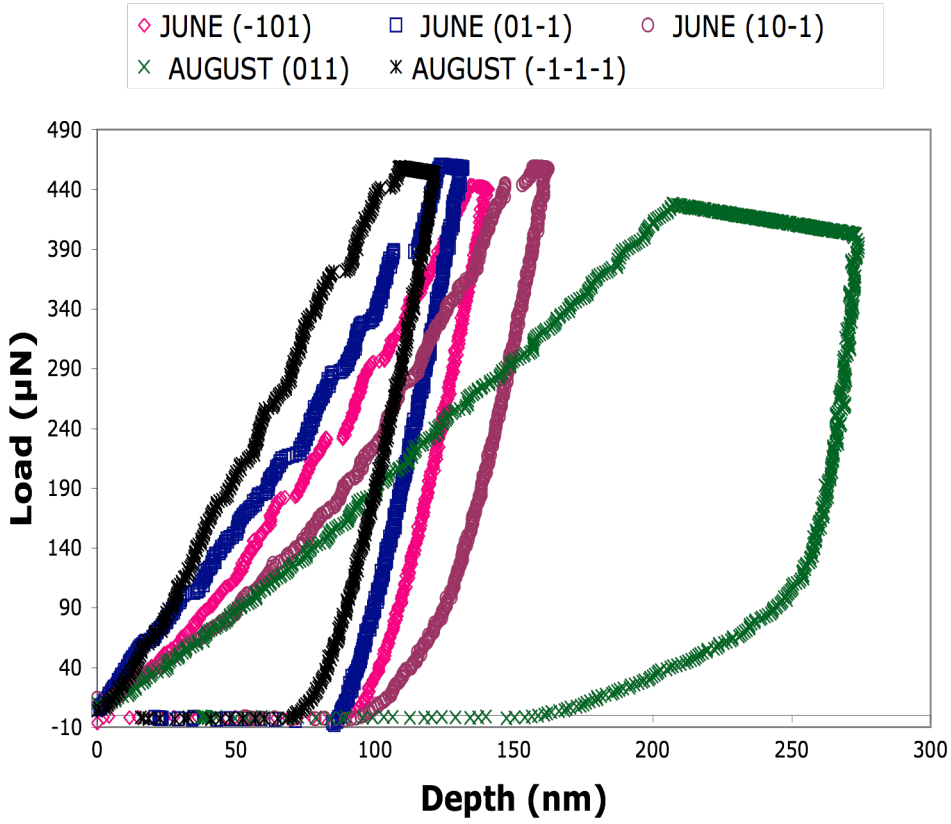


Figure 16: Loading curve of the multiple planes analyzed for APAP monoclinic Crystal 1. Planes (-101), (01-1) and (10-1) of crystal 1 were evaluted on JUNE 27,2011. Planes (011) was evaluted on AUGUST 4,2011 and (-1-1-1) was evaluted on AUGUST 8,2011of crystal 1. The ambient condition data for the JUNE evaluated planes is not recorded. The consitions for (011) planes: 80°F and 40% RH. Plane (-1-1-1) was evaluted at 80.3°F and 42% RH. Crystal was stored inside a covered pertri dish and was left in the office in beween testing. The nanoindentation was performed under maximum load of 500µN with a total of 10 indents per plane were made.

CRYSTAL 1 ANALYSIS						
APAP JUNE SINGLE CURVE STUDY at 500μN Load						
CRYSTAL PLANE	Er(GPa)	H(GPa)	Strain Rate (Δnm/sec)	Pmax(μN)	A(nm^2)	hc(nm)
(10-1)	14.7	0.7	0.0068	456.3	666660.3	136.4
(01-1)	19.0	1.1	0.0130	457.0	423145.9	106.2
(-101)	16.8	0.9	0.0097	438.8	489256.6	115.1
APAP AUGUST SINGLE CURVE STUDY at 500μN Load						
CRYSTAL PLANE	Er(GPa)	H(GPa)	Strain Rate (Δnm/sec)	Pmax(μN)	A(nm^2)	hc(nm)
(011)	CONDITION OF EVALUATION: 80°F 40% RH					
	10.3	0.2	0.0590	401.4	2176613.6	258.9
(-1-1-1)	CONDITION OF EVALUATION: 80.3°F 42% RH					
	20.3	1.3	0.0247	452.4	361065.6	97.3

Table 7: Crystal 1 nanoindentation single JUNE curve data for the curves selected in figure 16.

The variability in mechanical properties is reflected in the graphical analysis of the two single APAP monoclinic crystals. In Figure 16 and Figure 17, the loading- unloading curves naturally rank themselves based on their hardness. The planes are arranged from steepest to shallowest in the following order:

(-1-1 -1)>(01-1)>(-101)>(10-1)>(011). In Figure 16, the highest hardness value is assigned to plane (-1 -1 -1) with 1.3 GPa. Plane (01-1) has a hardness of 1.1Gpa; plane (-101) has hardness of 0.9; plane (10-1) has hardness of 0.7 GPa; and the smallest being 0.2 GPa associated with (011) plane. It should be noted that (011) and (-1-1-1) plane of APAP crystal 1 were tested in August under different ambient conditions. Both (011) and (-1-1-1) planes were evaluated early in the morning starting

around 10am, however they were analyzed on separate days. The (011) plane was evaluated on August 4 and the (-1-1-1) plane was evaluated on August 8. The crystal was enclosed in a plastic Petri dish that was covered and left in the office in between testing periods. The (01-1), (-101), and (10-1) were tested together in June 27, 2011 on the same day starting. They were evaluated starting in the early morning around 10am also. Prior to testing they were enclosed in the same conditions: inside a plastic Petri dish and covered.

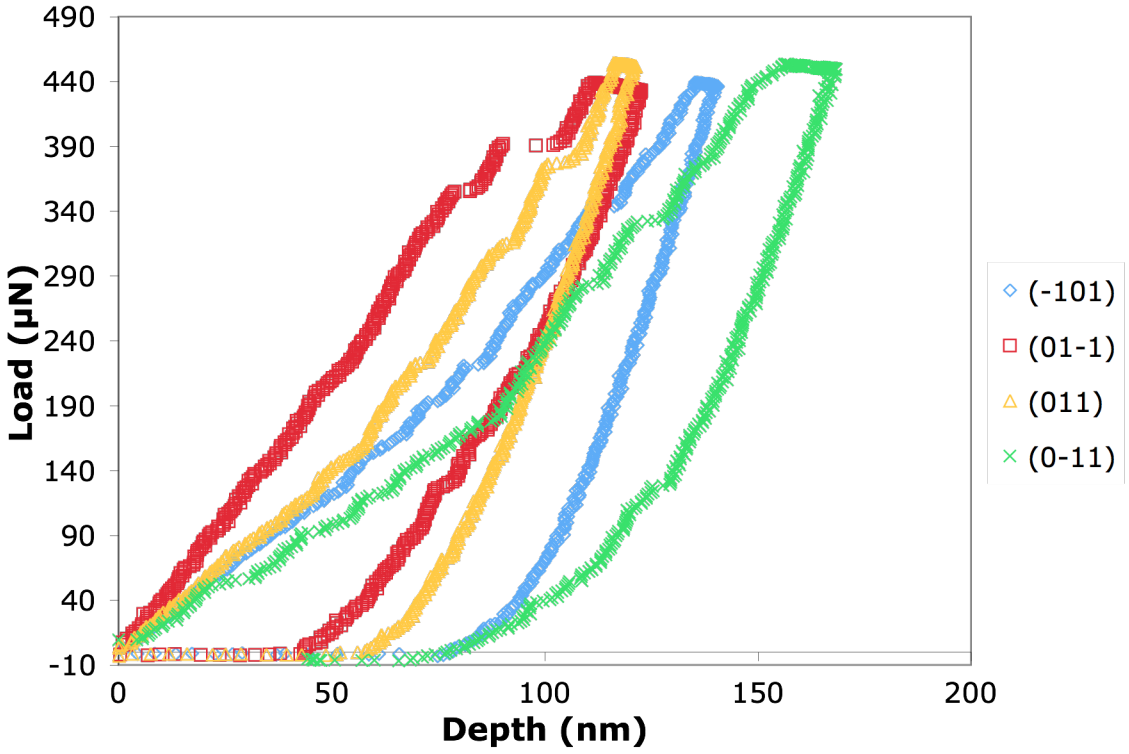


Figure 17: Loading depth curves of different planes from JULY APAP monoclinic crystal. All the planes were evaluated on JULY 6,2011. A total of 10 indents were performed per plane at maximum load of 500µN.

CRYSTAL 2 ANALYSIS						
APAP JULY SINGLE CURVE STUDY at 500μN Load						
Face	Er(GPa)	H(GPa)	Strain Rate (Δnm/sec)	Pmax(μN)	A(nm^2)	hc(nm)
(0-11)	12.2	0.7	7.7	448.6	678695.3	137.7
(01-1)	14.2	1.5	9.7	431.1	292811.5	86.5
(-101)	15.0	0.9	3.7	434.4	460556.5	111.3
(011)	18.0	1.4	4.3	450.7	321834.5	91.2

Table 8: Nanoindentation single curve data pertaining to the curves selected for Figure 17.

Similarly, Figure 17 follows the same trend relying on hardness to order the curves. In the single APAP crystal tested in July, the planes are ranked as follows: (01-1)>(011)>(-101)>(0-11). Their associated hardness values respectively are: 1.5GPa>1.4GPa>0.9GPa>0.7Gpa. Table 8 summarizes the hardness values of the curves from the July tested single APAP crystals.

Crystals are not immune to internalized disorder. And these imperfections can control mechanical properties and even reactivity of a crystalline product. Defects are grouped as either point or lattice defects. Point defects are intrinsically imperfections that manifest as extra atoms or vacancies in the crystal lattice. Linear defects, or dislocations are also intrinsically presents in the crystal as it forms. Dislocations are distortions or discontinuities in the ordered crystal lattice. They can be categorized based on orientation as either edge or screw dislocations. Regardless, these discontinuities are thermodynamically unstable and

are contingent upon elastic strain and core energy. This localized energy coupled with chemical potential energy at the core makes they highly reactive. Furthermore, in terms of mechanical property, increase concentration of dislocations have been correlated with decrease in hardness. Their mobility is reasoned to be the cause of the plasticity observed in the material.

Most authors cite dislocations as the means by which organic crystals handle applied stress. Dislocations move one slip plane at a time, so as to reduce the energy needed to break atomic bonds and still effectively propagate through the crystal. Usually, planes with shortest Burger's vector correspond to lowest energy where plastic deformation would be highly favorable. Slip systems, slip planes and slip direction in which dislocations like to move are usually of high packing density. In this way the distance between the planes perpendicular to the dislocation is greater and they can slip past each other.

The unusual increase in hardness and elastic modulus measure in crystal 2 (01-1) plane and crystal 1 (-1-1-1) plane at the same applied load can be attributed with crystal work hardening. Crystals have multiple slip systems and when a crystal is placed under stress it could induce dislocations to move and intersect with one and another. This accumulation would require a much larger force to move the dislocation further, thus making the crystal harder and more resistant to deformation. However, Duncan-Hewitt's microindentation study stated

that Paracetamol crystal has a restricted number of slip system from which it can accommodate the applied stress [24]. This restricted slip systems results in higher stress concentrations and results in brittle fracture. The (01-1) plane does collaborate the idea of brittle fracture by the large discontinuity in its loading curve. In either case, it should be noted that both these planes were the smaller sides of the crystal, and are considerably more densely packed than on the larger face of the respective crystals. The shallowest curves that reflect the lower values of hardness and elastic modulus are not always associated with the largest crystal face. The (0-11) plane of crystal 2 does have the largest face, however crystal 1 plane (011) is a much smaller plane. The larger faces provide molecular mobility and therefore, permanent deformation is not as appreciable. As the indenter tip penetrates the surface of the crystal, molecules are able to flow and accommodate the excess force. This mobility is also reflected in the depth of penetration. A more ductile sample will have a larger depth of penetration; meanwhile a brittle substance will have comparatively smaller depth of penetration. This effect is observed in the shallowest curves of crystal 2, the (-101)>(0-11). The depth of penetration for (-101) plane is 111nm and for plane (0-11) is 138nm. In the same manner the (011) plane in crystal 1 has indentation depth of 259nm, and indentation depth of plane (-1-1-1) is reported to be 97nm. The study on breakage behavior of Aspirin notes a similar trend, where it was concluded that plastic flow is more readily observed on the

(001) plane than (100) plane. This conclusion was based upon the application of force and resulting depth penetration. A force of 70mN was applied on the latter plane to reach the same depth of penetration that was reached on the former plane, which required substantially less load of 30mN [30].

Figure 18: Load displacement curves from Aspirin study that evaluated planes (001) and (100) [30].

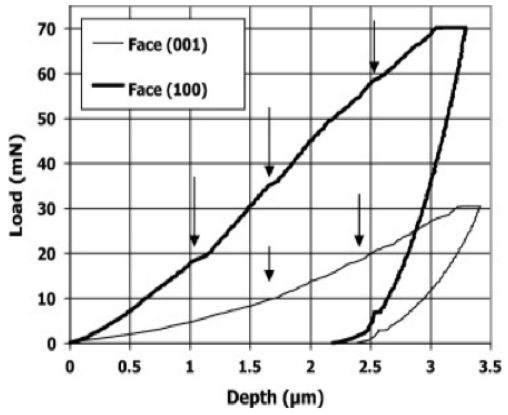


Fig. 9.

Load–displacement curves from indentation on faces (0 0 1) and (1 0 0) of ‹Aspirin› carried out at loading rates of 5 mN/s and at similar depths. 'Pop-ins' can be observed on both curves, indicated by the arrows.

The loading curve communicates the reaction of the APAP crystal as the Berkovich indenter tip is penetrating its surface. Plasticity is conveyed through discontinuities or pop-in events. This feature, which results in increases in depth, is indicative of onset of plasticity and indicates formation of a crack. For the shallower curves, the pop-in events are more frequent and evenly fragment the loading curve, and the discontinuities are not as deep. This observation was also noted by the breakage behavior study of aspirin that utilized nanoindentation analysis

in discerning deformation sensitivity of aspirin [30]. To validate this observation, concentration of pop-in events were quantified and conveyed through the below histogram graph. The graph evaluates the frequency of the pop-in event and the severity of the event in terms of displacement.

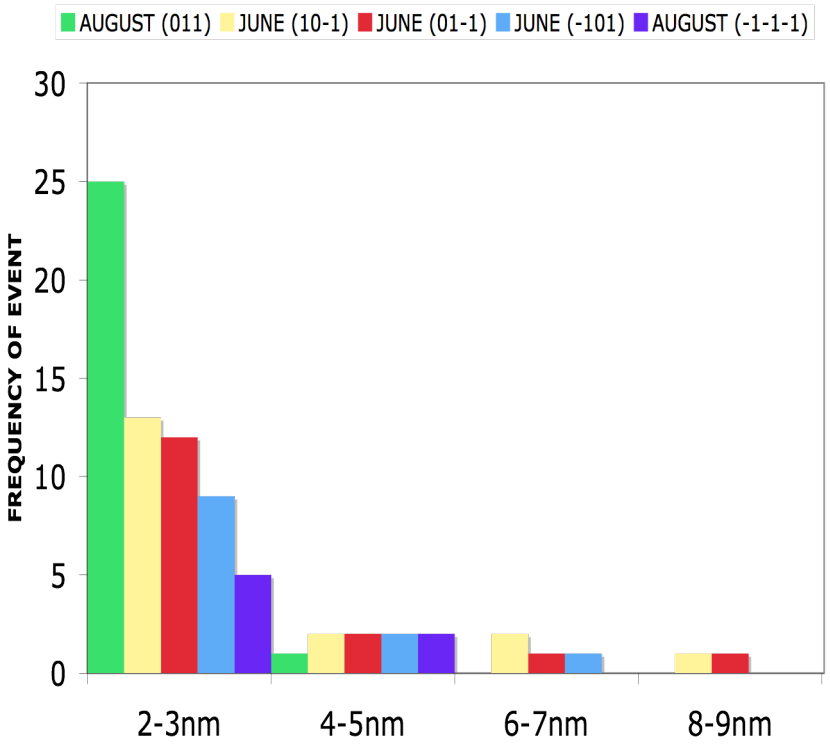


Figure 19: Histogram for crystal I that measures the frequency of pop-in events with respect to change in depth.

CRYSTAL 1 ANALYSIS						
APAP JULY CUMULATIVE STUDY at 500μN Load						
CRYSTAL PLANE	Er(GPa)	H(GPa)	Strain Rate (Δnm/sec)	Pmax(μN)	A(nm^2)	hc(nm)
(10-1)	14.9±0.2	0.8±0.02	0.0098±0.0009	452.3±2.5	587599.2±16660.7	127.1±2.0
(01-1)	18.2±0.6	1.1±0.1	0.0086±0.0010	452.5±3.5	423584.8±24135.2	105.9±3.2
(-101)	18.8±0.5	1.1±.04	0.0080±0.0007	435.5±2.0	399936.9±16958.8	102.7±2.4
APAP AUGUST CUMULATIVE STUDY at 500μN Load						
CRYSTAL PLANE	Er(GPa)	H(GPa)	Strain Rate (Δnm/sec)	Pmax(μN)	A(nm^2)	hc(nm)
(011)	CONDITION OF EVALUATION: 80°F 40% RH					
	12.1±0.4	0.3±0.02	0.0595±0.0008	402.6±6.0	1471450.9±118363	208.5±8.7
(-1-1-1)	CONDITION OF EVALUATION: 80.3°F 42% RH					
	25.0±2.2	1.5±0.3	0.0262±0.0016	457.8±6.0	309503.6±33857.9	88.7±6.0

Table 9: The complete nanoindentation analysis for APAP crystal 1

The data from the July and August histogram is in agreement with expected result with the smaller displacement range of 2-3nm. The August analyzed (011) plane has the highest frequency of smaller pop-in events. This was assumed to be the case because the (011) plane behaves in a more ductile manner in comparison to other planes. A ductile material is not as rigid and therefore the molecules have greater mobility and can disperse under stress. The rest of the planes experience frequency of pop-in's in proportion to their hardness values within the 2-3nm category. The hardest plane, (-1-1-1) experienced the least frequent amount of pop-ins in this range because it was able to absorb the energy from the added stress at low levels. This absorption of energy should culminate into higher frequencies of discontinuities at a greater depth of

displacement. However the data does not collaborate that theory. A possible reason could be that the plane, which exhibits the greatest degree of brittle behavior, has not experienced the adequate amount of pressure that would translate into large discontinuities. Which makes sense because brittle materials have higher yielding stress.

Table 10: The complete nanoindentation analysis for APAP crystal 2

CRYSTAL 2 ANALYSIS						
APAP JULY CUMULATIVE STUDY at 500μN Load						
Face	Er(GPa)	H(GPa)	Strain Rate (Δnm/sec)	Pmax(μN)	A(nm^2)	hc(nm)
(0-11)	13.5±0.4	0.9±0.04	0.0130±0.0007	453.8±1.9	543706.0±27913.4	121.6±3.4
(01-1)	13.1±0.5	0.9±0.1	0.0144±0.0011	430.6±10.8	537649.86±41431.1	120.5±5.4
(-101)	16.3±0.4	1.1±0.1	0.0069±0.0004	432.7±2.6	401139.5±19596.8	102.8±2.8
(011)	17.6±1.3	1.2±0.1	0.0090±0.0004	437.9±12.7	373601.2±28481.9	98.8±4.3

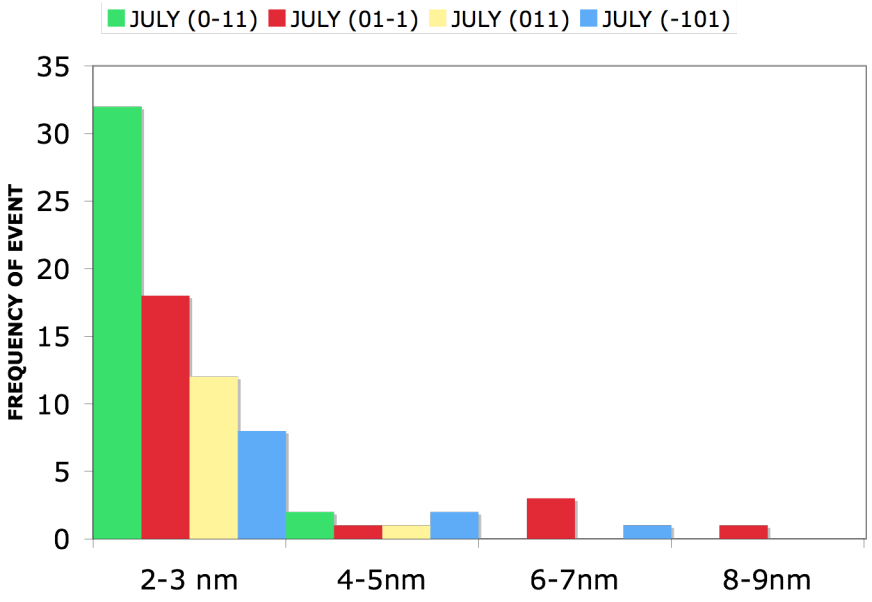


Figure 20: Histogram for crystal 2 that measures the frequency of pop-in events with respect to change in depth.

The data was from the July study is also somewhat in agreement with the observation that the curves with the steepest slopes would have the most pronounced discontinuities. The results aren't as strictly categorized because the observed hardness values are very close to one and another. However, the curves with lower hardness values over all experienced greater frequency of smaller pop-in events. Though, it was curious to see that the plane (01-1) experienced pronounced discontinuity despite the lower hardness values.

Pop-in events can indicate that the sample has been irreversible deformed and possibly formed a crack in the material. Likewise, pop-out events communicate the reaction of the product as the tip is withdrawn from the sample. The unloading curve, though assumed to be a completely elastic event, can signify plastic deformation via discontinuities. Pop outs, can signify stress induced phase transformation. However, in this instance we can only infer that pop out only signify deepening of cracks.

The slope of the unloading curve equates to contact stiffness measurement, from which elastic modulus is derived using the Oliver Pharr method [36]. Young's modulus conveys the intrinsic propensity of a material to deform under applied load. A high Young's modulus would indicate low inclination to deform. It should also be noted that Figure 16 and Figure 17, shows planes with the noticeably high-reduced modulus: (-1 -1 -1) and (0 1 -1) have slightly steeper loading curves behavior

opposed to their counterparts. This steeper feature is symptomatic of the loading curves exhibiting predominately plastic behavior. The remaining planes have a lower reduced modulus and alluding to a higher tendency to behave elastically.

Another event that can insinuate divergence from purely elastic behavior is the elongation of the nose of the load displacement curve, otherwise called creep. This behavior is observed when a hold is placed before unloading. Time dependent deformation behavior is observed in both graphical analyses. Though it is exceptionally present in the (011) plane in the August tested APAP single crystal 1.

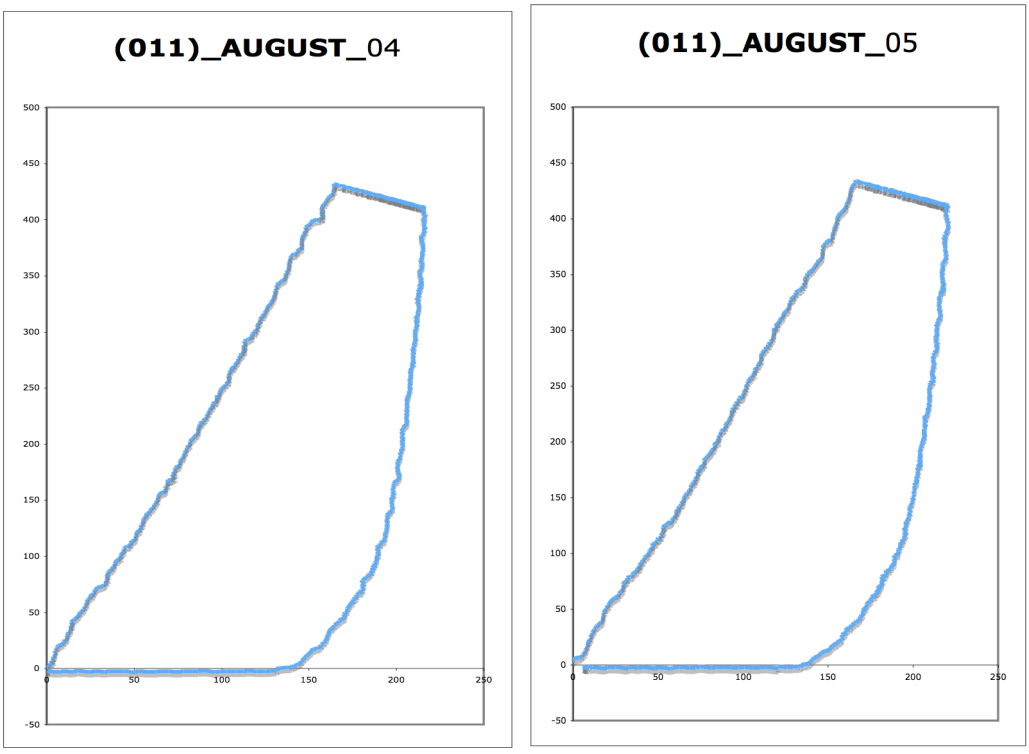


Figure 21: Load depth curves from fourth and fifth indent from Plane (011) from AUGUST analysis of APAP crystal I

The shape of the loading depth curve for (011) is identical and pervasive throughout the ten indentation data. Two more curves are added to as evidence of the observed effect. Because Acetaminophen is polar and has hydrophilic tendencies, the change in environment could have initiated, and prolonged the time dependent deformations observed. This is in line with behavior of creep, which is not an intrinsic material property. It manifests in variable degrees due to its high dependence on environmental factors. It is our contention that the modulation in relative humidity could have caused excess moisture to be present inside the nanoindentation-testing unit. This would have initiated a reaction whereby the hydrophilic nature of the molecules would have absorbed the moisture in the environment. The forging and breaking of hydrogen bonds over time could have resulted in the increase of observed creep.

This time depended deformation also distorts the unloading curve by producing an elbow effect. This effect is the thought to be the recovery of the elastic behavior from the time depended plastic deformation. As the stress was lifted the material gradually recovers.

The data did provide a correlation between creep strain rate and the elastic modulus. One would have hoped that low elasticity would translate into higher creep potential. This trend was observed in both the June and July crystals. As the elastic modulus increased there was a decrease in the observed strain rate. Creep is time depended permanent deformation, and so its logical that in a more ductile material, the

deformation is slower. At a molecular level the bonds are gradually being strained to the breaking point.

Plasticity was also observed during the hold period, as depicted in Figure 22. As the dwell time initiated, creep eventually resulted into fragmenting the nose of the load displacement curve. This fragmentation is most likely the result of crack propagation on the surface of the specimen. At a molecular level, some bonds are tightly bound, and their reaction to applied stress is analogous to a spring system. They purport instantaneous recovery after the stress has been lifted. Because they are restricted in their movement and they cannot slip past each other to recover. This is not the case for loosely bound molecules that are privy to greater mobility. They can slip past each other, leading to a time dependent deformation. And breakage behavior in this case is an amalgam of time independent and time dependent deformation.

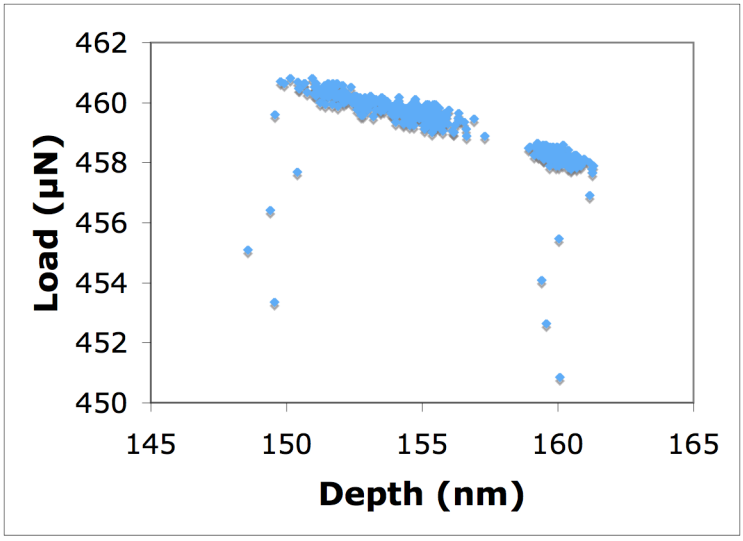


Figure 22: Plasticity observed during creep. This feature was prevent in both JUNE and JULY analysis

Variability between the crystal planes of two monoclinic individual single APAP crystals were discussed earlier, in terms of their features in the graphical analysis. The following discussion will take into account the variability of identical planes between the two crystals.

Structural deviations in a crystal lattice arise from internal energy fluctuations. These fluctuations give rise to the variety in structures. As a result, it would be permissible to conclude that a material's properties of an organic anisotropic substance would reflect this diversity. This has been the case thus far. At the same time, there is an implication that planes of the same family, or faces with similar internal energies should have identical material properties.

Both the crystals share three planes: (-101), (011) and (01-1), yet the associated data for the planes vary tremendously in some case and hardly at all for others. This inconsistency between crystals from the same batch is of some concern. The irregularity in behavior can be rationalized by their variable growth rates. It has been observed in Paracetamol crystals, that despite being grown under identical constant conditions of supersaturation, temperature and fluid dynamics, crystals have different crystalline properties [8]. This is hypothesized to be caused by the arbitrary manner in which dislocations manifest in crystals. And in particular their dependence on the orientation and direction of the dislocation.

Two mechanisms are proposed to understand the dynamic nature of irregular crystal growth between crystals form the same batch. The first insinuates that the there is a distribution of crystal growth, where individual crystals cultivate at a constant rate. But the rate of growth is variable and so two nuclei will be intrinsically different. The second gives credence to the idea that over an average period of time the two crystals will develop into similar structures. So, at one moment in time they maybe different, but over an average period of time they will come to share similar crystalline properties.

The planes (01-1) and (0-11) were concluded to be of the same family from the July study. These planes displayed identical values of the elastic modulus, hardness, creep, the depth of penetration and even the contact area.

The family of planes in the June study does not show such complimentary data. Planes (-101) and (10-1) have most noticeable distinction in their respective values of elastic modulus and hardness. This variability is not too substantial, but at the same time it deviates far more from the results in the June. Plane (-101) from both the June and July study are graphically in sync with each other. The hardness values for both the crystals were 0.9GPa. The elastic modulus varied from June to July, 16.8GPa to 15GPa respectively. The contact depth in range between 111nm to 115nm from July to June study respectively. The pop-in events incredibly, are also placed in similar positions and severity as

well. The only distinction arises in the change in the slope of the unloading curve.

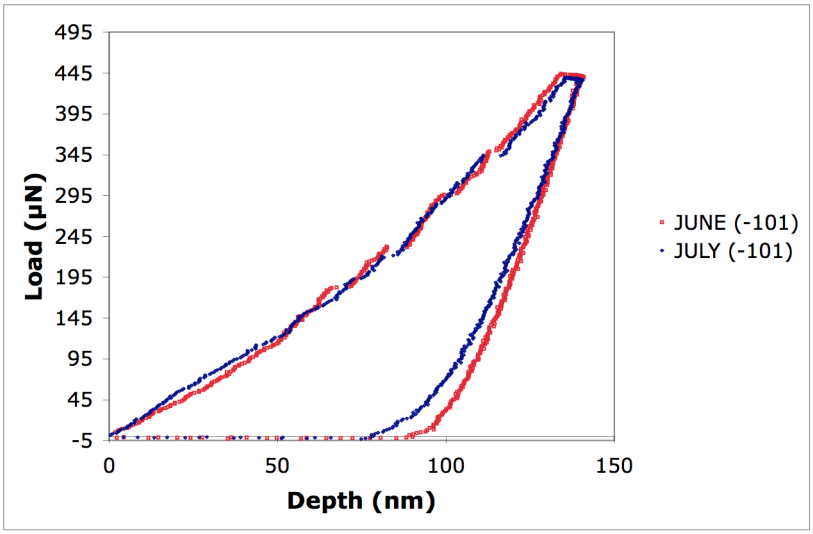


Figure 23: Loading depth curve comparison between (-101) plane of crystal 1 evaluated in JUNE and (-101) plane of crystal 2 evaluated in JULY

The divergence between the two crystals is more apparent in plane (01-1). This is important to understand because, both crystals were extracted from the same batch of crystals, and are of the same crystal structure and furthermore of the same plane. Still, there is a visible variability. It has seen theorized that a possible mechanism molecular mobility could be through crack propagation. If varying mechanisms are active with in the crystal then this could account for some of the variability observed. Crack propagation would rely on moisture content in the atmosphere. This increase in moisture would provoke the hydrophilic moieties to be attracted to the water molecules. An initially

stress induced crack would increase as more of the hydrophilic molecule was exposed.

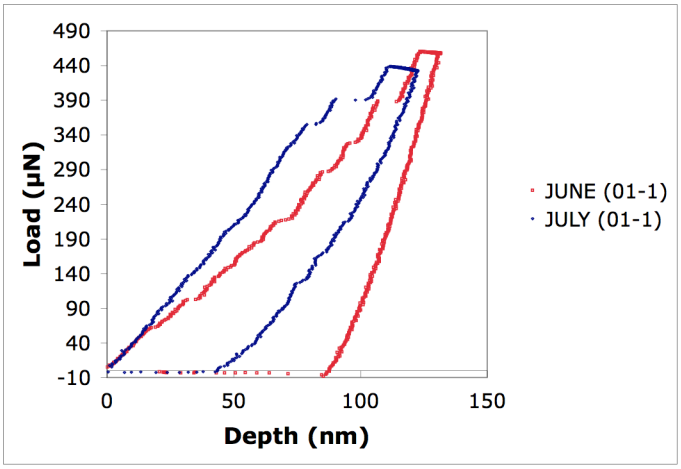


Figure 24: Loading depth curve comparison between (01-1) plane of crystal I evaluated in JUNE and (01-1) plane of crystal 2 evaluated in JULY

The greatest change between the same planes of the two crystal is illustrated in the (011). This change however, can be rationalized by the earlier discussion of modulation of the environment.

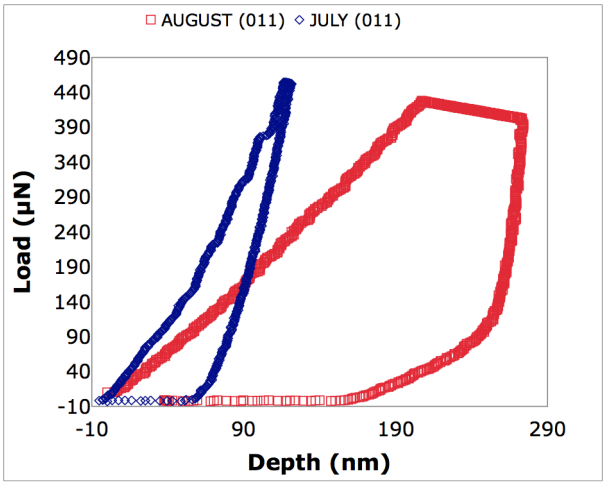


Figure 25: Loading depth curve comparison between (011) plane of crystal I evaluated in JUNE and (011) plane of crystal 2 evaluated in JULY

The cumulative study results of the average values of the ten indents show a characteristic size indentation effect. Indentation size effect is a phenomenon whereby the decrease in the depth of penetration results in increase in indentation hardness, and the effect can extend to the values of elastic modulus as well [30,40]. This effect is present in both the studies. The hardness values of the June and August study reveal a trend of increase in hardness over decrease in depth, only (-1-1-1) planes shows dramatic sudden changes. A sudden change can be seen inspecting the (-1-1-1) plane. The harness value of (-1-1-1) is 1.5GPa, with a contact depth of 88nm. The July study illustrates the ISE as well, thought it's graph of ten indentations of a particular plane translates into a more pronounced linear trend. This trend in increasing hardness with respect to planes can be seen in Figure 27. Though, if Figure 26, excludes the August study, it also shows this linear trend.

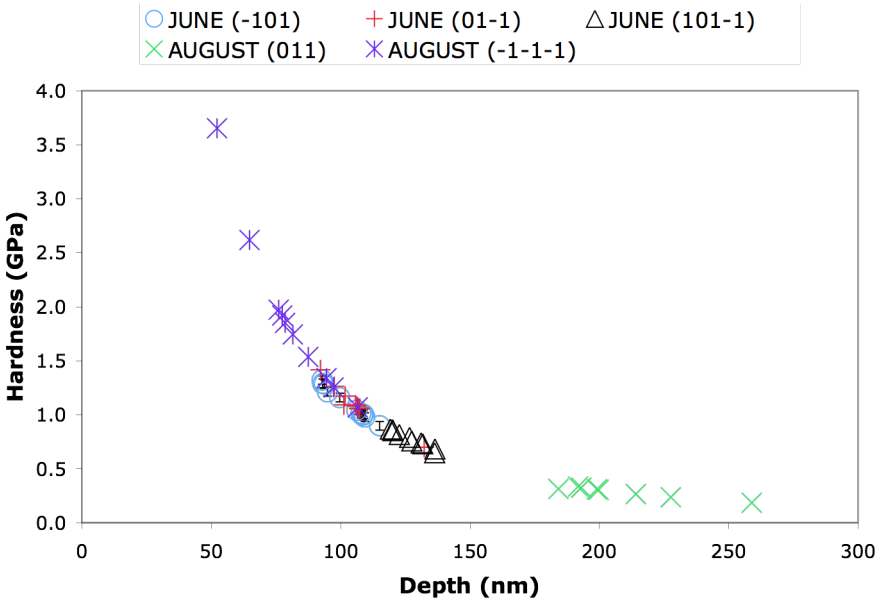


Figure 26: Indentation Size Effect observed in APAP crystal I

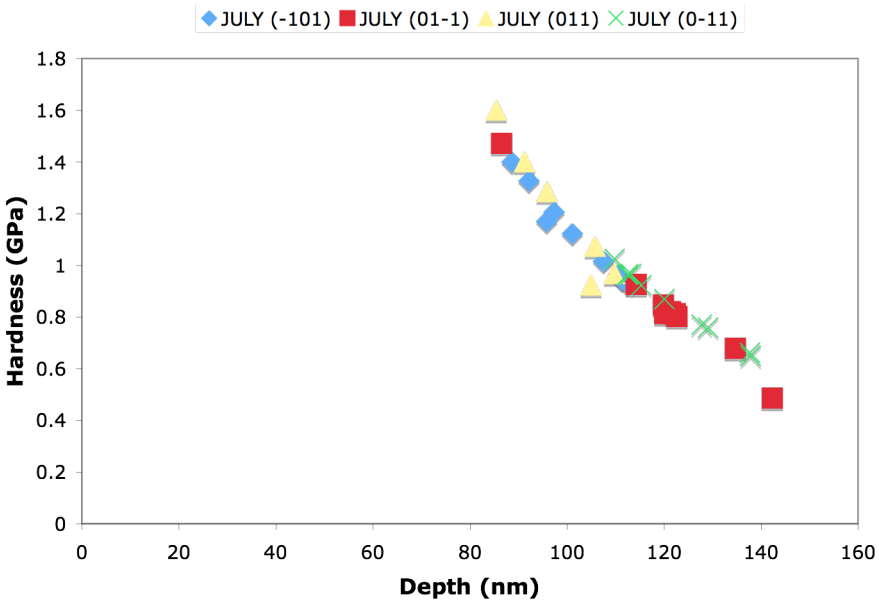


Figure 27: Indentation Size Effect observed in APAP crystal 2

Indentation size effect is hypothesized to arise due to several factors. Some considerations that produce in ISE are: friction between the indenter tip and the sample, surface layers, oxides and chemical contamination, and nucleation of dislocation with in the plastic zone [20,40].

Sudden variance in hardness and elasticity, as observed in August study in plane (-1-1-1) could be a consequence of work hardening. Work hardening is a by-product of nucleation of dislocation. As the intrinsic linear defects propagate simultaneously on multiple slip planes, there is a hardening effect. This is because the planes interlock as they shift in the crystal, making it difficult to induce plasticity. And so, there is an increase in the effective yield strength that needs to be traversed in order to break the semi brittle sample. Thereby increasing the hardness of the specimen. Creep should be in proportion to the level of strain hardening [20]. The same study also reflects decrease in hardness with increase in depth with (011) plane, and that could be in response to an analysis transition from surface to bulk properties [30]. And at the same time, decrease in hardness could be indicative of crack growth in the sample. In the case of plane (011) plane, it would seem that there was a transition from surface to bulk analysis since there was no fragmentation on the loading depth curve to suggest onset of plasticity. Moreover, both these planes are outliers in the June study, one is especially hard and the other especially weak. These affects are attributed to the change in

ambient conditions since the crystal were evaluated a little over a month after the other faces of the same crystal were evaluated. The variability of the two planes are attributed to environmental factors such as relative humidity and general history of the crystal.

Adhesion was also observed in the study, and below figure show instances where it was observed. Adhesion as discussed earlier is an attractive force between two different surfaces. It is mainly a result of the long-range Van der Waal's forces that are present in all materials. As the two materials decrease in distance there is a quantum mechanics effect that in turn creates fluctuations in dipole moments. These fluctuations vary the charge distribution within the sample and become polarized, which is why there is heavy dependence of moisture content associated with adhesion. Especially with respect to Acetaminophen crystals, which have hydrophilic functional groups.

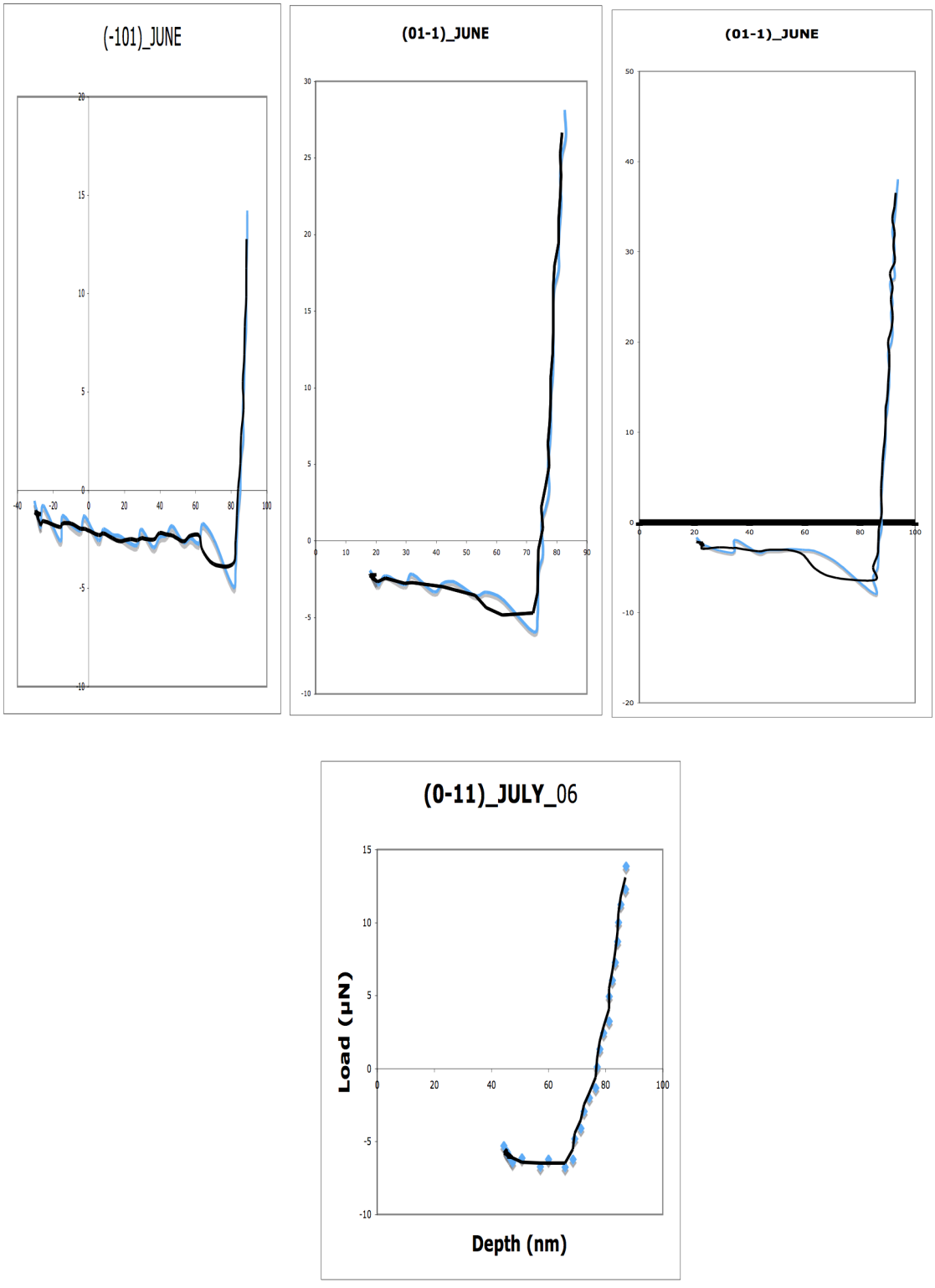


Figure 28: Examples of adhesion observed in JUNE and JULY APAP crystals

Anisotropy of the Acetaminophen crystals analyzed in June and July is shown in the below Figures 29 and 30. These graphs both show minimal variability between the hardness of different planes, however there is a distinct fluctuation in the Young's modulus between the various planes. This observation was also noted in the Sucrose. The hardness was deemed isotropic, and the Young's modulus was noted to be anisotropic.

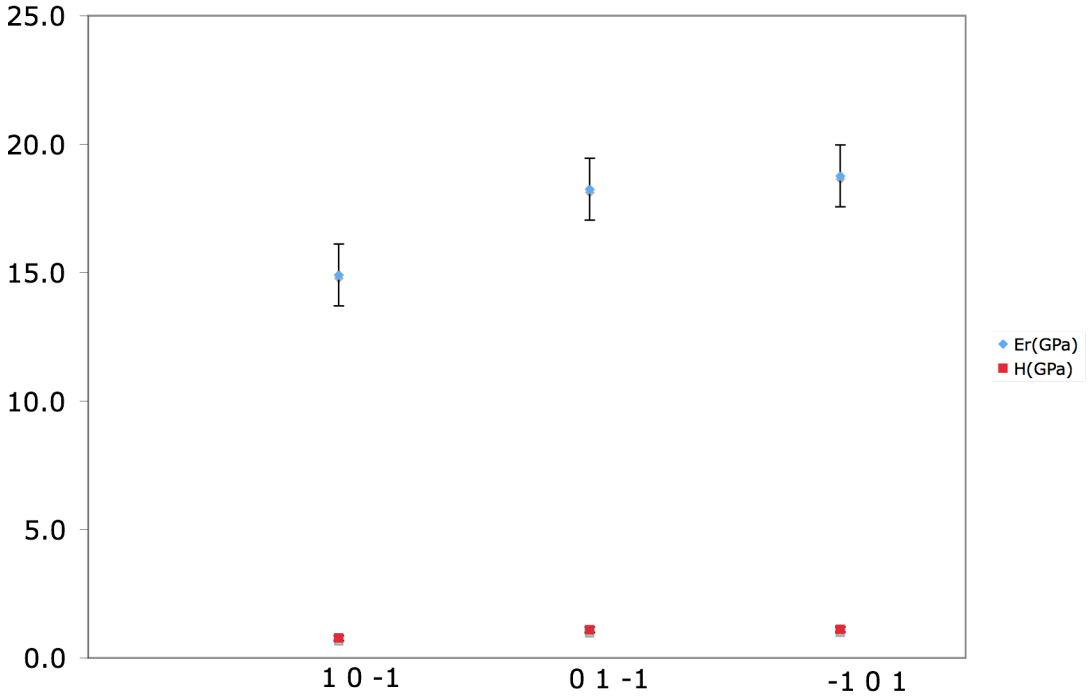


Figure 29: The variability in Hardness and Young's Modulus observed in the different planes of Acetaminophen crystal 1 (JUNE). The error bars for hardness could not be seen because the standard error was are 0.1.

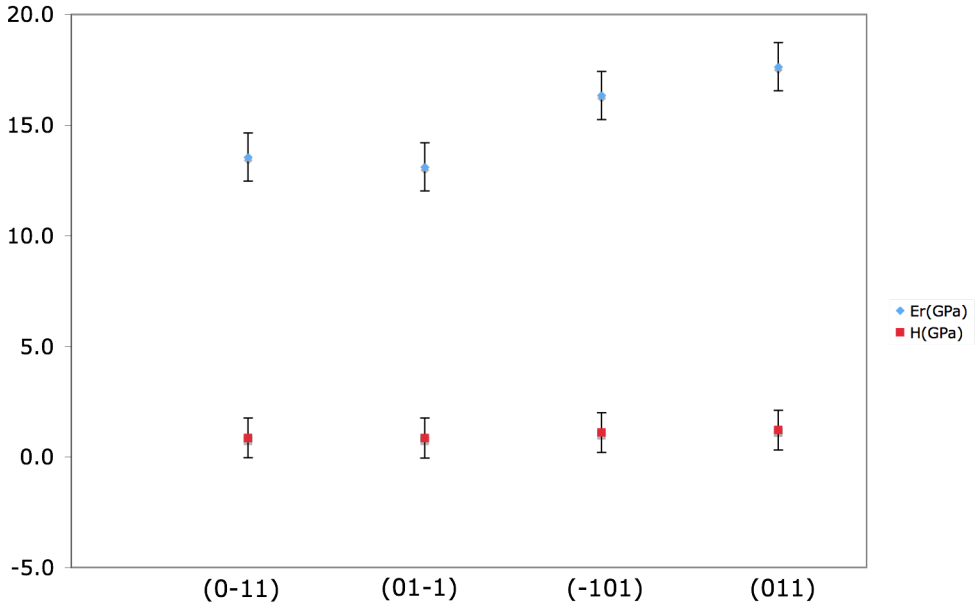


Figure 30: The variability in Hardness and Young's Modulus observed in the different planes of Acetaminophen crystal 2 (JULY).

5. CONCLUSION

Nanoindentation technique was used to analyze the multiple planes of two single Acetaminophen crystals. The results of the study were derived from the qualitative and quantitative analysis of the loading depth curve.

The loading depth curve revealed the following:

- Acetaminophen crystals have directional dependent Young's modulus and hardness
- The single crystals observed permanent, time independent deformation that were presented through discontinuities on the loading depth curve
- The crystal planes experienced time dependent deformation conveyed through elongation of the nose
- Adhesion forces were found as the tip retracted from the sample
- Considerable differences were observed from the two crystals that have matching crystal planes
- Indentation size effect was observed in both crystals

Histograms clarified that the frequency of the pop-in events were contingent upon the associated Young's modulus of the plane. It was observed that planes with smaller modulus had a higher frequency of small discontinuities.

The study also noted that Acetaminophen was sensitive to environmental conditions, like relative humidity and temperature. These sensitivities manifested through adhesion, creep and variability between the crystals.

Partial dislocations and propagation of defects through crack growth was a possible suggestion for the atomic motion observed through the loading depth curve.

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