URIC ACID CRYSTALS

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ABSTRACT

Uric acid is a crystalline component in kidney stones and gout deposits. In the work described herein, a variety of approaches are adopted to (1) create more soluble forms of uric acid, (2) examine the effects of additives on the crystallization of uric acid, and (3) compare the properties of urine-grown and water-grown samples.

Cocrystallization studies with pyridine and pyrimidine derivatives generated six new phases with hydrogen-bonding patterns inspired by DNA base pairing. Most of the new phases showed a modest increase in solubility compared to pure uric acid, though cocrystals with 2-aminopyridine had >2X higher solubility. Cocrystallization studies also yielded some unexpected results, including a gel and an amorphous film. New polymorphs of 2-amino-5,6-dimethyl-4-hydroxypyrimidine and 2,6-diaminopyridine were additionally identified.

Growth studies in the presence of both a urinary pigment, urorosein (Ur) and 2,4-diaminopyridine (24-DAP) were performed. The structure of Ur was determined from synchrotron X-ray diffraction. This helped to complete our understanding of how Ur can include in anhydrous uric acid (UA) and uric acid dihydrate (UAD) crystal matrices during growth. Solutions containing 4:1 ratios of uric acid:24-DAP yielded pyramidal UAD crystals with thin anhydrous UA layers in their centers. Over time the UAD to UA transformation in solution resulted in an unusual kind of “inside-out” epitaxial growth. AFM revealed dramatic topological differences in the UA (100) surface when exposed to acetate buffer and in buffered solutions containing 24-DAP.
Finally, prior work suggested that UAD crystals grown from urine (UAD-U) and water (UAD-W) have different levels of molecular disorder. Quasi-Elastic Neutron Scattering performed at NIST-NCNR enabled the water dynamics in both samples to be measured. UAD-U was found to have a small but statistically significant higher diffusion rate, which is consistent with a higher degree of molecular disorder in the solid.
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Chapter 1. Introduction

1.1 Uric Acid

Work described in this thesis focuses on developing a better understanding of the crystallization and properties of uric acid in its varied solid state forms. Uric acid is a natural metabolic product but perhaps better known for its role in kidney stones and gout (Figure 1.1). Uric acid levels are an important diagnostic for many health conditions. Elevated levels \textit{in vivo} have been linked to metabolic\textsuperscript{1} and cardiovascular diseases,\textsuperscript{2,3} as well as renal insufficiencies.\textsuperscript{4} Several studies have found it to be a marker for hypertension, diabetes, non-alcoholic fatty liver disease, and chronic kidney disease.\textsuperscript{5-7}

![Figure 1.1. The uric acid molecule.](image)

In the solid state, it can exist in neutral form as anhydrous uric acid (UA, refcode: URICAC),\textsuperscript{8} uric acid monohydrate (UAM, refcode: GEJQAO),\textsuperscript{9} and uric acid dihydrate (UAD). UA and UAD are the most commonly observed forms in stones. The 2D layer structures in these two phases are very similar, with the addition of water molecules in UAD between (001) layers of uric acid molecules (Figure 1.2). The cell parameters reflect their similarity. UA is monoclinic (P2\textsubscript{1}/a: \(a = 14.464\ (3)\text{Å}, b = 7.403\ (2)\text{Å}, c = 6.208\ (1)\text{Å}, \text{and} \beta = 65.10\ (5)\text{o}\)) with densely packed layers in the (100) plane. The dihydrate structure was initially reported in 1965 by Ringertz as orthorhombic
at room temperature.\textsuperscript{10} Three-dimensional coordinates were later reported by Artioli in 1997.\textsuperscript{11} In 1998, Parkin redetermined the structure at 120K and found it to be monoclinic (refcode: ZZZPPI02),\textsuperscript{12} with both molecular disorder and twinning. Parkin’s UAD structure, which we refer to throughout this thesis, is (P2\textsubscript{1}/c: a = 7.237 (3) Å, b = 6.363 (4) Å, c = 17.449 (11) Å, and β = 90.51 (4)°). The cell parameters of UAD are refined around a c-glide plane, and an a-glide plane in UA. The equivalent axes of UA : UAD are b : a and c : b. The c-axis in UAD is longer than the a axis of UA, due to the addition of water molecules that separate uric acid layers in UAD.

Uric acid, a weak acid, can also exist in a singly or doubly deprotonated state. The first hydrogen lost is at the N3 position to form urate (pKa\textsubscript{1} = 5.4),\textsuperscript{13,14} followed by loss of the hydrogen at the N9 position to form diurate (pKa\textsubscript{2} = 10.3).\textsuperscript{15} Both neutral and monoanionic phases are present \textit{in vivo}, but diurate is not relevant under physiologic conditions.\textsuperscript{16} Several urate salts have been isolated with counter ions including magnesium (refcode: BADTEX/BADTEX10),\textsuperscript{17,18} calcium (YODJAE),\textsuperscript{19} lead (DITKEX),\textsuperscript{20} methylene blue (UGEXIN),\textsuperscript{21} and guanidinium (XANDEV).\textsuperscript{22}
The physiologic salt most commonly found is monosodium urate monohydrate, MSU (NAURAT).\textsuperscript{23} There is only one example of a neutral uric acid complex in the CSD database (Version 5.39), which occurs with potassium (refcode: PABRIW).\textsuperscript{24}

Syntheses of urate salts were reported as early as 1913 (pre X-ray structure determination) by Schade and Boden who prepared gels of urate with NH\textsubscript{4}\textsuperscript{+}, Li, Na, K, Ba, Ca, Sr, and traces of Fe hydroxides.\textsuperscript{25} The physical properties of LiU were examined more closely in 1934 by Henstock.\textsuperscript{26} In the 1980’s, uric acid hydrate was synthesized with small amounts of Li, Na, K, Rb, Cs, NH\textsubscript{4}\textsuperscript{+}, NMe\textsubscript{4}\textsuperscript{+}, Tl, Mg, Ca, and Sr urate distributed uniformly throughout the crystal lattice as solid solutions and studied by powder x-ray diffraction. Zn urate was the only complex found in solid solution with potassium urate and uric acid hydrate.\textsuperscript{27} Since the late 1990’s, many more metal urate complexes have been synthesized with some incomplete characterization data indicating their coordination geometries. Kovatchoukova et al\textsuperscript{28} reported complexes of urate with Zn\textsuperscript{2+}, Hg\textsuperscript{2+}, Cu\textsuperscript{2+} and Ag\textsuperscript{+} which form five membered rings by coordinating to O6-N7 of urate. Cd\textsuperscript{2+} coordinated to O8-N9 of the imidazole ring. All solid state complexes were dihydrates, except for mercury which was a hexahydrate.\textsuperscript{28} Koksharova inferred from IR data that structures of urate in the less dominant hydroxo form with Mn, Fe, Co and Ni coordinated at the O8-N9 position. Again all reported structures were thought to be hydrated.\textsuperscript{29} Two additional reports suggested hydrated structures of urate with Mn(II), Fe(II), Co(II), Ni(II), Cu(II), Zn(II), Al(III), Cr(III), Fe(III), Cd, UO\textsubscript{2}, Na and K, with coordination to O2-N3-O9 for the trivalent iron and O2-N3 for the alkali metals.\textsuperscript{30,31} None of the urate salts described here were characterized by single crystal x-ray diffraction, most likely due to the difficulty of urate crystal growth. Therefore, no structures are available to confirm or dispute the suggested geometries.
1.2 Kidney Stones and Gout

Much has been written about the history of uric acid stones and gout. In 1776, uric acid (then referred to as “lithic acid”) was extracted from kidney stones for the first time by Carl Wilhelm Scheele. The first large scale investigation into the composition of kidney stones was conducted in 1795 by George Pearson, who found that 194 out of 200 stones contained uric acid in some concentration. In 1848, the link between uric acid serum levels and gout was established by Garrod.

MSU is the sole crystalline phase of uric acid identified in gout deposits. Kidney stones primarily contain UA and UAD, though minor amounts of other urate salts have been observed. Urate stones are also common in dogs, especially Dalmatians. The pH difference of the growth media in which these crystals form largely explains their protonation states. Typical human urine pH ranges from 5-7, but can be as low as 4.5 or as high as 8.5. Uric acid stone formers typically have a low urinary pH of less than 5.5. In a study by Grases, 80% of patients with uric acid stones had a urinary pH <5.5. However, pH alone is not a reliable indicator, since 20% of uric acid stone formers have normal urinary pH values and many with low pH urine do not develop stones. Gout crystallizes in interstitial fluid in joints which has a more neutral pH. Figure 1.3 plots the fractional composition of uric acid as a function of pH. At low pH, neutral uric acid is the major form in solution, and can crystallize in anhydrous or hydrated forms. Above the pKa₁ and at neutral pH, urate (U⁻) is the dominant species in solution. Solutions with a pH > pKa₂ would primarily contain diurate (U²⁻), but such basic conditions are not physiologically relevant.
Figure 1.3. Fractional composition of uric acid (green), urate (red) and diurate (blue) as a function of pH. Reproduced with permission from reference 245.

Uric acid kidney stones are just one type of crystalline deposit that forms *in vivo*. Many different crystalline species have been identified in human stones, some being extremely rare and others very common.\(^{42-47}\) Figure 1.4 is a pie graph based on data reported by Grases\(^{40}\) which illustrates the occurrence rates of these stones. Calcium oxalate stones, by far the most common, can occur as a monohydrate or dihydrate, or combine to form mixed stones with hydroxyapatite and uric acid.\(^{40}\) Genetics and/or environmental factors likely play a role, since the prevalence of different stone types varies by population. While uric acid is the major component in about 10% of kidney stones in the United States,\(^{44}\) it is the major component of 29% in Israel.\(^{48}\) No two uric acid stones are identical. Images in Figure 1.5 from the Louis C. Herring lab\(^ {49}\) reflect the individual differences.
Figure 1.4. Distribution of crystalline components identified in large scale compositional studies of kidney stones. The pie graph was prepared based on data reported by Grases in reference 40.

Figure 1.5. Images of uric acid stones reproduced with permission from the Louis C. Herring lab.49
1.3 Evolutionary Aspects of Uric Acid Regulation

The generation of uric acid in vivo comes from the metabolism of purines, including proteins, ATP depletion, and the degradation of nucleic acids (Figure 1.6). The molecule xanthine, which is formed from all three of these pathways, is oxidized to uric acid by the enzyme xanthine oxidase. Uric acid is the end product for humans, hominoid primates, new world monkeys, birds, and reptiles. While most is excreted to the urinary system (80%), the remainder is brought to the intestines. Elevated levels of uric acid in urine (hyperuricosuria) or in the blood (hyperuricemia) is a necessary but not exclusive precondition that can lead to kidney stones and gout. It is not known why some hyperuricemic patients develop stones and/or gout deposits while others do not.

Mammals other than primates and old world monkeys do not exhibit elevated uric acid levels. During evolution, these species retained the use of an enzyme called uricase that further oxidizes uric acid to allantoin. Uricase was mutationally silenced in higher primates from hominoid...
evolution. Allantoin is much more soluble than uric acid and therefore does not crystallize in vivo.\textsuperscript{52,53} In fact, many other species can oxidize uric acid as well as its oxidation products. For example, marine invertebrates are able to oxidize uric acid all the way down to ammonia and carbon dioxide.\textsuperscript{50}

Avian and reptilian species also produce uric acid as the end product of purine metabolism. However, they are able to excrete solid uric acid. These species excrete nitrogen in the form of uric acid in order to conserve water, which is required by the uricase enzyme to oxidize uric acid to allantoin.\textsuperscript{38} This need for water conservation evolved in order for these amniotes to survive on dry land and avoid the risk of desiccation.\textsuperscript{54} Uric acid dihydrate is the main component found in bird urine.\textsuperscript{55,56} Urate salts with sodium, potassium, ammonium, nitrogen, calcium, sulfur, chlorine, and phosphate were identified in the excrement of other avian species and reptiles such as lizards, snakes, and desert tortoises.\textsuperscript{57-59} Interestingly, uric acid precipitates from these species appear as spherical particles, though it is not clear what factors lead to this morphology.

Solid uric acid may have entirely different uses in other contexts. For example, it is also found as a pigment in the wings of butterfly where it is incorporated into the black and yellow scales, in quantities that can vary by sex.\textsuperscript{60} Other studies have identified uric acid deposits within the skin of larvae including the armyworm and the domesticated silkworm.\textsuperscript{61,62} It is possible that the presence of uric acid in these seemingly unexpected locations could be due to its potential role as a food or energy reserve since the molecule is rich in nitrogen. Uric acid is seen to accumulate rapidly in the pupae of the swallowtail butterfly right before emergence\textsuperscript{60} when fuel would be most needed.
1.4 Solubility

The precipitation of uric acid in vivo is due in large part to the molecule’s poor aqueous solubility (0.1505 mM at 18°C). The solubility of uric acid increases with increasing temperature and also increasing pH as uric acid is deprotonated to the more soluble urate. Figure 1.7 shows a solubility curve of anhydrous uric acid in a 0.03M LiCl solution (solid line) at 25°C (square markers) and 37°C (dots). The dotted lines represent the solubility of uric acid in 0.15-0.30M NaCl solutions. Dashed, dash-dot and dash-dot-dot lines are the solubility of sodium hydrogenurate in 0.30M NaCl, KCl and LiCl solutions, respectively. After converting the y-scale units to molarity, the concentration of soluble UA at pH = 4 is 0.16 mM. When pH is increased to 6, the solubility increases exponentially to 1 mM. This large increase in solubility as a function of pH is the basis for some therapies used to treat uric acid kidney stones.

Figure 1.7. Solubility curve of anhydrous uric acid in 0.03M LiCl solution (solid line) at 25°C (squares) and 37°C (dots). Reprinted by permission from Springer Nature: Springer, Monatshefte für Chemie/Chemical Monthly, Solubility of Uric Acid in Lithium Chloride Solutions, Galina Sadovska, Ivan Kron, Erich Konigsberger, Copyright 2002.
1.5 Current Therapies for Stones and Gout

Reports of kidney stone formation date back to almost 2500 years ago where lithotomy, or “cutting for the stone,” was described in ancient texts of the Greeks, Romans, Persians, and Hindu to relieve the pain caused by stones. Surgery remains one of the most common treatments for these deposits. While effective, it is a very intrusive method that is not a long term solution, as stone recurrence occurs on average every 27.8 months. Extracorporeal shockwave lithotripsy is another common therapy that works by using shockwaves positioned at the affected area to break up the large deposits into smaller ones. The goal is for the smaller crystals that break off to naturally pass. However, if any small crystal fragments remain in the kidneys they can act as seeds that can initiate the growth of additional stones. Oral alkalinization therapy uses the relationship between increased solubility of uric acid with increased pH. Sodium bicarbonate, potassium citrate, and other basic compounds are administered to patients with the intent of raising the pH of urine. By increasing the urine pH, uric acid can be ionized to urate and portions of the stones may dissolve back into solution, essentially shrinking the stones sizes. The challenge with this therapy is that tuning urine pH is challenging and raising urinary pH too high can cause the precipitation of other phases such as calcium phosphate or struvite.

The main pharmaceutical used to treat gout is allopurinol. This is a xanthine oxidase inhibitor, which blocks the enzyme from converting xanthine to uric acid in the purine metabolism pathway (Figure 1.6). By preventing the formation of uric acid, it effectively lowers the concentration present in the body. However, reports have identified this drug as having potentially toxic side effects like Allopurinol Hypersensitivity Syndrome (AHS) in patients with poor renal function. Since the drug is excreted by the kidneys, it may not be tolerated well in patients with
Chronic Kidney Disease (CKD). In addition, the drug has failed to sufficiently lower the levels of urate in some cases, making it ineffective at preventing its crystallization.

1.6 Cocrystallization as a Strategy

Designing molecular crystals via cocrystallization is a strategy that has gained much popularity in recent years. In the pharmaceutical industry especially, this technique has been used to improve the solubility and dissolution of active pharmaceutical ingredients (API). Since only a small fraction of a tablet is API, doubling the volume in a tablet is an acceptable way to effect increased bioavailability. Others have engineered pharmaceutical cocrystals to improve characteristics such as elasticity, improved stability, and new optoelectronic properties.

The generation of cocrystals is typically guided by the formation of complementary hydrogen bonds, or hydrogen-halogen bonds. In the absence of hydrogen bond functionalities, methods based on charge transfer or structure activity relationships have been employed to design cocrystals. Some cocrystals have lower solubility than the single component crystals. This may be desirable in some cases, but most often it is not. For example, cyanuric acid and melamine when combined form a very insoluble product. In our study, complimentary hydrogen bonding is used to guide the formation of uric acid cocrystals, with the goal of achieving improved solubility.

1.7 Crystal Growth

The Classical Nucleation Theory (CNT) is a two-step process where a primary nucleation event forms a critical nucleus that then grows monomer-by-monomer to generate a bulk crystal. A competing theory (non-classical nucleation) suggests nuclei of higher orders form first, which then migrate and attach to the growing crystal surface. These higher order nuclei can be liquids,
amorphous particles, or even nanocrystals.\textsuperscript{95} Although these two different crystallization pathways both ultimately generate a single crystal, it is plausible to assume that some differences may exist between the solid state phases due to their different assemblies.

Non-classical nucleation is especially common in biological systems where crystallization occurs in highly complex media.\textsuperscript{96} The medium in which a crystal grows can affect its physical and chemical properties. Additives can be intentionally incorporated into growth media in order to direct crystal growth to display desired properties.\textsuperscript{97} In some cases this can be to encourage or inhibit crystal growth,\textsuperscript{98,99} and in others to model specific growth conditions.\textsuperscript{100,101} Here, uric acid crystals were grown from pure aqueous solutions, and from aqueous solution containing additives to mimic urine conditions. Differences in their assemblies were probed with neutron scattering.

1.8 Project Outline

In the work described herein, a variety of approaches are adopted to (1) create more soluble forms of uric acid, (2) examine the effects of additives on the crystallization of uric acid, and (3) compare the properties of urine-grown and water-grown samples. The results of these studies suggest potential new therapeutic approaches for treating uric acid deposition \textit{in vivo}, as well as other types of crystal deposition diseases.

In Chapter 2, the growth of UA and UAD is examined in the presence of a biological pigment. The study of colorants inclusions in crystals has been studied by others in the past,\textsuperscript{102-116} with several previous reports of dye inclusions from our group on uric acid.\textsuperscript{21,117-121} For the first time, a urinary pigment (urorosein) potentially present in kidney stones was synthesized and characterized by synchrotron single crystal x-ray diffraction. The urorosein structure determination confirmed its molecular composition and conformation, which was not yet known from previous
studies. The pigment stability was studied by visible spectroscopy and its inclusion in UAD and UA single crystals was observed with optical microscopy.

In Chapter 3, efforts to generate more soluble phases of uric acid through cocrystallization are described. The idea was to identify molecules that can bind strongly to uric acid so they can be employed as growth inhibitors or solubilizing agents for pre-existing deposits, or cocrystallize with uric acid to generate phases with improved aqueous solubility. A variety of potential coformer molecules, mostly aromatic pyridine and pyrimidine derivatives, were chosen based on their potential complementary hydrogen bonding motifs. Evidence for six cocrystals was found through a combination of single crystal, synchrotron and powder x-ray diffraction work, as well as differential scanning calorimetry. Two of the coformer molecules are drugs already approved by the Food and Drug Administration for other uses.122,123 Cocrystals of 2-aminopyridine were more than twice as soluble as pure uric acid.

Some cocrystallization attempts failed, but yielded various unexpected outcomes that are described in Chapter 4. New polymorphs of two coformer molecules were obtained, one of which was generated from sublimation and the other as a recrystallization product. Non-crystalline products were also formed in two cases, an amorphous film and a gel. The gel properties were examined by scanning electron microscopy which revealed fibers as well as small crystals of triamterene throughout. Additionally, a cone-plate rheometer showed a higher storage modulus than loss modulus for all frequencies tested.

Chapter 5 describes the unusual effects of 2,4-diaminopyrimidine in low concentrations on the crystallization of uric acid. Scanning electron microscopy revealed a kind of “inside-out epitaxy” growth where UA in the center of pyramidal UAD crystals grows as UAD dissolves. This
was the first time this kind of growth pattern had been observed with uric acid, and was not observed with any other additives. The UA (100) surface was investigated with *in situ* atomic force microscopy in the presence and absence of this pyrimidine derivative leading to observable changes in the surface nanostructure.

Chapter 6 is a stand-alone study which employed neutron spectroscopy to examine differences in the water dynamics in uric acid dihydrate crystals grown from water (UAD-W) and from urine (UAD-U). Work by other members of the Swift group previously determined that microstructure differences result from different assembly mechanisms in solution. UAD crystals grown from urine have a higher degree of molecular disorder owing to their non-classical assembly. High flux backscattering experiments performed at NIST-NCNR (Gaithersburg, MD) with generous help from Dr. Timothy Prisk were used to measure the water dynamics in the channels of UAD-U and UAD-W, enabling quantification of material properties due to differing levels of order/disorder.
Chapter 2. Inclusion of a Urochrome Pigment in Uric Acid Crystals†

2.1 Introduction

Kidney stones are composites of microscopic crystals embedded in an amorphous organic material called the “matrix,” which constitutes 2-3 weight %. The matrix composition may or may not depend on the stone type. Its suggested functions include controlling the growth or inhibition of different crystal forms, and/or acting as an epitaxy mediator between them. It has also been suggested to act as a glue that holds the crystalline composites together, but its possible roles are not well understood. Molecules of the matrix are usually thought to reside in the intercrystalline spaces of the composite, however many biomineralization studies have shown that any number of molecules can become trapped within a growing crystal lattice when they adsorb on the crystal surface.

Identifying molecular impurities in the crystal lattice can be extremely challenging due to their typically low concentration which limits spectroscopic quantification and characterization. Impurity identification is much easier when they are visible to the naked eye. Starting as early as the 1850s, research has been conducted investigating the inclusion of chromophores like dyes within host crystals. It was discovered that the size and shape of the impurity need not correlate with that of the host crystal for inclusion to be successful, debunking previous notions that the two needed to be nearly isomorphic. It was also revealed later that the inclusion of dye impurities could initiate changes to the typical growth and morphology of the host crystals. Kahr et al. are recognized for their expansive research in both the understanding and applications of this field.

From compositional analysis of a vast number of kidney stones, uric acid was found to be the major component in ~10% of stones in the United States,\textsuperscript{42,44} as high as ~29% in Israel,\textsuperscript{48} and a secondary component in an even larger number of mixed stones. The two most readily identified forms of uric acid found in kidney stones are anhydrous uric acid (UA) and uric acid dihydrate (UAD) whose structures have both previously been determined.\textsuperscript{8,12} Many other solid state phases of this molecule exist, including salts of urate, the deprotonated form of uric acid. When grown from aqueous solutions in a laboratory, UA and UAD typically crystallize as colorless rectangular plates. When found \textit{in vivo}, uric acid crystals have been identified in a wide variety of morphologies and colors typically in the orange, red and brown families (Figure 1.5). While the individual identities of these colored impurities are unknown, it is plausible that they belong to a category of pigments from the urochrome family, which are found in urine.\textsuperscript{141} Previously reported urochrome pigments include urobilin, urohematoporphyrin, uroporphyrin, uroproporphyrin, urohematin, urocyanin, indirubin, indigotin, uroerythrin, purpurin, skatol red, nephrorosein, urorosein and urocine.\textsuperscript{141-144} Since their characterization and isolation is difficult,\textsuperscript{145-148} it is not clear if these reported pigments are all separate species, or if some names refer to the same molecule.\textsuperscript{149-157}

Previous work in the Swift lab conducted by Ryan Sours,\textsuperscript{158} in conjunction with an undergraduate Honors student Kristin Cox, identified the preferred orientation of the red urochrome pigment urorosein (Ur, Figure 2.1) when included in uric acid crystals. This was done using microspectroscopy techniques where the transition dipole of the molecule’s lowest energy conformation was calculated using a method developed by Kahr et al.\textsuperscript{107,112,140} In our more recent work, the synthesis of Ur was re-examined and the solution conditions for crystallization were optimized. The first crystal structure of Ur was unambiguously determined from single crystal
synchrotron x-ray diffraction data. Visible absorption spectroscopy was used to monitor the properties of urorosein in uric acid solution and to confirm that this pigment can exist in both the inter- and intracrystalline sites of kidney stones. This work revises and expands the conventional definition of where “matrix” is located in kidney stone composites.

![Molecular structure of urorosein.](image)

**Figure 2.1. Molecular structure of urorosein.**

2.2 Previous Studies

2.2.1 Inclusion of Colorants in UA and UAD Crystals

Pure UA and UAD crystals typically precipitate from aqueous solutions at 37°C and room temperature, respectively, as colorless rectangular plates. Dimensions rarely exceed 300 µm in any direction. UA crystals have a large (100) face and are bounded by (210), (201), (001), and sometimes (121) faces. UAD crystals have a large (001) face and are bounded by (011), (102), and sometimes (210) faces (Figure 2.2). The UAD cell convention corresponds to that reported by Parkin. When grown from aqueous solutions containing methanol in concentrations as high as 10%, crystals appeared identical to pure aqueous solutions, though clustering/epitaxial growth tended to occur at higher methanol concentrations.
The inclusion of colorants in uric acid crystals began with Gaubert\textsuperscript{160} in the 1930s when synthetic dyes were added to growth solutions. More dye studies were conducted with uric acid in the 1970s by Kleeberg.\textsuperscript{161,162} Our lab has been responsible for a large majority of more recent work, demonstrating that a wide variety of neutral and cationic dyes are capable of including into UA and UAD crystals, typically with high specificity.\textsuperscript{21,117-120} Inclusion of cationic dyes requires charge balance to be achieved by the simultaneous inclusion of a deprotonated uric acid molecule (urate). Specificity of inclusion varies in the two systems, for example methylene blue and various other dyes include exclusively in the \{001\} and \{201\} growth sectors of UA, but in UAD methylene blue is seen throughout the whole crystal.\textsuperscript{21,118} Some dyes like acriflavine neutral, which includes in both UA and UAD specifically,\textsuperscript{119} can systematically change the growth morphology with a change in solution dye concentration.

2.2.2 Inclusion of Urorosein (Ur) in UA and UAD Crystals

UAD crystals with included Ur (UAD-Ur) were prepared from warm supersaturated uric acid solutions containing small amounts of 4-7 mM Ur stock solution in methanol, resulting in

\textbf{Figure 2.2. Schematic of typical UAD (left) and UA (right) single crystals with associated Miller Indexes.} Adapted with permission from Hall, V. M., Cox, K. A., Sours, R. E., and Swift, J. A. (2016) Urochrome Pigment in Uric Acid Crystals. \textit{Chem. Mater.} 28, 3862-3869. Copyright 2016 American Chemical Society.
final concentrations ranging from 370-850 µM Ur. Crystals that precipitated at room temperature were mostly colorless, however a few were found in each batch with distinct red coloration. Quantification of dye included in each UAD crystal was not possible, however from previous work, [dye]_{crystal} is usually 1-2 orders of magnitude less than [dye]_{solution}, with [dye]_{crystal} typically not exceeding 0.1 weight %. Although not optically visible, it is likely that some dye is also included in the crystals that appear colorless.

Visibly colored UAD-Ur crystals were analyzed and found to display a variety of inclusion patterns, including an hourglass shape with colored \{102\} growth sectors, color spread throughout the whole crystal, and also others with color in isolated regions (Figure 2.3). Crystals with colored inclusions in isolated regions may have been a result of trapped solvent pockets within the crystal. In all cases, the size and morphology of the crystals remained unchanged as compared to typical pure UAD growth.

**Figure 2.3. Photomicrographs of UAD-Ur grown from water/methanol solutions.**

[U]_{solution} = 560 µM (left), 590 µM (center), 1.70 mM (right). Scale bars = 100 µm.


UA also included Ur (UA-Ur) when grown in solutions at 37°C. UA-Ur typically grew in clusters of 2 or 3 crystals with UAD or other UA crystals in contact face to face. Observation of UA-Ur as a standalone crystal occurred just once, where it was seen to have an hourglass inclusion pattern. Previous research showed UA can nucleate epitaxially on dissolving UAD crystals in some
but perhaps not all solutions.\textsuperscript{163} The transformation kinetics have been reported for UAD to UA in solution by a dissolution-recrystallization mechanism.\textsuperscript{164} It is not fully understood why this epitaxial growth is so readily observed in the presence of Ur, but it is notable compared to many other additives tested for this effect.

Inclusion patterns observed in UA-Ur were limited to an hourglass shape or Maltese cross (X-shaped) (Figure 2.4). The growth sectors affected in the Maltese cross could not be identified, however the hourglass pattern in UA was previously observed with a number of other colorants.\textsuperscript{118} Hourglass inclusion affects the \{001\} and \{201\} growth sectors, most likely because of kinetic factors; growth along the $\pm c$-axis is 10X faster than along the $\pm b$-axis.\textsuperscript{165}

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{UA-Ur_grown_from_water_methanol_solutions.png}
\caption{Photomicrographs of UA-Ur grown from water/methanol solutions. Opaque centers are partially dissolved UAD-Ur crystals. \([\text{Ur}]_{\text{solution}} = 560 \, \mu\text{M} \) (left), \textbf{1.70 mM (right)}. Scale bars = 100 \, \mu\text{m}. Adapted with permission from Hall, V. M., Cox, K. A., Sours, R. E., and Swift, J. A. (2016) Urochrome Pigment in Uric Acid Crystals. \textit{Chem. Mater.} 28, 3862-3869. Copyright 2016 American Chemical Society.}
\end{figure}

\textbf{2.2.3 Determination of Urorosein Orientation in UAD-Ur}

Orientation of synthetic dyes trapped in crystals has been determined previously and the method was repeated here to determine the orientation of Ur in UAD-Ur crystals. Determination of orientation in UA-Ur was not possible because of the epitaxial growth that was present in all otherwise viable crystals. A modified Olympus BX50 polarizing microscope was used with an
Ocean Optics PC2000-ISA spectrometer that was optimized for the visible region to measure the visible absorption spectra of the UAD-Ur crystals displaying an hourglass inclusion pattern. Single crystals were mounted with a small amount of mineral oil onto the tip of a sewing needle that passed through a plastic vial cap epoxied to a glass microscope slide. Measurements were taken along two orthogonal directions at a time (a- and b-, b- and c-, and a- and c-axes), and intensity corrections were applied using the Vuks’ correction method\textsuperscript{166} to account for differences in the refractive indices of the three crystal directions. Baseline corrected absorption spectra were plotted from the best fit of three Gaussians whose integrated intensities were calculated (Figure 2.5). A more detailed description is provided in the dissertation of Ryan Sours.\textsuperscript{158}

From visible absorption spectra, the $\lambda_{\text{max}}$ of UAD-Ur (~520nm) was red-shifted from the $\lambda_{\text{max}}$ of Ur in solution (~485nm), which is consistent with previous data for dyes trapped in crystals relative to those in solution. The consistency of $\lambda_{\text{max}}$ for the three sets of UAD-Ur absorption data suggest that the dye molecules may be oriented 45° to the two positions on the faces of the crystal, which is again consistent with previous data for synthetic dyes trapped in UAD affecting the same growth sectors.\textsuperscript{117} Other possible explanations for this $\lambda_{\text{max}}$ trend could indicate random orientation

![Figure 2.5. Baseline corrected absorption spectra of UAD-Ur crystals. Polarized light incident along a-axis (left), b-axis (middle), c*-axis (right). Adapted with permission from Hall, V. M., Cox, K. A., Sours, R. E., and Swift, J. A. (2016) Urochrome Pigment in Uric Acid Crystals. Chem. Mater. 28, 3862-3869. Copyright 2016 American Chemical Society.](image-url)
of the dye molecules, as well as a few highly oriented Ur molecules that saturate the linear dichroism.

Using the calculated dipole moment and spectroscopy measurements, a model was constructed for the orientation of Ur in the UAD lattice (Figure 2.6). Based on the calculated lowest energy conformation of Ur, the molecule was planar, with the visible transition dipole (calculated with the Zindo module of CAChe V5.0 (Fujitsu, Beaverton OR)) parallel to the molecule’s long axis. Ur is larger than uric acid, and presumably replaces 2-3 uric acid molecules within a given (001) layer and into the next adjacent layer. This protrusion may serve as a point for new molecules or crystals to attach, as was demonstrated in UA-Ur by the consistent observation of exptaxial growth. Ur is assumed to be cationic under these growth conditions, so one urate ion is likely included for every one dye molecule. In other conformations it is possible for Ur to fit within a single channel of uric acid, but there is no evidence to support or refute that Ur would adapt a higher energy conformation in the crystal. As it sits in the model, Ur does not cause a large disturbance to its neighboring molecules and is able to form hydrogen bonds and π-π stacking with neighboring molecules.

2.3 Synthesis and Characterization of Ur orosein

Although references to Ur in literature date back to over a hundred years, reports of its structure have varied, with only a characteristic absorption band of 530-540 nm used to suggest its presence. It is likely synthesized in vivo from tryptophan oxidation products indole-3-carboxaldehyde or indole-3-acetic acid when in the presence of acid. A few different syntheses of urorosein have been reported in literature, but the one used in this work was the Harley-Mason method. 120 mL sulfuric acid (Avantor Performance Materials), 280 mL water (purified by passage through a Barnstead deionizing cartridge then distilled) and 0.5g of indole-3-carboxaldehyde (Aldrich, CAS# [487-89-8]) were stirred for 1 h at 70 °C. This resulted in an initial precipitate thought to be urorosein bisulfate (Ur\(^+\)(HSO\(_4^−\))). \(^1\)H NMR was used to confirm the identity of the product, and the results matched those of previously published data (Figure 2.7). An acetic acid solvate was formed upon vacuum filtering the precipitate, dissolving in boiling glacial acetic acid (Fisher) and allowing it to recrystallize, which formed very fine red needle-like crystals (Figure 2.8).

![Figure 2.7. \(^1\)H NMR (CD\(_3\)OD) of Ur needles with peaks at 9.36 (s, 1H), 9.14 (s, 2H), 8.26 (d, 2H), 7.73 (d, 2H) and 7.57 (m, 4H).]
Thermal characterization was performed using differential scanning calorimetry (DSC). Closed pans with 2 mg samples were heated at a rate of 10 °C/min to 300 °C in a TA Instruments 2920 Modulated differential scanning calorimeter. While thermal analysis of these two products showed the initial precipitate to lose >50% of its weight by 180 °C with the solvate only loosing ~12% in the same temperature range, NMR revealed them to be identical. The acetic acid solvate had an additional singlet at 2ppm, likely indicating the presence of acetic acid. DSC displayed an endothermic peak at 124 °C owing to the desolvation of the solvate, as well as a decomposition peak at 238 °C (Figure 2.9). This data is consistent with the recrystallized product having one acetic acid molecule per Ur⁺(HSO₄⁻) unit. The solvate could be converted back to Ur⁺(HSO₄⁻) by heating at 150 °C for 30 min.

Products isolated at various phases of the synthesis were identified with powder X-ray diffraction (PXRD). Data was collected at room temperature with a 2θ range of 5-40° on a Rigaku Ultima IV X-ray diffractometer (Cu Kα radiation, 40 kV, 44 mA current). Jade v9.0 software was used for analysis. PXRD data was also collected on the recrystallized needles (Figure 2.8) by Dr. Maren Pink using an EMPYREAN diffractometer at Indiana University (Cu Kα radiation, 45 kV, 40 mA current).

Figure 2.8. Needles of Ur after recrystallization in glacial acetic acid. Scale bar = 200 µm.
2.4 Solution Characterization

Absorption spectroscopy was used to determine the stability of Ur in different methanol containing solutions. Data was collected on an Agilent 8453 UV-vis spectrophotometer from 200-800 nm. One set of solutions contained 0-5 mg uric acid powder in 20 mL water with 10 mL of 20 mg Ur / 100 mL methanol stock dye solution. A second set of solutions contained 0-5 mg Ur in 30 mL methanol. The solutions were stored at room temperature and measured in triplicate periodically over a number of days.

2.4.1 Urorosein in Methanol

Previous reports of urorosein dissolved in methanol have stated that decomposition of the dye molecule occurred, resulting in numerous indole-based products. UV-vis absorption was used to re-determine the stability of this dye in methanol (2.7 x 10^-4 M). Spectra were recorded over the course of 8 days (Figure 2.10), where the broad absorption band visible from 418-567 nm matched previously reported data. A blue shift of ~4 nm was observed in $\lambda_{\text{max}}$, along with a
decrease in intensity of ~83% by the 8\textsuperscript{th} day. Precipitation of black sediment was found at the bottom of the vial towards the end of the experiment.

![Graph showing UV-vis absorption of urorosein bisulfate in methanol](image)

**Figure 2.10. UV-vis absorption of urorosein bisulfate in methanol (2.7 x 10\textsuperscript{-4} M) collected over 8 days shows decomposition.** Adapted with permission from Hall, V. M., Cox, K. A., Sours, R. E., and Swift, J. A. (2016) Urochrome Pigment in Uric Acid Crystals. *Chem. Mater.* 28, 3862-3869. Copyright 2016 American Chemical Society.

2.4.2 *Urorosein in Methanol with Uric Acid*

While urorosein has proven to be unstable in methanol solutions, it was previously shown to remain stable in solutions of acetic acid. UV-vis absorption was monitored on urorosein bisulfate (2.7 x 10\textsuperscript{-4} M in 10 mL methanol) in solution with 1 mg uric acid in 20 mL water over several days. Spectra recorded over 4 days displayed almost no decrease in intensity, although the peak at longer wavelength did decay within a day (Figure 2.11). A slight increase in the pH of the solution between day 1 and 2 from 3.25 to 3.8 may be related to the disappearance of the longer wavelength peak, but it is not well understood. It is clear from these results that in the 1-2 days it takes for crystallization of uric acid to occur, the dye molecule would remain stable in typical growth solutions.
2.5 Crystal Growth Difficulty

Obtaining large X-ray quality single crystals of Ur proved difficult due to the limitations of slow evaporation, and the fact that urorosein decomposes in solvents like methanol and ethanol. Crystals were typically extremely thin and flexible, resulting in minimal to no diffraction. A solution of dissolved urorosein bisulfate in acetic acid was left undisturbed at 37 °C for over a month, yielding crystals with dimensions ~ 0.2 x 0.01 x 0.005 mm³, thick enough for diffraction to be observed via synchrotron XRD. PXRD was collected for each of these four products (Figure 2.12), which displayed different diffraction patterns. This suggests that the urorosein bisulfate acetic acid solvate (d) is likely a different phase than the recrystallized solvate (b).
2.6 Structure Solution of Urorosein

Structure solution was possible through synchrotron XRD at the Advanced Photon Source, ChemMatCARS sector 15. The frames had an exposure time of 1 s and were collected at 100 K in a Bruker platform three-circle goniometer using $\phi$ scans with fixed chi. The instrument was equipped with a CCD area detector ($\lambda = 0.41328\text{Å}$), an undulator beam, and a diamond [111] crystal with two mirrors in the beam path to exclude higher harmonics. SMART was used to carry out data collection and unit cell refinement; SAINT was used for data reduction. Structure solution was obtained by direct methods with SHELXS and refined with SHELXL by full-matrix least-squares on $F^2$. Nonhydrogen atoms were modeled anisotropically; hydrogen atoms were found from the difference Fourier map and modeled isotropically. The bisulfate hydrogen ion was
modeled as 50% occupancy over the H14 and H15 positions. The structure, now in the Cambridge Structure Database, has a refcode USADUP.

The single crystal structure belonged to the orthorhombic space group P\textit{bca} (Figure 2.13). The final R$_1$ value ($I > 2\sigma$) was 0.0479 with Z = 8 and cell parameters $a = 20.1106\text{Å}$, $b = 7.3879\text{Å}$ and $c = 24.510\text{Å}$. The asymmetric unit contained Ur$^+$, HSO$_4^-$ and CH$_3$COOH in a 1:1:1 stoichiometric ratio. The planar conformation of Ur$^+$ allowed it to align along the $b$-axis in one-dimensional π stacks, which were 3.69Å apart and related by a two-fold rotation. Hydrogen bonding occurred between Ur$^+$ and HSO$_4^-$ through N-H…O (1.887Å, 162.31° (N…O = 2.756Å)), and between Ur$^+$ and CH$_3$COOH through N-H…O (2.014Å, 153.07° (N…O = 2.806Å)). Hydrogen bonding between CH$_3$COOH and HSO$_4^-$ occurred through O-H…O (1.799Å, 172.58° (O…O = 2.690Å)). Interactions between neighboring bisulfate ions through O-H…O were able to be present as a result of modeling the hydrogen atom in bisulfate with 50% occupancy over two sites.

![Figure 2.13. Single crystal structure of urorosein bisulfate acetic acid solvate refcode: USADUP viewed down the $b$-axis.](image)

2.7 Conclusions

Previous members of our lab (Ryan Sours and Kristin Cox) began the work on urorosein inclusion in UA and UAD crystals. UAD-Ur and UA-Ur single crystals displayed distinct inclusion patterns, where the dye was visible in specific growth sectors. UAD-Ur crystals were analyzed with microspectroscopy, and the orientation of the dye molecule was able to be determined within the lattice. Included Ur is believed to be planar, aligned partially within the ribbons of uric acid molecules in the plane with some portion protruding out into the adjacent layer. We believe this may create a possible attachment or nucleation site which encourages epitaxial growth, as it was almost always observed in the growth of UA-Ur crystals.

My contribution to this project was a more detailed study of the dye molecule itself in solution and in the solid state. The synthesis and recrystallization of Ur was optimized, and the first definitive crystal structure was able to be obtained. The geometry of the molecule matched closely to what was assumed in microspectroscopy measurements and calculations. UV-vis absorption studies showed Ur decomposes in methanol solutions, but is stable in aqueous methanol solutions containing uric acid. This indicates Ur remains stable in the acid growth solutions used to grow the dye included UA-Ur and UAD-Ur crystals.

Previous notions have assumed kidney stone “matrix” resides in the intercrystalline sites of these composite materials. However, the research conducted here demonstrates the ability of this material to include in intracrystalline sites as well. The urochrome pigment urorosein was used in our work to exhibit this, however further research might address other compounds that are also capable of including in these intracrystalline regions.
Chapter 3. Uric Acid Cocrystals

3.1 Introduction

The crystallization of uric acid in humans in vivo has long been associated with the formation of kidney stones and gout deposits. Crystalline uric acid deposited under physiologic conditions can adopt a range of forms. The specific crystal form that grows in vivo is heavily dependent on pH. Uric acid kidney stone formers typically have low urinary pH, less than 5.5. Protonated forms are favored at low pH. Anhydrous uric acid (UA) and uric acid dihydrate (UAD) are the phases typically identified in kidney stones. A uric acid monohydrate form has also been identified, but is considered to be rare. With a pKa of 5.4, uric acid exists primarily as urate (U\(^-\)) with deprotonation occurring at the N3 position in higher pH solutions. Monosodium urate monohydrate (MSU) is the sole crystalline phase found in gout, since the pH of interstitial fluid is neutral and sodium is the most abundant cation. MSU and other urate salts have been identified in kidney stones as minor components. Urate has a pKa of 10.3, but diurate is not physiologically relevant due to the strongly basic conditions required for its formation.

The formation of any of these crystalline phases in vivo is due in part to the low aqueous solubility of uric acid, which is created from the metabolism of purines. In a standard reference artificial urine solution, UA has a solubility of 0.32 mM at a pH of 4, 37°C. Solubility increases slightly with pH, however is still only 2.5 mM at pH = 6, 37°C. While gout is usually treated effectively with the xanthine oxidase inhibitor allopurinol, pharmacological treatment of uric acid kidney stones are only modestly successful. Some current therapies attempt to address the solubility problem by increasing urine pH through oral alkalization therapy with Na\(^+\)(HCO\(_3\))\(^-\) or (K\(^+\))\(_3\)(C\(_6\)H\(_5\)O\(_7\))\(^3-\). This approach has shown limited success, and potential side effects can include the formation of calcium phosphate or struvite stones which form at higher pH values.
Extracorporeal shockwave lithotripsy treatment is used to break up large stone deposits so that they can be passed naturally, however smaller crystal fragments can act as seeds if they are not all excreted. Surgery is used as the last resort to retrieve impassable stones, though the invasive procedure is not ideal either for patient or economically when the recurrence rate for uric acid stones averages ~27.8 months.

Alternative methods which facilitate better management of high uric acid concentrations can be an effective way to avoid the symptoms associated with these crystal deposition diseases. Such strategies might include inhibiting uric acid crystal nucleation or growth, developing more effective solubilizing agents, or redirecting uric acid into more soluble forms. Though these strategies may not prevent the first incidence of kidney stones, they could be adopted as a preventative strategy to lower stone recurrence rates. Key to any of these strategies is identifying molecules capable of binding uric acid more strongly than uric acid binds to itself.

The strategy explored here takes inspiration from industry’s efforts to improve the solubility of active pharmaceutical ingredients through cocrystallization. Cocrystal design typically relies on strong complementary hydrogen bonding between two or more molecules. Although cocrystals can have higher, lower or similar solubility relative to the single component phase, here, we attempt to use cocrystallization as a way to identify more soluble forms of uric acid. The solubility of new uric acid multicomponent phases is then assessed against that of pure uric acid. Even a significant reduction in the growth rate would enable small crystals to be naturally excreted. This strategy would be in principle applicable to other types of pre-existing pathogenic crystals formed *in vivo*.
A search of the Cambridge Structural Database (CSD) Version 5.38 for uric acid complexes returned only four results. Complexes included lead (refcode: DITKEX), potassium (PABRIW), methylene blue (UGEXIN), and guanidinium (XANDEV), where three included hydrated forms of $U^-$ and one was a complex with UA. With only a limited number of cocrystals to use as a guide, we looked to the packing diagrams of UA and MSU (Refcodes: URICAC and NAURAT), whose structures have been reported previously.\textsuperscript{8,23} Both form strong bonds to neighboring molecules with the former displaying two bidentate bonds at N1-O2 and N7-O8, and four single H-bonds at the O6, N3, N9, and O8 positions. In MSU, each urate forms three bidentate bonds at the N1-O2, O6-N7 and O8-N9 positions (Figure 3.1). These structures suggest that the H-bond donor/acceptor (D/A) properties of the peripheral nitrogen/oxygen atoms play an important part in stabilizing the structure. Binding motifs which enable the formation of stronger bidentate or possibly tridentate bonds could lead to the realization of new cocrystal forms. The idealized interactions are shown in Figure 3.2. Uric acid (or urate) molecules can also interact with coformer molecules through $\pi-\pi$ stacking. However, it is less obvious how to engineer compatible $\pi-\pi$ stacking coformers.

Figure 3.1. Hydrogen bonding geometry surrounding a uric acid molecule in UA (left) and a urate molecule in MSU (right).
Based on this criteria, 12 coformer molecules with complementary binding motifs were chosen (Figure 3.3), five of which were unambiguously found to cocrystallize with uric acid (3, 4, 7, 9 (CCDC deposition numbers: 1841864-1841867), a second phase of 4, and 10). Potential coformer donor groups included S-H, N-H and O-H, however all successful coformers bonded through N-H; acceptor atoms were always nitrogen. Three of the six cocrystals included tridentate binding where three displayed a bidentate bond as its highest order ligand. Conformational flexibility of the coformer molecules varied, though only rigid coformers were successful. For hydroxyl-containing species, the ability to tautomerize in solution was originally not considered, but later became apparent (see Chapter 4). The six successful uric acid:coformer molecules are described in detail below.
3.2 Experimental

Uric Acid (99+%), 2-aminopyridine (2-AP, ≥99%), 2,4-diaminopyrimidine (24-DAP, 98%), 2,4,6-triaminopyrimidine (246-TAP, 97%), 2,6-diaminopyridine (26-DAP, 98%), trimethoprim (TMP, ≥98%), 2-amino-5,6-dimethyl-4-hydroxypyrimidine (ADP, 96%), melamine (99%), trimetoprim (TAT, ≥99%), 2-mercaptopyridine (2-MP, 99%), 2-hydroxypyridine (2-HP, 97%), biuret (98%), and 2-amino-4,6-dihydroxypyrimidine (AHP, 98%) were purchased from Sigma-Aldrich and used as received. Deionized water was filtered through a Barnstead deionizing cartridge and distilled prior to use. Coformers were ground with uric acid using a mortar and pestle.
in either 1:1 or 2:1 molar ratios and dissolved in boiling water. Crystals formed either from slow evaporation at ambient conditions or in sealed vials at elevated temperatures.

Model urine was made from an established recipe (pH=6.2)\(^{179}\) and used the following ingredients. Sodium sulfate anhydrous (Na\(_2\)SO\(_4\), 99%), magnesium sulfate heptahydrate (MgSO\(_4\)·7H\(_2\)O, 98%), potassium chloride (KCl, 99%), hydrochloric acid (HCl, 36.5%) and sodium hydroxide (NaOH, 97%) were purchased from EMD Millipore. Ammonium chloride (NH\(_4\)Cl, 99.5+%), sodium phosphate monobasic monohydrate (NaH\(_2\)PO\(_4\)·1H\(_2\)O, \(\geq99.0\)%)) and sodium phosphate dibasic (Na\(_2\)HPO\(_4\), \(\geq99\)%)) were purchased from Sigma-Aldrich. Sodium chloride (NaCl, 100.5%) was purchased from Fisher Scientific. All compounds were used as received.

3.2.1 Growth and Morphology of Uric Acid Cocrystals

3.2.1.1 2-Aminopyridine (2-AP)

A 1:1 molar ratio of uric acid:2-AP was prepared, and 15 mg were dissolved in 10 mL of boiling water. Vials were maintained at room temperature and the solvent was allowed to slowly evaporate. After several weeks, colorless blade-like crystals appeared in the dry vials. X-ray diffraction showed the crystals correspond to U\(^{\cdot}(3)^+\)·2H\(_2\)O. Typical dimensions of the cocrystal were 0.42 x 0.31 x 0.08 mm (Figure 3.4).

![Figure 3.4. Optical micrograph of U\(^{\cdot}(3)^+\)·2H\(_2\)O cocrystal in air. Scale bar = 100 \(\mu\)m.](image)
3.2.1.2 2,4-Diaminopyrimidine (24-DAP)

A 1:1 molar ratio of uric acid:24-DAP was prepared and 11 mg were dissolved in 10 mL of boiling water. Vials were sealed and placed in a 35°C water bath. Colorless needle-like crystals formed after about a week, which x-ray diffraction showed to be U⁺(7)⁺·3H₂O. Typical dimensions of the cocrystal were 0.40 x 0.20 x 0.05 mm (Figure 3.5).

![Figure 3.5. Optical micrograph of U⁺(7)⁺·3H₂O cocrystal in air. Scale bar = 100 µm.](image)

3.2.1.3 2,4,6-Triaminopyrimidine (246-TAP)

A 1:1 molar ratio of uric acid:246-TAP was prepared and 11 mg were dissolved in 10 mL of boiling water. Vials were sealed and placed in a 35°C water bath. Needle-like yellow crystals of U⁺(9)⁺·4H₂O formed after about two weeks. Typical dimensions of the cocrystal were 0.40 x 0.10 x 0.005 mm (Figure 3.6).

![Figure 3.6. Optical micrograph of U⁺(9)⁺·4H₂O cocrystal in air. Scale bar = 100 µm.](image)
3.2.1.4 2,6-Diaminopyridine (26-DAP)

A 1:2 molar ratio of uric acid:26-DAP was prepared and 30 mg were dissolved in 10 mL of boiling water. Vials were refrigerated for a week and then moved to room temperature. Slow evaporation over several weeks yielded brown block-like crystals of \( \text{U}^-:(4)^+ \cdot 1.5\text{H}_2\text{O} \). Typical dimensions of the cocrystal were 0.50 x 0.40 x 0.20 mm (Figure 3.7).

![Figure 3.7. Optical micrograph of \( \text{U}^-:(4)^+ \cdot 1.5\text{H}_2\text{O} \) cocrystal in air. Scale bar = 100 µm.](image1)

A 1:1 molar ratio of uric acid:26-DAP was prepared and 11.6 mg were dissolved in 10 mL of boiling water. Slow evaporation over several weeks yielded colorless blade-like crystals of \( \text{U}^-:(4)^+ \cdot 1.5\text{H}_2\text{O} \). Typical dimensions of the cocrystal were 0.22 x 0.19 x 0.01 mm (Figure 3.8).

![Figure 3.8. Optical micrograph of \( \text{U}^-:(4)^+ \cdot 1.5\text{H}_2\text{O} \) (II) cocrystal in air. Scale bar = 100 µm.](image2)
3.2.1.5 Trimethoprim (TMP)

A 1:1 molar ratio of uric acid:TMP was prepared and 5 mg were dissolved in 10 mL of boiling water. Vials were maintained at room temperature; slow evaporation led to the formation of colorless blade-like crystals of $\text{U}^{-}\cdot(10)^{+}\cdot\text{H}_2\text{O}$ after ten days. Typical dimensions of the cocrystal were $0.08 \times 0.1 \times 0.01$ mm (Figure 3.9).

![Figure 3.9. Optical micrograph of $\text{U}^{-}\cdot(10)^{+}\cdot\text{H}_2\text{O}$ cocrystal in air. Scale bar = 100 µm.](image)

3.2.2 X-ray Diffraction Characterization

3.2.2.1 Powder X-ray Diffraction

PXRD was performed on a Rigaku Ultima IV X-ray diffractometer with Cu Kα radiation ($\lambda=1.5418$ Å) at a tube voltage of 40 kV and a current of 30 mA. Data was collected over a 2θ range of 5–40° at a scan speed of 2.0°/min and analyzed with Jade v9.0 software. Cocrystal samples were ground and data was compared to PXRD of each molecular component. Simulated data from CIF files of reported crystal structures include UAD (refcode: ZZZPPI02), 2-AP (AMPYRD), 24-DAP (ESOYUI), 246-TAP (BEWHAM), 26-DAP (FOYLEK), and two TMP polymorphs (AMXBPM10 and AMXBPM13). Powder patterns were also simulated from the CIF files of the six new cocrystals and compared to experimental patterns (Figure 3.10) to assess phase purity.
3.2.2.2 Single Crystal X-ray Diffraction

Single crystal XRD data for $\text{U}^+ \cdot 2\text{H}_2\text{O}$, $\text{U}^+ \cdot 3\text{H}_2\text{O}$ and $\text{U}^+ \cdot 1.5\text{H}_2\text{O}$ were collected by Elizabeth Koch and data for $\text{U}^+ \cdot 1.5\text{H}_2\text{O}$ (II) was collected by Taylor Watts on a Siemens SMART 1000 CCD platform diffractometer. A full Ewald sphere was scanned using an APEX II CCD detector (Bruker-AXS). Graphite monochromated Mo Kα radiation was applied at 100K ($\lambda = 0.71073$). The structures were solved using SHELXS-Version 2013/1 and refined using SHELXL-2014/7 by Jeffrey Bertke.

The structures of $\text{U}^+ \cdot 2\text{H}_2\text{O}$, $\text{U}^+ \cdot 3\text{H}_2\text{O}$, $\text{U}^+ \cdot 1.5\text{H}_2\text{O}$, and $\text{U}^+ \cdot 1.5\text{H}_2\text{O}$ (II) were solved by direct methods and refined using full-matrix least-squares on $F^2$. Non-hydrogen atoms were modeled anisotropically while hydrogen atoms were treated by a mixture of
independent and constrained refinement parameters. The oxygen atom of the half occupied water molecule in U\textsuperscript{\textdegree}:(4)\textsuperscript{\textdegree}\cdot1.5H\textsubscript{2}O was disordered over a symmetry site (O5/O5B) and the hydrogen atoms were not able to be located in the difference map. In the U\textsuperscript{\textdegree}:(4)\textsuperscript{\textdegree}\cdot1.5H\textsubscript{2}O (II) structure, all hydrogen atoms were calculated. The hydrogen atoms on water molecules could not all be found and were therefore left out of the model. Water molecules O8-O11 are each half occupied.

Data for U\textsuperscript{\textdegree}:(10)\textsuperscript{\textdegree}\cdotH\textsubscript{2}O was collected on a Siemens SMART 1000 CCD platform diffractometer (full Ewald sphere) using an APEX II CCD detector (Bruker-AXS). Graphite monochromated Mo K\textalpha radiation was applied at 100K (\(\lambda = 0.71073\)). The structure was solved using SHELXS-Version 2013/1 and refined using SHELXL-2014/7. It was phased by direct methods and converged using full-matrix least-squares refinement on F\textsuperscript{2}. Due to the small crystal size and apparent polycrystallinity of the sample, both hydrogen and non-hydrogen atoms were modeled by a mixture of independent and constrained refinement parameters in order to limit non-positive definite atoms. Although the structure solves in the P-1 space group, it is likely that higher quality diffraction data might allow refinement in a higher symmetry space group as multiple molecular units are observed in the asymmetric unit.

Single crystal synchrotron data for U\textsuperscript{\textdegree}:(9)\textsuperscript{\textdegree}\cdot4H\textsubscript{2}O was collected at 173K at ChemMatCARS Sector 15 at the Advanced Photon Source. A Bruker platform three-circle goniometer with fixed chi diffractometer was used with a CCD area detector (\(\lambda = 0.518\)). Structure refinement help was provided by Michael Ruf (Bruker). Absorption data was processed with TWINABS V2012/1; structure solution and refinement were processed with SHELX-2014. The cocrystal was refined as a 2-component twin. It was phased by direct methods and successfully converged using full-matrix least-squares refinement on F\textsuperscript{2}. Non-hydrogen atoms were modeled anisotropically while
hydrogen atoms were treated by a mixture of independent and constrained refinement parameters. Crystallographic data appears in Table 3.1.

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<th>Table 3.1. Crystallographic data for uric acid cocrystals.</th>
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3.2.3 Thermal Characterization

Differential Scanning Calorimetry (DSC) and Thermogravimetric Analysis (TGA) were used to determine the % water content, dehydration temperature and melting points of the six cocrystals, when possible. A TA Instruments SDT_Q600 simultaneous TGA-DSC analyzer (New Castle, DE) was used for cocrystals of 3, 4, 7 and 9. A TA Instruments TGA_Q50 was used for cocrystals of 4 (II) and 10, and a 2920 Modulated DSC (New Castle, DE) was used for the cocrystal.
of 10. In all cases, 1-2 mg samples were sealed in aluminum pans (TA Instruments) and heated to 400°C at a ramp rate of 10 °C/min.

3.2.4 Solubility Measurements

The solubility of uric acid cocrystals, UA and MSU were measured using a Uric Acid Assay Kit (Cayman Chemical Company, Ann Arbor, MI), and a fluorescence detection method. Each well of a 96-well plate was filled with 105 µL diluted assay buffer, 15 µL fluorometric detector, 15 µL sample and 15 µL enzyme mixture. Crystal samples were allowed to dissolve in model urine for a specific time before being filtered and analyzed. After a 15 minute incubation of the plate at room temperature, a Spectra GeminiEM plate reader (Molecular Devices, Sunnyvale, CA) in the Roepe Lab was used to measure the fluorescence at an excitation wavelength of 530 nm and an emission wavelength of 590 nm. A standard calibration curve for uric acid first was generated from eight uric acid solutions with known concentrations ranging from 0 to 10 µM where the control sample was model urine without uric acid. These data values were collected prior to all cocrystal sample measurements. Samples were diluted 1:50 with assay buffer and measured in triplicate.

The three values for each sample were averaged and the error was found using equation 3.1.

\[
Error = \frac{std\text{e}a(3 \text{ values}}}{\sqrt{3}} \quad \text{Equation 3.1}
\]

Stdeva is the standard deviation function in Excel 2013. The control sample value was subtracted from all other values to obtain the corrected fluorescence (CF), and those errors were found from propagation of error.

To generate the standard curve, the eight standard solutions were plotted in Excel 2013. Errors were weighted using equation 3.2 where \(S_y\) is the error in the CF values.
Equation 3.2

\[
Error = \frac{1}{s_y^2}
\]

A software called Macrobundle12 (©Robert de Levi) was downloaded to Excel 2013 to calculate the weighted values of the y-intercept and slope of the standard curve.

Calculations of the uric acid concentration in each sample were determined as described in the included assay booklet, shown in equation 3.3.

\[
[UA] = \frac{CF-(y-int)}{slope} \times \text{dilution factor}
\]

Equation 3.3

The concentration was in µM and the dilution factor was 500. The error of these samples was calculated using propagation of error. Final concentration values for each sample with error bars indicated are shown in Figure 3.29.

3.3 Cocrystal Structures

All six cocrystal molecules were first identified by the presence of new diffraction peaks in PXRD (Figure 3.10). Thermal characterization (DSC and TGA) were also used to verify hydrates, as well as new melting/decomposition points when possible. Single crystal x-ray diffraction was used to unambiguously determine structures for \(U^\cdot(3)^+\cdot2H_2O\), \(U^\cdot(4)^+\cdot1.5H_2O\), \(U^\cdot(4)^+\cdot1.5H_2O\) (II), \(U^\cdot(7)^+\cdot3H_2O\), \(U^\cdot(9)^+\cdot4H_2O\), and \(U^\cdot(10)^+\cdotH_2O\). It is important to note that all five of these coformers are weak bases. This characteristic results in the presence of urate in the cocrystal complex instead of neutral uric acid, as well as a protonated coformer molecule to complete the charge balance.

3.3.1 Urate:2-aminopyridin-1-ium Dihydrate (\(U^\cdot(3)^+\cdot2H_2O\))

Blade-like cocrystals of urate and (3) (\(pK_b = -13.7\) in \(Me_2SO\)) were first identified by PXRD (Figure 3.10), which showed new diffraction lines at \(2\theta = 8.5, 12.4, 14.0, \) and 27.5. An 8.8% weight loss was observed by TGA (Figure 3.11) before 150°C, which is slightly less than
the 12.1% theoretical weight loss of two water molecules. An additional weight loss of 15.4% occurs between 150-250°C, which may be due to additional water loss and/or evaporation of 2-AP. No clear melting transition was observed.

Single crystal x-ray diffraction revealed a triclinic P-1 space group \((Z = 2)\) with cell parameters \(a = 6.892(7)\,\text{Å}, b = 9.076(2)\,\text{Å}, c = 11.057(3)\,\text{Å}, \alpha = 78.648(3)^\circ, \beta = 73.477(3)^\circ,\) and \(\gamma = 74.583(3)^\circ\). The asymmetric unit consists of one 2-AP cation, one \(U\) anion, and two water molecules. Face-to-face stacking of ions separated 3.45Å apart is apparent when viewed down the \(a\)-axis. H-bonding ions form nearly planar sheets in the \((212)\) plane (Figure 3.12).
Each urate ion is hydrogen bonded to two other urates, two 2-AP ions and four water molecules (Figure 3.13). Bidentate bonding to other urate molecules form $R_2^2(8)$ and $R_2^2(10)$ motifs.

Figure 3.12. Short $\pi-\pi$ stacking interactions form (212) sheets in the U$^\cdot$:(3)$^+ \cdot 2\text{H}_2\text{O}$ cocrystal.

Figure 3.13. H-bonding geometry around urate in U$^\cdot$:(3)$^+ \cdot 2\text{H}_2\text{O}$. 

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as described by graph set notation. The first between O2…N1-H (1.926Å) and the second between O6…N7-H (1.927Å). Both interactions are symmetrical about an inversion center. Urate H-bonds to 2-AP ions between O2…H-N (1.82Å) and N3…H-N (1.937Å) to form a bidentate $R_2^2(8)$ motif, and to the second 2-AP with a single H-bond between O8…H-N (2.08Å). H-bonds between urate and the two water molecules occur through O6, O8 and N-H9 (2Å, 1.98Å, 2.05Å and 1.893Å). All bond distances and angles can be found in Table 3.2. The N3-O2 bidentate geometry observed between the two charged components agrees well with predicted binding geometries for urate and monovalent metal ion coordination. The N1-H…O2 and N7-H…O6 urate-urate interactions are also seen in monosodium urate.¹⁸⁷

Table 3.2. H-bond geometry distances (Å) and angles (°) in U⁻(3)⁺·2H₂O.

<table>
<thead>
<tr>
<th>D—H⋯A</th>
<th>D—H</th>
<th>H⋯A</th>
<th>D⋯A</th>
<th>D—H⋯A</th>
</tr>
</thead>
<tbody>
<tr>
<td>N₁₁—I₁₁⋯O₂¹</td>
<td>0.856 (19)</td>
<td>1.926 (19)</td>
<td>2.7795 (15)</td>
<td>174.7 (17)</td>
</tr>
<tr>
<td>N₂₂—I₂₂⋯O₆²²</td>
<td>0.885 (19)</td>
<td>1.927 (19)</td>
<td>2.7851 (15)</td>
<td>163 (16)</td>
</tr>
<tr>
<td>N₉—I₉⋯O₉₂⁻²²</td>
<td>0.91 (19)</td>
<td>1.893 (19)</td>
<td>2.7957 (16)</td>
<td>170.8 (16)</td>
</tr>
<tr>
<td>O₉—I₉⋯O₈₂⁻²⁴</td>
<td>0.88 (2)</td>
<td>1.98 (2)</td>
<td>2.8458 (15)</td>
<td>168.6 (19)</td>
</tr>
<tr>
<td>O₉—I₉⋯O₈₂⁻²⁴</td>
<td>0.89 (2)</td>
<td>1.82 (2)</td>
<td>2.7003 (16)</td>
<td>169.5 (18)</td>
</tr>
<tr>
<td>N₁₁—I₁₁⋯N₃⁻³</td>
<td>0.94 (19)</td>
<td>1.937 (19)</td>
<td>2.8763 (16)</td>
<td>176.5 (16)</td>
</tr>
<tr>
<td>O₈—I₈⋯O₈₂⁻¹⁴</td>
<td>0.92 (2)</td>
<td>2.05 (2)</td>
<td>2.8995 (15)</td>
<td>153.1 (17)</td>
</tr>
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<td>O₈—I₈⋯O₈₂⁻¹⁴</td>
<td>0.89 (2)</td>
<td>2 (2)</td>
<td>2.8545 (16)</td>
<td>162.5 (18)</td>
</tr>
<tr>
<td>N₂₁—I₂₁⋯O₈⁻¹⁴</td>
<td>0.89 (2)</td>
<td>2.08 (2)</td>
<td>2.961 (16)</td>
<td>169.4 (17)</td>
</tr>
<tr>
<td>N₁—I₁⋯O₂²⁻³</td>
<td>0.99 (2)</td>
<td>1.82 (2)</td>
<td>2.8024 (16)</td>
<td>176.1 (17)</td>
</tr>
</tbody>
</table>

Symmetry codes: (i) -x+1, -y+2, -z+2; (ii) -x+2, -y+2, -z+1; (iii) x+1, y, z; (iv) x-1, y-1, z; (v) x, y, z-1; (vi) x-1, y, z; (vii) x, y+1, z.
3.3.2 Urতe:2,4-diaminopyrimidin-1-ium Trihydrate (U:(7)+·3H₂O)

Colorless needles of urate and (7) (pK₆ = 6.74) were identified by new diffraction lines observed in PXRD (Figure 3.10) at 2θ = 7.5, 13.1 and 16.1. TGA displayed a 15.2% weight loss before 180°C which matched closely with the theoretical weight loss of three water molecules (16.2%). The DSC spectrum showed three small endothermic transitions < 180°C which likely corresponded to water loss, and a large endothermic peak at 318°C which we assume to be melting and/or decomposition (Figure 3.14).

The single crystal x-ray structure fit to a monoclinic P2₁/c space group (Z=4) with cell parameters \( a = 7.136(0)\text{Å} \), \( b = 23.598(6)\text{Å} \), \( c = 8.650(2)\text{Å} \), and \( \beta = 106.602(4)^\circ \). The asymmetric unit contains one 24-DAP cation, one U anion, and three water molecules. A view down the \( c \)-axis reveals the corrugated sheets in the (100) plane. Molecular ions in adjacent layers are separated by 3.568Å (Figure 3.15).
Each urate (Figure 3.16) is coordinated to two 24-DAP molecules, two other urates and three water molecules. One of the 24-DAP molecules coordinates through a tridentate bonding motif with O2…H-N (1.96Å), N1-H…N (2.04Å) and O6…H-N (1.98Å). The second 24-DAP ion

![Figure 3.15. Corrugated sheets in the bc plane π–π stack along the a-axis in the U:(7)$^+$·3H$_2$O cocrystal.](image)

![Figure 3.16. H-bonding geometry around urate in U:(7)$^+$·3H$_2$O.](image)
coordinates through a bidentate bonding motif with O2…H-N (1.933 Å) and N3…H-N (1.92 Å). Single H-bonds to other urate ions also form between O8…H-N7 and N7-H…O8 (each bond is 2.19 Å). Five water molecules are H-bonded to each urate, one at N9-H (1.86 Å), two at O8 (1.89 Å and 1.91 Å), and two at O6 (1.97 Å and 1.94 Å). Bond distances and angles can be found in Table 3.3. Infinite 1D chains of H-bonded waters run parallel to the c-axis with H…O distances ranging from 1.752 to 1.767 Å. This was the first cocrystal to display tridentate binding between components which importantly provided confirmation that this geometry could be achieved.

3.3.3 Urate:2,4,6-triaminopyrimidin-1-ium Tetrahydrate (U⁻:(9)⁺·4H₂O)

Yellow needles of the urate and (9) (pKₐ = 7.3)¹⁸⁹ cocrystal showed new diffraction lines from PXRD (Figure 3.10) at 2θ = 5.7, 7.1, and 25.8. DSC showed the cocrystal to melt at 293°C. TGA displayed a 27% weight loss below 200°C where 15% was lost after 150°C. Two small
endothermic peaks are visible from DSC at 154°C and 196°C which correspond to these step transitions. With a theoretical weight loss of 37%, these are attributed to the loss of three water molecules, where the remaining water was most likely lost upon melting/decomposition (Figure 3.17).

Due to the extremely thin nature of the needles, the single crystal XRD structure was solved from synchrotron diffraction data collected at the Advanced Photon Source Sector 15 ChemMatCARS. Structure refinement was aided considerably by Mike Ruf (Bruker), and ultimately led to a monoclinic $P2_1/c$ space group ($Z=4$) with cell parameters $a = 3.609(2)\text{Å}$, $b = 30.989(3)\text{Å}$, $c = 13.511(2)\text{Å}$, and $\gamma = 91.418(3)^\circ$. The asymmetric unit contains one 246-TAP cation, one U anion and four water molecules. Short $\pi-\pi$ stacking distances of 3.609Å can be viewed down the $a$-axis. Planar (102) sheets formed from hydrogen bonding between the ions and water molecules (Figure 3.18).

**Figure 3.17.** DSC(red)/TGA(black) of thermal transitions in U :$(9)^{+}\cdot4\text{H}_2\text{O}$ cocrystal.
The H-bonding surrounding each urate (Figure 3.19) includes coordination to two 246-TAP molecules via tridentate bonding through O2…H-N (2.05Å), N1-H…N (2.05Å) and O6…H-N (2.07Å) and bidentate bonding through N3…H-N (2.19Å) and O2…H-N (1.94Å), which is similar to the 24-DAP cocrystal. An $R_2^2(8)$ motif forms from urate to a neighboring urate through

Figure 3.18. Short π–π stacking interactions between (102) sheets in the U:(9)$^+$·4H$_2$O cocrystal.

Figure 3.19. H-bonding geometry around urate in U:(9)$^+$·4H$_2$O.
a part of N9-H…O bonds (1.99Å). Four water molecules bind to urate at N7-H (2.02Å), two at O6 (1.97Å and 1.87Å) and at O8 (1.96Å). Bond distances and angles can be found in Table 3.4.

Table 3.4. H-bond geometry distances (Å) and angles (°) in U⁺:(9)⁺·4H₂O.

<table>
<thead>
<tr>
<th>D—H···A</th>
<th>D—H</th>
<th>H···A</th>
<th>D····A</th>
<th>D—H···A</th>
</tr>
</thead>
<tbody>
<tr>
<td>N1—H1···NCl⁻</td>
<td>0.91(4)</td>
<td>2.05(5)</td>
<td>2.955(5)</td>
<td>176(4)</td>
</tr>
<tr>
<td>N7—H7···Ow2</td>
<td>0.83(5)</td>
<td>2.02(5)</td>
<td>2.842(5)</td>
<td>173(4)</td>
</tr>
<tr>
<td>N9—H9···O2⁻</td>
<td>0.81(5)</td>
<td>1.95(5)</td>
<td>2.799(4)</td>
<td>171(5)</td>
</tr>
<tr>
<td>N2⁺—H2⁺···O2⁻</td>
<td>0.81(5)</td>
<td>1.94(5)</td>
<td>2.727(4)</td>
<td>164(4)</td>
</tr>
<tr>
<td>N2⁺—H2⁺···O2⁻</td>
<td>0.93(5)</td>
<td>2.36(5)</td>
<td>3.09(1)(5)</td>
<td>136(4)</td>
</tr>
<tr>
<td>NCl⁺—HCl⁺···O6⁻</td>
<td>0.82(5)</td>
<td>2.07(5)</td>
<td>2.87(1)(5)</td>
<td>166(5)</td>
</tr>
<tr>
<td>NCl⁺—HCl⁺···N3⁻</td>
<td>0.85(5)</td>
<td>2.19(5)</td>
<td>3.03(4)(5)</td>
<td>171(4)</td>
</tr>
<tr>
<td>N—H—Ow3</td>
<td>0.93(5)</td>
<td>2.09(5)</td>
<td>2.99(8)(5)</td>
<td>167(4)</td>
</tr>
<tr>
<td>NCl⁺—HCl⁺···O2⁻</td>
<td>0.88(7)(19)</td>
<td>2.05(2)</td>
<td>2.93(6)(4)</td>
<td>179(5)</td>
</tr>
<tr>
<td>N—H—Ow4⁺</td>
<td>0.82(5)</td>
<td>2.18(5)</td>
<td>3.00(2)(5)</td>
<td>176(5)</td>
</tr>
<tr>
<td>Ow1—Hw1···O6⁻</td>
<td>0.88(4)(19)</td>
<td>1.97(3)</td>
<td>2.82(4)(4)</td>
<td>163(6)</td>
</tr>
<tr>
<td>Ow1—Hw1···O6</td>
<td>0.88(6)(19)</td>
<td>1.87(3)</td>
<td>2.72(5)(5)</td>
<td>159(6)</td>
</tr>
<tr>
<td>Ow2—Hw2···Ow4⁺</td>
<td>0.87(2)</td>
<td>2.06(5)</td>
<td>2.66(8)(9)</td>
<td>126(6)</td>
</tr>
<tr>
<td>Ow3—Hw3···O8⁻</td>
<td>0.89(4)(19)</td>
<td>1.96(2)</td>
<td>2.84(5)(4)</td>
<td>171(5)</td>
</tr>
<tr>
<td>Ow3—Hw3···Ow2⁺</td>
<td>0.87(6)(19)</td>
<td>2.06(3)</td>
<td>2.78(9)(5)</td>
<td>140(4)</td>
</tr>
<tr>
<td>Ow4—Hw4···Ow3</td>
<td>0.89(2)</td>
<td>2.14(8)</td>
<td>2.79(6)(6)</td>
<td>130(9)</td>
</tr>
<tr>
<td>Ow4—Hw4···Ow1⁺</td>
<td>0.90(2)</td>
<td>1.78(3)</td>
<td>2.66(5)(6)</td>
<td>170(11)</td>
</tr>
</tbody>
</table>

Symmetry codes: (i) x, −y+3/2, z−1/2; (ii) −x+1, −y+1, −z+1; (iii) x+1, y, z; (iv) x, −y+3/2, z+1/2; (v) x, y, z+1; (vi) −x+1, y, z; (vii) x, y, z−1; (viii) −x+2, −y+1, −z+1.

3.3.4 Urate:2,6-diaminopyridin-1-ium Sesquihydrate (U⁺:(4)⁺·1.5H₂O)

Cocrystals of urate and (4) (pKb = -0.56 in acetonitrile)¹⁹⁰ formed brown blocks that showed new diffraction lines in PXRD (Figure 3.10) at 2θ = 7.5, 11.7, and 18.2. TGA showed a 7.6% weight loss below 200°C which is attributed to loss of 1.5 water molecules (theoretical weight loss = 8.8%). DSC showed endothermic peaks at 118°C and 239°C where the former is
attributed to the water loss and the later melting and/or decomposition of the cocrystal (Figure 3.20).

![DSC and TGA plot]

Figure 3.20. DSC(red)/TGA(black) of thermal transitions in U^+:(4)^−·1.5H_2O cocrystal.

Single crystal XRD of the cocrystal fit to an orthorhombic space group Fdd2 (Z = 16) with cell parameters \(a = 15.5627(15)\text{Å}, b = 22.236(2)\text{Å}, \text{and} c = 30.257(3)\text{Å}\). The asymmetric unit consists of one 26-DAP cation, one U anion and one and a half water molecules where the half occupied water molecule is disordered over a symmetry site. Figure 3.21 shows mixed π stacks made from alternating urate and (4) molecules within the (001) plane which have an average face-face separation of 3.39 Å.
Urate hydrogen bonds (Figure 3.22) to two 26-DAP ions through O6…H-N (2.21Å, 2.08Å). A bidentate bond forms through N3…H-N (1.976Å) and O2…H-N (1.865Å), which forms an $R_2^2(8)$ motif. Urate-urate hydrogen bonding coordination occurred through bidentate bonds at N1-H…O (1.92Å), O2…H-N (1.92Å) and N7-H…O (1.983Å), O8…H-N (1.983Å). Four water

Figure 3.22. H-bonding geometry around urate in $U^\cdot(4)^+\cdot1.5H_2O$. 
molecules are also hydrogen bonded to urate at O6 (1.852Å), two at O8 (2.05Å, H is missing in structure) and at N9-H (1.932Å). Bond distances and angles are found in Table 3.5.

Table 3.5. H-bond geometry distances (Å) and angles (°) in U⁻ : (4)⁺ · 1.5H₂O.

<table>
<thead>
<tr>
<th>D—H···A</th>
<th>D—H</th>
<th>H···A</th>
<th>D···A</th>
<th>D—H···A</th>
</tr>
</thead>
<tbody>
<tr>
<td>N₃—H₃···O₂</td>
<td>0.898 (17)</td>
<td>1.865 (17)</td>
<td>2.76 (2)</td>
<td>174 (2)</td>
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<tr>
<td>N₃—H₃···N₃</td>
<td>0.902 (17)</td>
<td>1.976 (18)</td>
<td>2.878 (2)</td>
<td>178 (3)</td>
</tr>
<tr>
<td>N₃—H₃···O₆⁺</td>
<td>0.872 (17)</td>
<td>2.21 (2)</td>
<td>3.025 (2)</td>
<td>156 (3)</td>
</tr>
<tr>
<td>N₄—H₄···O₆⁺</td>
<td>0.887 (17)</td>
<td>2.08 (2)</td>
<td>2.887 (2)</td>
<td>151 (3)</td>
</tr>
<tr>
<td>N—H···O₆</td>
<td>0.874 (17)</td>
<td>2 (2)</td>
<td>2.829 (12)</td>
<td>158 (3)</td>
</tr>
<tr>
<td>N—H···O₆</td>
<td>0.874 (17)</td>
<td>2.22 (2)</td>
<td>3.013 (7)</td>
<td>151 (3)</td>
</tr>
<tr>
<td>N—H···O₆</td>
<td>0.874 (17)</td>
<td>2.36 (2)</td>
<td>3.179 (8)</td>
<td>156 (3)</td>
</tr>
<tr>
<td>N—H···O₆⁺</td>
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<td>1.92 (17)</td>
<td>2.812 (2)</td>
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<tr>
<td>N₂—H₂···O₈⁻</td>
<td>0.891 (17)</td>
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<td>N₉—H₉···O₁⁻</td>
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<td>O₆—H₆···O₆</td>
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<td>1.852 (19)</td>
<td>2.723 (2)</td>
<td>173 (3)</td>
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<tr>
<td>O₁⁻—H₁⁻···O₈⁻</td>
<td>0.888 (18)</td>
<td>2.05 (2)</td>
<td>2.885 (2)</td>
<td>157 (3)</td>
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</table>

Symmetry codes: (i) x+1/4, -y+1, z+1/4; (ii) -x+5/4, y, -z+5/4; (iii) x, -y+5/4, -z+5/4; (iv) -x+1/2, -y+1, -z+3/2.

A second cocrystal of urate and (4) formed colorless blades with new diffraction lines in PXRD (Figure 3.10) at 2θ = 5.18 and 10.56. DSC showed endothermic peaks at 81.14°C, 116.66°C and 247.02°C where the first may be due to a mixture of UAD in the sample, the second to water loss, and the third to melting/decomposition of the cocrystal (Figure 3.23).
Single crystal XRD of the cocrystal again fit to an orthorhombic space group P2\(_1\)2\(_1\)2\(_1\) (Z=4) with cell parameters \(a = 12.4511(14)\)Å, \(b = 32.796(4)\)Å, \(c = 6.7835(7)\)Å. The asymmetric unit consists of two 26-DAP cations, two U anions and five water molecules where four of the water molecules are half occupied. Figure 3.24 shows the pairs of π stacks made between urate and (4) stacked along the (100) plane with centroid…centroid distances as small as 3.385Å.

The H-bonding surrounding each urate (Figure 3.25) includes three bidentate bonds where one is to 26-DAP through O2…H-N (1.85Å) and N3…H-N (2.07 Å), and the other two to urate
molecules through N1-H…O (2.06 Å), O6…H-N (1.94 Å) and N7-H…O (2.08 Å), O8…H-N (1.99 Å). Four single H-bonds to water molecules occur at O6, O2, O8, and N9-H. Bond distances and angles are found in Table 3.6.
Table 3.6. H-bond geometry distances (Å) and angles (°) in U⁻·(4)⁺·1.5H₂O (II).

<table>
<thead>
<tr>
<th>D—H···A</th>
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<th>H···A</th>
<th>D···A</th>
<th>D—H···A</th>
</tr>
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<td>N₁₂—H₁₂···O₈’</td>
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<td>1.99</td>
<td>2.790(12)</td>
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<td>0.88</td>
<td>1.95</td>
<td>2.828(12)</td>
<td>178.6</td>
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<td>N₁₁—H₁₁···O₆</td>
<td>0.88</td>
<td>1.94</td>
<td>2.773(12)</td>
<td>157.3</td>
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<td>N⁷—H⁷···O₆’”</td>
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<td>2.08</td>
<td>2.887(11)</td>
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<td>N₁—H₁···O₁’</td>
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<td>2.06</td>
<td>2.913(12)</td>
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<td>N⁹—H⁹···O₆’’’</td>
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<td>1.86</td>
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<td>1.81</td>
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<td>N—H···O₁’’</td>
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<td>2.59</td>
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<td>N—H···O₁”</td>
<td>0.88</td>
<td>2.49</td>
<td>3.26(2)</td>
<td>146.2</td>
</tr>
<tr>
<td>N—H···O’’’</td>
<td>0.88</td>
<td>2.31</td>
<td>3.17(2)</td>
<td>164.9</td>
</tr>
<tr>
<td>N—H···N₁’</td>
<td>0.88</td>
<td>2.05</td>
<td>2.927(14)</td>
<td>178.3</td>
</tr>
<tr>
<td>N—H···O₁’’’</td>
<td>0.88</td>
<td>2.08</td>
<td>2.933(13)</td>
<td>164.4</td>
</tr>
<tr>
<td>N₁’—H₁’···O₂</td>
<td>0.88</td>
<td>1.85</td>
<td>2.698(12)</td>
<td>161.6</td>
</tr>
<tr>
<td>N₁’—H₁’···N₃’</td>
<td>0.88</td>
<td>2.07</td>
<td>2.93(14)</td>
<td>165.1</td>
</tr>
<tr>
<td>N₁’—H₁’···O’’’</td>
<td>0.88</td>
<td>2.29</td>
<td>3.11(2)</td>
<td>155.9</td>
</tr>
<tr>
<td>N₁’—H₁’···O₁’’’</td>
<td>0.88</td>
<td>2.17</td>
<td>3.028(12)</td>
<td>166.3</td>
</tr>
<tr>
<td>N₁’—H₁’···O₂</td>
<td>0.88</td>
<td>2.36</td>
<td>3.083(12)</td>
<td>140.2</td>
</tr>
</tbody>
</table>

Symmetry codes: (i) x−1/2, −y+3/2, −z+1; (ii) −x+1, −y+2, z; (iii) x+1/2, −y+3/2, −z+1; (iv) −x+1, −y+1, z; (v) x+1, y, z; (vi) −x+1/2, −y+1/2, −z+1; (vii) x−1, y, z.

3.3.5 Urate:2,4-diamino-5-(3,4,5-trimethoxybenzyl)pyrimidin-1-ium Monohydrate (U⁻·(10)⁺·H₂O)

Urate and (10) (pK₉ = 6.9)¹⁹¹ cocrystals formed as colorless blades that displayed new PXRD diffraction lines at 2θ = 6.9, 8.7, and 12.1 (Figure 3.10). TGA showed a gradual weight loss totaling 6.17% up until 200°C which is larger than the theoretical weight loss of one water molecule per U⁻·TMP⁺ unit (theoretical weight loss = 3.8%). After 200°C, a large drop in weight
is correlated to a large endothermic peak in DSC at 200°C, followed quickly by an exothermic event, and then finally decomposition around 292°C (Figure 3.26).

![DSC and TGA graph showing thermal transitions in U^+(10) \cdot H_2O cocrystal](image)

**Figure 3.26.** DSC(red)/TGA(black) of thermal transitions in U^+(10) \cdot H_2O cocrystal.

Single crystal XRD was extremely difficult due to the small size of the crystals. Slightly larger crystals resulted from solutions seeded with the small particles, however from x-ray analysis they were shown to probably be multiple crystals that grew together. A likely structure was ultimately able to be extracted, however data quality is poor. The asymmetric unit shows four TMP cations, four U anions, and four water molecules. Higher symmetry than the triclinic P-1 space group (Z=8) that resulted is unable to be found potentially due to poor data quality but presumably does exist. Cell parameters of the structure as it is currently solved are \( a = 8.590(3) \)Å, \( b = 22.132(8) \)Å, \( c = 24.994(9) \)Å, \( \alpha = 113.485(7)^\circ \), \( \beta = 90.039(8)^\circ \), and \( \gamma = 91.857(8)^\circ \). Figure 3.27 is viewed down the \( a \)-axis where short interactions are seen.
Hydrogen bonding around each urate (Figure 3.28) shows coordination to two TMP\(^+\) molecules through a tridentate bond O2…H-N, N1-H…N, O6…H-N and a bidentate bond through N3…H-N, O2…H-N, similar to the 24-DAP and 246-TAP cocrystals. An \(R_2^2(8)\) motif between neighboring urates form at N7-H…O, O8…H-N. Three water molecules are also hydrogen bonded to urate at N9-H, O6, and O8. Bond lengths may be inaccurate due to the data quality and are
therefore excluded from this description. Most hydrogen atoms observed in Figure 3.28 were calculated based on hydrogen bonding patterns that dictate the arrangement of molecules in the asymmetric unit. Although the quality of this data is lacking, the existence of this cocrystal is significant since TMP is an FDA approved drug that would not have issues in possible in vivo applications mentioned previously.

3.4 Cocrystal Solubility

Fluorescence spectroscopy was used to determine the solubility of five of the six cocrystals. Uric acid from the cocrystal was oxidized to allantoin by the uricase enzyme, which additionally produced carbon dioxide and peroxide. 10-Acetyl-3,7-dihydroxyphenoxazine (ADHP) was used as a substrate for horseradish peroxidase to detect the amount of peroxide produced in the initial reaction, which then formed the fluorescent molecule resorufin. At the appropriate excitation and emission wavelengths, resorufin can detect peroxide concentrations as small as 5 pmol per 100 µl sample. This was how the amount of uric acid in each sample solution was detected.

The solubility of the cocrystals containing (3), (4), (7), (9), and (10) were tested and compared to UA and MSU (Figure 3.29). The potential in vivo applications of these samples would cause them to be flushed from the body in a much shorter time span than what it takes to reach equilibrium (~24 hours). Therefore, 1 minute and 2 hours were chosen as the experimental time scales for these measurements. In order to ensure accuracy of these values, samples of UA and MSU were measured after equilibrating in model urine solution for 24 hours and compared to literature values. The concentrations measured for these samples (993±108µM and 436±80µM, respectively) matched very closely to reported literature values, indicating that the values obtained for the cocrystal samples determined using the same methods should be equally accurate.
Uric acid concentrations of the 2-AP, 26-DAP, 24-DAP, 246-TAP and TMP cocrystals after 1 minute were 1245±167µM, 8±76µM, 815±104µM, 643±112µM, and 189±69µM, respectively. Measurements of cocrystals (3), (4), (7), (9) and (10) after 2 hours were 1470±139µM, -23±87µM, 709±159µM, 251±81µM, and 595±76µM, respectively. All values can be found in Table 3.7. In both cases, the most soluble cocrystal contains the most basic coformer that interestingly took the longest amount of time to precipitate from solution (2-AP, pK$_b$ = -13.7 in DMSO, >30 days of growth).

Compared to the concentrations in the MSU samples (174±104µM after 1 minute and 220±72µM after 2 hours), four of the five cocrystals showed improved solubility. Pure UA samples (606±104µM after 1 minute and 724±96µM after 2 hours) were more soluble than the 26-DAP and TMP cocrystals, but were either equally or less soluble than the remaining three. The most notable result was the 2-AP cocrystal which showed an increase of over 100% compared to UA.

**Figure 3.29.** Bar graph displaying concentration of uric acid/urate in UA, MSU, and cocrystals containing 2-AP, 26-DAP, 24-DAP, 246-TAP, and TMP. Samples were dissolved in model urine for 1 minute (solid bars) and 2 hours (striped bars).
and over 600% compared to MSU. The reason behind this is not apparent, however some differences between this coformer compared to the others include lower melting point and solely bidentate hydrogen bonding.

### Table 3.7. Solubility measurements of cocrystals containing 2-AP, 26-DAP, 24-DAP, 246-TAP and TMP compared to MSU and UA.

<table>
<thead>
<tr>
<th></th>
<th>1 Minute (μM)</th>
<th>2 Hours (μM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>MSU</td>
<td>173.63 ± 104</td>
<td>220.19 ± 72</td>
</tr>
<tr>
<td>UA</td>
<td>605.59 ± 104</td>
<td>724.38 ± 96</td>
</tr>
<tr>
<td>2-AP</td>
<td>1244.55 ± 167</td>
<td>1470.25 ± 139</td>
</tr>
<tr>
<td>26-DAP (II)</td>
<td>8.09 ± 76</td>
<td>-23.35 ± 87</td>
</tr>
<tr>
<td>24-DAP</td>
<td>814.89 ± 104</td>
<td>709.33 ± 159</td>
</tr>
<tr>
<td>246-TAP</td>
<td>643.01 ± 112</td>
<td>250.55 ± 81</td>
</tr>
<tr>
<td>TMP</td>
<td>189.45 ± 69</td>
<td>594.60 ± 76</td>
</tr>
</tbody>
</table>

### 3.5 Conclusions

Crystallization of uric acid *in vivo* has long been known to lead to the formation of kidney stones and gout. We hope to advance current therapies for uric acid stones by finding molecules that can bind strongly to uric acid/urate and improve its solubility. Some examples that are capable of the former are guanidinium and methylene blue, which form cocrystals with urate (Refcodes: XANDEV\(^{22}\) and UGEXIN).\(^{21}\) A uric acid/melamine complex was found from infrared spectroscopy data\(^{196}\) and a host receptor was synthesized for binding purposes.\(^{197}\) Biological components such as hemoglobin\(^{198}\) and various proteins\(^{199}\) are capable of this task as well.

Six new cocrystals were synthesized, which serve to illustrate that it is possible to disrupt the strong hydrogen bonds that typically form in UA and MSU crystals. Predicted binding motifs were accurate, illustrating that hydrogen bonds readily form from uric acid to complementary
motifs on various coformer molecules. PXRD and single crystal XRD elucidated the structures of these hydrated urate cocrystals containing the protonated coformer molecules 2-AP, 24-DAP, 246-TAP, 26-DAP, and TMP. DSC and TGA indicated new melting points and verified dehydration of water molecules. While most cocrystals displayed an increase in solubility as compared to MSU and UA, the 2-AP cocrystal showed the biggest change with an increase of over 100%. While it is not obvious the exact solubility that is needed to be achieved \textit{in vivo} to avoid crystallization, factors that would need to be considered are total uric acid levels and the percent that binds to the coformer. Although none of these new cocrystals may be the exact compound needed to prevent and/or dissolve uric acid kidney stones, we were able to show that specific molecules can recognize uric acid in solution and interrupt its crystallization. Future directions of this project may include working to make the cocrystals more favorable for \textit{in vivo} applications.
Chapter 4. Crystal Growth Oddities: Part II*

4.1 Introduction

In the previous chapter, coformer molecules that successfully cocrystallized with uric acid were presented and discussed. Here, we present some of the various outcomes observed when cocrystallization failed. The most common outcome of a failed crystallization experiment was the crystallization of the less soluble component in the mixture, which was identified by PXRD. Anhydrous uric acid crystals precipitated out of solutions containing 1:1 ratios with 2-mercaptopyridine (2-MP, 1), 2-hydroxypyridine (2-HP, 2), biuret (B, 6), and 2-amino-4,6-dihydroxypyrimidine (AHP, 11) (Figure 3.3, Figure 4.1). Although there were no cases of the coformer crystallizing first from aqueous growth solution, new coformer phases were observed in two different cases: 2,6-diaminopyridine (26-DAP, 4) (Figure 4.4) and 2-amino-5,6-dimethyl-4-hydroxypyrimidine (ADP, 8) (Figure 4.7). Also, a gel formed in the presence of triamterene (TAT, 12) (Figure 4.12) and an amorphous film in the presence of melamine (M, 5) (Figure 4.10).

4.2 26-DAP

2,6-Diaminopyrimidine (26-DAP, 4) is a strong base (pKb = -0.56 in acetonitrile)\textsuperscript{190} often used in the synthesis of azo dyes, including hair colorants and textiles. From a search of the Cambridge Structural Database, over 70 cocrystal and/or solvate structures include 26-DAP as a component. It is protonated as either pyridinium\textsuperscript{+1} or pyridinediammonium\textsuperscript{+2} in all but 5 cases (Refcodes: DEDMAD,\textsuperscript{200} DEDMEH,\textsuperscript{200} IRUGIM,\textsuperscript{201} PEJQEC,\textsuperscript{202} UMIWET).\textsuperscript{203} A cocrystal of

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{figure4.1.png}
\caption{Simulated PXRD patterns of UAD, UA, and coformers when available from CIF files and experimental PXRD patterns of remaining coformers and precipitate from failed cocrystals.}
\end{figure}
26-DAP with uric acid was reported in Chapter 3.3. The 26-DAP used in the cocrystal study was purchased from Sigma-Aldrich. Though it had a listed purity of 98%, 26-DAP as received was a brown powder with a broad melting range of 117-122°C. The presence of an initially unknown component in the $\text{U}^+(4)^+\cdot 1.5\cdot \text{H}_2\text{O}$ cocrystal prompted us to reexamine its purity. The crystal structure of 26-DAP was previously determined from neutron diffraction data (Refcode: FOYLEK),\textsuperscript{204} which we now refer to as Form I. Form I crystallized in the orthorhombic space group $\text{P2}_1\text{2}_1\text{2}_1$ with cell parameters $a = 5.397(2)$, $b = 7.337(3)$, and $c = 13.597(7)$. It was described as “a black truncated triangular fragment” and must have been quite large as it was solved from single crystal neutron diffraction.\textsuperscript{204} However, the authors do not describe how the crystal was grown.

Hot stage microscopy of the brown powder revealed sublimation occurs at ~100°C. Single crystal x-ray diffraction of the colorless needles obtained by sublimation revealed them to be a polymorph of 26-DAP (Form II). Form II also resulted from recrystallization of the starting material from acetone (≥99.9%), ethyl acetate (99.5+%), and toluene (≥99.5%).

The new polymorph was solved in the orthorhombic space group by single crystal x-ray diffraction. A Bruker APEX DUO/APEXII CCD diffractometer with Mo Kα radiation was used at 100K ($\lambda = 0.71073$). The structure was determined by Jeffrey Bertke using SHELX programs. All hydrogen atoms on nitrogen atoms were found in difference maps and their distances were set to 0.88Å (esd 0.02). Displacement parameters were assigned as 1.5 times the carrier atoms. Remaining hydrogen atoms were included as riding atoms with their displacement parameters assigned as 1.2 times the carrier. Experimental details can be found in Table 4.1.
The asymmetric unit contains one 26-DAP molecule which packs in a layered structure with a herringbone motif when viewed in the \( ab \) plane (Figure 4.2). Centroid-centroid distances for face...face and edge...face interactions are 5.122(7) Å and 5.397(5) Å, respectively. Hydrogen bonds between neighboring amine groups along the \( a \)-axis are enabled by the projection of N ends.
of all heterocycles to the same side of the $ab$ plane within each herringbone layer (N2…H-N3). Bilayer stacks along the $c$-axis are created by the contact between N-rich sides of adjacent layers. These bilayers are bridged by hydrogen bonds between pyridyl and amino groups (N1…H-N3), creating a 2-dimensional network of hydrogen bonding (C(4) and $R_2^+(16)$ by graph set notation). N-H distances can be found in Table 4.2.

Figure 4.2. (a) Asymmetric unit, (b) layered packing diagram, and (c) herringbone motif in $ab$ plane of Form II. Reproduced with permission of the International Union of Crystallography.

<table>
<thead>
<tr>
<th>$D$—H—$A$</th>
<th>$D$—H</th>
<th>H—$A$</th>
<th>$D$—$A$</th>
<th>$D$—H—$A$</th>
</tr>
</thead>
<tbody>
<tr>
<td>N3—H3A⋯N1$^i$</td>
<td>0.902(18)</td>
<td>2.16(2)</td>
<td>3.054(4)</td>
<td>171(3)</td>
</tr>
<tr>
<td>N3—H3B⋯N2$^{ii}$</td>
<td>0.891(18)</td>
<td>2.29(2)</td>
<td>3.118(4)</td>
<td>155(3)</td>
</tr>
</tbody>
</table>

Symmetry codes: ($i$) $x+1/2$, $y$, $-z+1/2$; ($ii$) $-x+1/2$, $y+1/2$, $z$.

In comparison, the Form I structure has two types of hydrogen bonds, one between amino groups and one between pyridine and amino groups (N2—H⋯N3 and N1—H⋯N3, respectively) which form a 3-dimensional network ($R_6^2(20)$ by graph set notation). Compared to the hydrogen
bonding in Form II, both are slightly shorter (N2…H = 2.077 Å; N…N = 3.066 Å between amino groups and N1…H = 1.976 Å; N1…N3 = 2.982 Å between pyridine and amino groups). π-π stacking interactions reveal centroid-centroid distances as close as 4.971Å and 5.397Å for edge…face and offset face…face, respectively. Crystal Explorer software\textsuperscript{205,206} was used to generate Hirshfield surfaces for the two polymorphs of 26-DAP, which define the environment surrounding each molecule in the crystal. From these surfaces, fingerprint plots were generated for each polymorph, which summarize all intermolecular interactions (Figure 4.3). The pair of light blue spikes pointing towards the bottom left are due to N-H interactions, which are the shortest contacts in both Forms. An additional spike is observed in the fingerprint plot of Form II between the light blue spikes, which is due to close H…H contacts in adjacent herringbone layers from the C-rich sides.

![Figure 4.3. Fingerprint plots of 26-DAP Forms I and II. Reproduced with permission of the International Union of Crystallography.](image)

Figure 4.4 displays the powder x-ray diffraction data for Forms I and II simulated from the single crystal CIF files, as well as experimental data from the brown starting material, and the
crystals grown from sublimation and recrystallization from toluene, ethyl acetate, and acetone. Form I appears to be the major component in the bulk commercial powder, however low intensity lines near $2\theta = 8.0$ and 16.0 are likely from (002) and (004) reflections of Form II, present as a phase impurity. Phase pure Form II was obtained from sublimation, as well as recrystallization from toluene and acetone. Recrystallization from ethyl acetate indicated a mixture of both Forms I and II.

When Form II is heated, a solid state transformation to Form I occurs at 62°C, which then sharply melts at 120°C as observed from DSC. A TA Instruments 2920 Modulated differential scanning calorimeter was used with sample sizes of 2 mg. A ramp rate of 10 °C/min was used to heat substances to 300°C. PXRD of the heated sample as well as its melting temperature indicated

![Figure 4.4. PXRD patterns of 26-DAP simulated from single crystal CIF files of Forms I and II, and experimental data from the bottle (as received), sublimed, and recrystallized from toluene, ethyl acetate, and acetone. Reproduced with permission of the International Union of Crystallography.](image)
that it was phase pure Form I. A Rigaku Ultima IV diffractometer was used with Cu Kα radiation (λ=1.5418Å), a tube voltage of 40 kV, and a current of 30 mA. Data collected from 2θ = 5-40° at a scan speed of 1.0°/min was analyzed with Jade v9.0 software.

### 4.3 ADP

2-Amino-5,6-dimethyl-4-hydroxypyrimidine (ADP, 8) was the second system that resulted in a new coformer phase (Figure 3.3). 4-Hydroxypyrimidines are a class of biomolecules that include nucleobases and pharmaceuticals. They can undergo keto-enol tautomerization in solution to form a pyrimidin-4-one, with either protonation at the (1H) or (3H) position. The relative tautomer stability depends on the position and functionality of substituents on the ring. While the enol tautomer is less stable than the keto forms, the hope was that cocrystallization with uric acid, which has a complementary hydrogen bond motif, might stabilize the enol form. This approach has been used in the past as a way of isolating less stable tautomeric forms.

Here, crystallization of an equimolar ratio of uric acid and ADP (Sigma-Aldrich, 96%) resulted in the isolation of a (1H) keto tautomer. Varying the molar ratio of the two components to 1:4 resulted in the generation of a second pyrimidin-4-one polymorph which contained both (1H) and (3H) tautomers.

Crystallization of aqueous solutions at room temperature of equimolar ratios of uric acid and ADP ground using a mortar and pestle yielded Form I. Form I powder was also generated from recrystallization of pure ADP in either water or ethanol (190 proof, Warner-Graham Company). Molar ratios of 1:4 uric acid:ADP resulted in prismatic monoclinic crystals of Form II when recrystallized from aqueous solution. Melting/decomposition was observed for both materials above 300°C from differential scanning calorimetry, with no other observable phase changes in the spectrum. A Nicolet 380 FT-IR spectrometer (Thermo Electron Corporation) was
used with removable KBr optics over a range of 4000 to 400 cm$^{-1}$. Spectra were averaged over 16 scans with a 1 cm$^{-1}$ resolution. Data was analyzed with the OMNIC (Thermo Nicolet Analytical Instruments, Madison, Wisconsin) software package.

ADP crystals of Form I were refined from single-crystal x-ray diffraction data as triclinic, with one 2-amino-5,6-dimethylpyrimidin-4(1H)-one molecule in the asymmetric unit. All H atoms on N atoms were found in difference maps and refined to well suited H-bonding locations, with the exception of N1-H13 which was restrained to 0.96(2) Å. Optimization of methyl H-atoms occurred by rotation around Me-C bonds where ideal distances were obtained for C-H, Me-H, and H...H. Displacement parameters were assigned as 1.5 times the carrier atoms for methyl and amine H atoms. Remaining H atoms were assigned as 1.2 times the carrier value.

The packing diagram in Figure 4.5 shows molecules form 1-D hydrogen bonded ribbons in the [100] direction. An $R_{1}(6)$ motif is formed from hydrogen bonding between oxygen atoms and H-N1 as well as H$_2$N(C2) groups, which was also part of a C(6) motif along the $a$ axis. Cyclic

![Figure 4.5](image-url)

**Figure 4.5.** (a) Asymmetric unit, and (b) packing diagram displaying H-bonded ribbons in the [100] direction of ADP Form I. Reproduced with permission of the International Union of Crystallography.
$R_2^2(8)$ dimers are formed from molecules in adjacent antiparallel C(6) chains between N3 and the H$_2$N(C2) group. Adjacent ribbons along the $b$ axis π-π stack with a centroid…centroid distance of 3.680 (18) Å.

Although the displacement ellipsoids on C, N, and O atoms, as well as the carbonyl bond length of 1.259 (4) Å in Form I indicate the presence of a single tautomer, IR data shows evidence of a second. The IR spectrum of ADP recrystallized from water in Figure 4.6 shows a broad signal around 2900-3300 cm$^{-1}$ which must correspond to O-H stretching. This intense O-H stretching signal likely arises from intermolecular H-atom transfer from N1-H…O to N1…H-O (N1-H13…O9 in Table 4.3). This was the only method that detected this phase impurity, as the PXRD pattern of CIF simulated Form I matched closely to patterns of ADP recrystallized from water, ethanol, and also unrecrystallized powder, with no additional diffraction lines present (Figure 4.7). The data collection temperature of FT-IR (298K) could have played a role in picking up the impurity, as single crystal x-ray diffraction data was collected at 100K.

![Figure 4.6. FT-IR from KBr pellet of ADP powder recrystallized from water. Reproduced with permission of the International Union of Crystallography.](image-url)
Altering the molar ratios of uric acid and ADP resulted in a second pyrimidin-4-one tautomer of ADP. All H atoms on N atoms were found in differences maps and refined to well suited H-bonding locations. Optimization of methyl H-atoms occurred using the same method as in Form I. H atoms on methyl and amine groups were assigned as 1.5 times the carrier atom. All experimental details can be found in Table 4.1.

<p>| | | | | |</p>
<table>
<thead>
<tr>
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</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>D—H···A</td>
<td>D—H</td>
<td>H···A</td>
<td>D···A</td>
</tr>
<tr>
<td>N1—H13···O9$^i$</td>
<td>0.96 (2)</td>
<td>1.87 (2)</td>
<td>2.784 (4)</td>
<td>159 (3)</td>
</tr>
<tr>
<td>N10—H11···O9$^j$</td>
<td>0.94 (5)</td>
<td>2.04 (4)</td>
<td>2.837 (4)</td>
<td>142 (3)</td>
</tr>
<tr>
<td>N10—H12···N3$^{ii}$</td>
<td>0.87 (4)</td>
<td>2.09 (4)</td>
<td>2.956 (4)</td>
<td>173 (3)</td>
</tr>
</tbody>
</table>

Symmetry codes: (i) x+1, y, z; (ii) −x+1, −y+1, −z+1.

Table 4.3. ADP Form I Hydrogen bond geometry (Å, °). Reproduced with permission of the International Union of Crystallography.

Figure 4.7. PXRD patterns from simulated CIF structures of ADP Forms I & II and experimental data recrystallized from water, ethanol, and as received. Reproduced with permission of the International Union of Crystallography.
Form II contains two 4(1\(H\))-tautomers and two 4(3\(H\))-tautomers in the asymmetric unit (Figure 4.8 (a), molecules B/C and A/D, respectively). This 50/50 ratio of each tautomer allows for tridentate hydrogen bonding between pairs of A and B molecules (or C and D molecules) due to the donor-acceptor-acceptor groups and acceptor-donor-donor groups in the 4(1\(H\))- and 4(3\(H\))-tautomers, respectively (Figure 4.8 (b) and Table 4.4). The tridentate bonding consists of two hydrogen bonds between (C4)O…H-N(C2) and a third between N3…H-N3.

![Figure 4.8. (a) Asymmetric unit of ADP Form II and (b) H-bonding surrounding the (010) layers. Reproduced with permission of the International Union of Crystallography.](image)

The (0\(k\)0) layers contain tridentate hydrogen bonded A and B or C and D molecules that form a 2-D network of \(\pi-\pi\) stacks. Centroid-centroid distances between A and B rings is 3.8162 (16) Å. The centroid…centroid distance between C and D rings is 3.4844 (16) Å. The \(\pi\)-stack direction is parallel to (101) in A/B layers and parallel to (100) in C/D layers, which alternates in adjacent layers. Adjacent A/B and C/D layers are connected by O…H-N1 and O…H-N(C2) hydrogen bonds along the \(b\) axis, which form the 3-D hydrogen-bond network.
All donor and acceptor atoms in both forms of ADP are fully satisfied, with a slightly greater density in Form I (157.3Å³ per molecule) than in Form II (164.50Å³ per molecule). No transformations between phases were observed from DSC before decomposition around 300°C.

A search of the Cambridge Structural Database as of November 2015 (Version 5.37) yielded 12 entries for 4-hydroxypyrimidines, 42 entries for pyrimidin-4(1H)-ones, and 168 entries for pyrimidin-4(3H)-ones after limiting the search to exclude large heterocycles. Of these entries, 63% contain an amino group at the C2 position like ADP. Of the pyrimidin-4(3H)-one and 4-hydroxypyrimidine tautomers in the CSD, 96/168 (57%) and 6/12 (50%) have an amino group at C2, respectively. In contrast, 40/42 (95%) of the pyrimidin-4(1H)-one tautomer structures in the CSD have an amino group in the 2-position (only CERZEF213 and TAGVIG214 do not). This data suggests that the presence of an amino group at this position allows for complementary H-bonding.

### Table 4.4. ADP Form II Hydrogen bond geometry (Å,°).

<table>
<thead>
<tr>
<th></th>
<th>D—H</th>
<th>H···A</th>
<th>D···A</th>
<th>D—H···A</th>
</tr>
</thead>
<tbody>
<tr>
<td>N3A—H13A···N3B⁺</td>
<td>0.94 (3)</td>
<td>1.94 (3)</td>
<td>2.885 (3)</td>
<td>179 (3)</td>
</tr>
<tr>
<td>N10A—H11A···N1D⁺</td>
<td>0.89 (3)</td>
<td>2.18 (3)</td>
<td>3.030 (3)</td>
<td>160 (3)</td>
</tr>
<tr>
<td>N10A—H12A···O9B¹</td>
<td>0.88 (3)</td>
<td>1.99 (3)</td>
<td>2.860 (3)</td>
<td>174 (3)</td>
</tr>
<tr>
<td>N1B—H13B···O9D</td>
<td>0.90 (3)</td>
<td>1.90 (3)</td>
<td>2.762 (3)</td>
<td>160 (3)</td>
</tr>
<tr>
<td>N10B—H11B···O9C</td>
<td>0.87 (3)</td>
<td>2.04 (3)</td>
<td>2.841 (3)</td>
<td>153 (3)</td>
</tr>
<tr>
<td>N10D—H12D···O9A¹</td>
<td>0.91 (3)</td>
<td>1.93 (3)</td>
<td>2.834 (3)</td>
<td>179 (3)</td>
</tr>
<tr>
<td>N1C—H13C···N1A³⁺</td>
<td>0.82 (3)</td>
<td>2.19 (3)</td>
<td>2.997 (3)</td>
<td>170 (3)</td>
</tr>
<tr>
<td>N10C—H11C···O9B⁺</td>
<td>0.91 (3)</td>
<td>1.94 (3)</td>
<td>2.804 (3)</td>
<td>158 (3)</td>
</tr>
<tr>
<td>N10C—H12C···O9D⁶</td>
<td>0.91 (3)</td>
<td>1.98 (3)</td>
<td>2.893 (3)</td>
<td>178 (3)</td>
</tr>
<tr>
<td>N3D—H13D···N3C¹⁺</td>
<td>0.89 (3)</td>
<td>2.01 (3)</td>
<td>2.898 (3)</td>
<td>179 (3)</td>
</tr>
<tr>
<td>N10D—H12D···O9C⁵</td>
<td>0.89 (3)</td>
<td>1.95 (3)</td>
<td>2.830 (3)</td>
<td>176 (3)</td>
</tr>
</tbody>
</table>

Symmetry codes: (i) -x+1, -y+1, -z+1; (ii) x, -y+1/2, z+1/2; (iii) x+1, -y+1/2, z+1/2; (iv) -x+1, y-1/2, z+1/2; (v) x, -y+1/2, z+1/2.
between 1H- tautomers, or between 1H- and 3H- tautomers that isn’t possible with a different arrangement of functional groups. There are additionally 13 entries that contain both 1H- and 3H-tautomers within the same lattice (Refcodes: CERZEF, ICYTIN, ICYTIN01, LEJLAN, LEJLOB, LEJMES and LEJMIW, MECXUP, MINVIP01, OQURAU and OQUREY, QOBCIV and ZERMIS), 11 of which exhibit the same tridentate hydrogen bonding observed in Figure 4.8 (b). The two outliers are refcodes: CERZEF and ZERMIS, where the former does not contain an amino group at C2 and the latter does not report 3-D coordinates.

4.4 Melamine

Failed cocrystallization attempts generated non-crystalline material with two coformers. The first was observed with melamine (5, Figure 3.3), which is a compound that has a high nitrogen content (66.7% by mass). For this reason, it has been involved in many global health scares. Protein content in food is determined by testing for nitrogen content, without regard for its source. A few years ago it was discovered that some companies were illegally adding melamine to dog food and infant formula, in order to increase the apparent protein content. Mixtures of melamine and cyanuric acid rapidly precipitate as a 1:1 complex which has really low aqueous solubility. When both are present in food substances, the complex crystallizes out in vivo leading to kidney illnesses in those who ingested these contaminated foods. It was chosen as a coformer in our studies with uric acid due to its ability to form extensive hydrogen bonding, as displayed in the melamine cyanuric acid cocrystal.

Melamine (99%, Sigma-Aldrich) and uric acid were combined in equimolar ratios, ground with a mortar and pestle and recrystallized from aqueous solution. A film precipitated out of solution that was characterized with optical microscopy (Figure 4.9). The substance did not appear to melt via hot stage microscopy when heated to 350°C. PXRD of the dried film showed a single
broad peak at $2\theta = 27.48$, indicating it was amorphous. This peak appears in the predicted PXRD pattern of a high energy uric acid : melamine complex.\textsuperscript{226} The ground powder was also analyzed with PXRD and appeared to be a combination of the two components. After aging more than 1 year, the dried film crystallized and peaks aligned with those in the ground powder (Figure 4.10). Due to the non-crystalline phase of this material, structural analysis was not able to be performed and the microstructure of this film remains unknown.

![Figure 4.9. Optical micrograph of dried film from 1:1 UA:M precipitate.](image)

![Figure 4.10. PXRD patterns of simulated UA and melamine, experimental 1:1 molar ratio of UA:M ground with mortar and pestle, the dried film obtained from recrystallization in water, and the dried film after aging >1 year.](image)
4.5 Triamterene

Triamterene (12, Figure 3.3) (Drug bank # DB00384) is a Food and Drug Administration approved substance, and was the second system that produced non-crystalline material in our investigation. It is used as a diuretic for the treatment of high blood pressure and issues with fluid retention.\footnote{227} It is typically administered alone, or in combination with other potassium-sparing diuretics like hydrochlorothiazide. The main drawback of its use in pharmaceuticals is its poor aqueous solubility. Attempts at improving this characteristic have been attempted with cocrystals, salts and prodrugs to name a few.\footnote{228} Its single crystal structure was deposited in the Cambridge Structural Database in 2007 (deposition number 668589) as a private communication from authors M. Tutughamiarso and M. Bolte.\footnote{229} A search of the database returned 9 results for the triamterene molecule. It is neutral in 3 cases (Refcodes: FITZAJ01,\footnote{229} GOLGOF,\footnote{230} SEWZOJ),\footnote{231} protonated at the N1 position in 3 cases (GOLDUG,\footnote{232} KUTJAM, KUTJIU),\footnote{233} and doubly protonated in the remaining 3 cases. Two are at the N1 and N3 positions (NUTQAW, NUTQEA)\footnote{228} and one is at the N3 and N8 positions (HEDSEO)\footnote{234} (Figure 4.11).

![Triamterene molecule with atoms labeled in red](image)

**Figure 4.11.** Triamterene molecule with atoms labeled in red.
Our initial goal was to cocrystallize uric acid and TAT to improve their solubility. Equimolar ratios of uric acid and triamterene (≥99%, Sigma-Aldrich) ground using a mortar and pestle appeared to produce a new phase from PXRD (Figure 4.12). Compared to the simulated patterns of anhydrous uric acid and triamterene, the experimental powder displayed new peaks, most notably around $2\theta = 7.80$ and 25.16. Adding to this evidence of a new phase was DSC data that showed the experimental powder had a melting point different than either of its components (Figure 4.13). While uric acid decomposes above 400°C and TAT melts around 330°C, the ground 1:1 powder showed a sharp melting point at 320°C, followed by decomposition around 360°C.

![Figure 4.12. PXRD of simulated UA and TAT and experimental ground powder of equal molar uric acid and TAT.](image)
Recrystallization of the 1:1 powder from water in a 2.5 mM concentration led to the formation of an amorphous gel. When hot, the substance appeared colorless but turned yellow upon cooling to room temperature. A Zeiss SUPRA55-VP scanning electron microscope was used to examine fibers and crystals throughout the gel with secondary electrons at 1kV. Fibers with dimensions over 1 mm in length and slightly under 50 µm in width with a very small thickness (~5 µm) were identified (Figure 4.14). Crystals were found as well (Figure 4.15), which were large
enough to be used for single crystal x-ray diffraction. The unit cells of these crystals matched that of triamterene, which was likely their identity.

Figure 4.14. (a) Fiber found in amorphous substance from uric acid and TAT [2.5 mM] (b) zoomed in on fiber.

Figure 4.15. Crystal found in amorphous substance from uric acid and TAT, later identified as TAT.
The gel was analyzed with a MCR302 Anton Paar cone-plate rheometer. A small amount of gel was placed on the bottom plate and the top piece with a 25 mm diameter came down to sandwich it to a width of 0.104 mm. Excess gel was whisked away with a wipe. Keeping the angular frequency constant at 1 rad/s, the strain on the gel was varied from 0.08 to 100% and the storage (G’) and loss (G”) moduli were measured. These represent the amount of solid and liquid-like behavior contained in the gel, respectively. Within the linear viscoelastic region (LVR) of the shear strain sweep (0.1 to 5%), G’ remained higher than G” (Figure 4.16). After choosing a constant 1% strain which fell within the LVR region for the shear frequency sweep, the frequency was varied on the gel from 100 to 0.05 rad/s. Again, G’ remained higher than G” for all frequencies tested (Figure 4.16). This allows the substance to be characterized as a true gel, which implies that the material contains more solid than fluid-like behavior.²³⁵-²³⁸
4.6 Conclusions

The previous chapter was spent analyzing cocrystals that formed from uric acid molecules combining with coformer molecules in stoichiometric ratios to produce new material. Here, we investigated the substances that formed when cocry stallization failed, which proved just as interesting. After identifying the systems that simply precipitated out the less soluble component (uric acid in the case of 2-MP, 2-HP, B and AHP), the remaining four systems had more unexpected results. Two resulted in crystal precipitates; 26-DAP formed a new polymorph.

Figure 4.16. (a) Log-log shear strain sweep of 1:1 UA:TAT gel [2.5mM] with frequency of 1 rad/s (b) log-log shear frequency sweep with strain of 1%.
from sublimation, and ADP crystallized in two different keto tautomeric forms from different molar ratios of ADP and uric acid. Melamine and TAT formed non-crystalline material precipitates that were identified as an amorphous dried film in the former case and a gel in the latter. It’s important to be thorough with observations and analysis, as they may provide insight for future experiments.
Chapter 5. Effects of 24-DAP on Uric Acid Crystal Growth

5.1 Introduction

Uric acid crystals grown *in vivo* can appear physically different than those grown from pure aqueous solutions. Both UA and UAD crystallize as colorless rectangular plates when grown from pure aqueous solutions, while those grown *in vivo* are colored and can have non-rectangular morphologies. The micrographs in Figure 5.1 compare uric acid crystals that were grown under physiologic conditions and synthetic crystals grown in lab. As was described in Chapter 2, the color apparent in the physiologically derived samples is due to the inclusion of low concentrations of impurities included during biomineralization. The causes of the morphological differences in the natural and synthetic samples are more difficult to ascribe unambiguously, but are typically attributed to molecular recognition between other solution components and growing crystal surfaces. Such impurity-surface interactions can be either specific or non-specific, but are usually thought to decrease the relative growth rate normal to that surface. Consequently, the area of a crystal face is inversely proportional to the relative growth rate normal to it. However, in a fluid

![Figure 5.1](image1)

**Figure 5.1.** (left) Optical micrograph of uric acid crystals grown *in vivo*. Reproduced with permission from the Louis C. Herring Lab. (right) Synthetic crystals of UAD grown from aqueous solution at 24 ± 1 °C. Image courtesy of Frank Liu. Scale bar = 200 µm.
as chemically complex as urine, it is difficult to know which specific component(s) might induce morphology changes.

In this chapter we describe the growth of uric acid crystals in the presence of low concentrations of 24-DAP (7, see Figure 3.3). 24-DAP, along with various derivatives, have been previously studied for their reactivity in biochemical synthesis. Although readily absorbed \textit{in vivo} and proven to be active in biological systems as antibiotics and antimalarials, they are not metabolized or utilized to synthesize nucleic acids.\cite{refcode:ESOYUI} Its crystal structure was recently reported by Hützler et al (refcode: ESOYUI).\cite{240} In Chapter 3, we showed that supersaturated aqueous solutions of 1:1 uric acid and (7) yielded cocrystals with the anticipated tridentate binding motif (Figure 3.15). The solubility of the U\textsuperscript{-}:(7)\textsuperscript{+}\cdot3H\textsubscript{2}O complex was found to be higher than both UA and MSU in the one minute solubility tests. Curiously, the measured solubility of the cocrystal after two hours (closer to equilibrium conditions) was \textit{lower} than it was at one minute (Table 3.6). One expects the measured solubility after two hours to be the same or \textit{higher} than the measurement taken after one minute, which was the case for UA, MSU and all other cocrystals examined, in the absence of parallel phase transformation processes. Although optimal growth conditions for U\textsuperscript{-}:(7)\textsuperscript{+}\cdot3H\textsubscript{2}O required a 1:1 molar ratio of the two components, solutions containing other molar ratios exhibited some very unusual crystallization behavior, particularly those with higher uric acid:(7) ratios. Here we examine how the presence of varying concentrations of 24-DAP in the growth solution affect uric acid crystallization.

\textbf{5.2 Experimental}

\textit{5.2.1 Materials}

Water was either passed through a Barnstead deionizing cartridge followed by distillation or acquired from a Milli-Q Integral Water Purification System at 18.2 mΩ. Glacial acetic acid was
purchased from Fisher. Uric acid (Aldrich, 99+%), 24-DAP (Aldrich, 98%), and methylene blue (Aldrich, 98%) were used as received.

Model urine was made from an established recipe\textsuperscript{185} and used the following ingredients. Sodium sulfate anhydrous (Na\textsubscript{2}SO\textsubscript{4}, 99%), magnesium sulfate heptahydrate (MgSO\textsubscript{4}·7H\textsubscript{2}O, 98%) and potassium chloride (KCl, 99%) were purchased from EMD Millipore. Ammonium chloride (NH\textsubscript{4}Cl, 99.5+%), sodium phosphate monobasic monohydrate (NaH\textsubscript{2}PO\textsubscript{4}·1H\textsubscript{2}O, ≥99.0%) and sodium phosphate dibasic (Na\textsubscript{2}HPO\textsubscript{4}, ≥99%) were purchased from Sigma-Aldrich. Sodium chloride (NaCl, 100.5%), sodium citrate dihydrate (Na\textsubscript{3}C\textsubscript{6}H\textsubscript{5}O\textsubscript{7}·2H\textsubscript{2}O, certified), and urea (certified ACS) were purchased from Fisher Scientific. All compounds were used as received.

5.2.2 Crystal Growth

Pure anhydrous uric acid (UA) and methylene blue doped UA (UA-MB) crystals were prepared as described previously in a 37°C water bath, and with an overall dye concentration of 50 µM.\textsuperscript{21} UA-MB crystals were added to saturated model urine solutions as seeds and stored at room temperature (21°C).

Stock powders of uric acid:(7) were prepared in molar ratios ranging from 4:1 to 20:1. Each stock powder (8-10 mg) was dissolved in 40 mL H\textsubscript{2}O by heating and allowed to recrystallize at room temperature (21°C). Crystals of UAD precipitated after one day.

5.2.3 Characterization Methods

Optical and hot stage microscopy were conducted on an Olympus BX-50 polarizing microscope with an HCS302 optical hot-stage (INSTEC, Inc., Boulder, CO).

A Zeiss SUPRA55-VP Scanning Electron Microscope (SEM) was used at an acceleration voltage of 2kV with secondary electrons to view detailed crystal features. Crystals from solutions
were pipetted onto adhesive carbon circles and allowed to dry. Carbon circles were attached to a conductive metal plate and placed into the vacuum chamber.

Powder X-ray diffraction (PXRD) data was collected on a Rigaku Ultima IV X-ray diffractometer (Cu Kα radiation, 40 kV, 44 mA current) at room temperature over 2θ = 5–40°, and analyzed with Jade v9.0 software.

Miller Indices were assigned to individual crystals using a whole data set collection for UAD and from a unit cell determination for UA with APEX3 software. Morphology models were made using WinXMorph software (Version 1.54).241,242

5.2.4 Atomic Force Microscopy

A Nanoscope IIIa Multimode instrument (Digital Instruments, Santa Barbra, CA) operated in contact mode with an attached fluid cell was used to acquire AFM topographs. The piezoelectric J-scanner stage used had a maximum scan area of 30 x 30 µm², as limited by the o-ring. The Si₃N₃ cantilevers had a manufacturer reported spring constant of ~0.6 N/m. The 2 mL reservoir containing the growth solution that feeds directly into the fluid cell (Tres in Figure 5.2) was maintained at 45°C and the temperature controlled heating stage under the sample puck (Tcell) was set to 37°C (Digital Instruments, Santa Barbara, CA). A constant flow rate of 1 mL/hr was achieved by pulling the solution through the fluid cell with a syringe pump (KD Scientific model KDS210, New Hope, PA) containing two 10 mL Hamilton gastight syringes. All AFM images presented were collected under fluid and are depicted in deflection mode.
Pure UA single crystals used in *in situ* AFM experiments were secured to 15 mm diameter glass coverslips with 5-minute epoxy. Coverslips were attached to stainless steel sample pucks, and the crystals were oriented such that their *c*-axis was aligned perpendicular to the X-scanning direction. Supersaturated growth solutions were prepared by dissolving 24 mg solute (either pure uric acid or a 25:1 uric acid:(7) mixture) in 0.1M acetate/acetic acid buffer, which resulted in a pH of 4.8. The solutions were heated to effect dissolution, maintained at 50°C, and gravity filtered prior to entering $T_{\text{res}}$.

![Figure 5.2. Schematic representation of the AFM apparatus with parameters labeled.](image)


### 5.3 Results and Discussion

#### 5.3.1 Uric Acid Crystals Obtained from 20:1, 16:1, 10:1 and 4:1 Uric Acid:(7) Solutions

Pure supersaturated uric acid solutions maintained at room temperature typically crystallize as UAD. UAD single crystals have a rectangular morphology defined by a (001) plate face and (011) and (102) side faces. Note: These Miller Indices reference to the monoclinic cell convention
reported by Parkin.\textsuperscript{12} Crystals obtained from the 4:1 solution of uric acid:(7) had PXRD patterns consistent with UAD (Figure 5.3), where >100 crystals were sampled. There is some variety in the sizes/shapes of these crystals, as well crystals from all other ratios (UA:(7) 10:1, 16:1, and 20:1). Representative crystals obtained from each solution type are shown in Figure 5.4. The UAD crystals from the 20:1 mixtures appear as plates with (001) as the major face, though the side faces correspond to \{21-2\}. Small \{011\} side faces are always present. This is different than the side faces seen in previous work where UAD was grown in the presence of assorted dye molecules. Crystals grown from 16:1 and 10:1 are smaller than those grown from 20:1 and have more elongated morphology. UAD grown from 4:1 solutions are the smallest of all solutions. They appear hexagonally shaped and slightly elongated along the $a$-axis. A schematic of a UA:(7) 4:1 crystal from face indexing is shown in Figure 5.5, compared to that of pure UAD. The (001) major face and \{212\} and \{011\} side faces are labeled.

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{figure5_3.png}
\caption{Simulated PXRD of (a) UAD, (b) UA, (c) $U^+(7) \cdot 3H_2O$ cocrystal, and experimental PXRD of (d) 4:1 UA:(7) crystals.}
\end{figure}
Previous work has shown that UAD crystals transform in solution to UA through a
dissolution-recrystallization mechanism.\textsuperscript{164} This transformation is mediated by the epitaxial
relationship between UAD (001) and UA (100) surfaces.\textsuperscript{11} Epitaxy can be visualized by the
appearance of one or more UA crystals which nucleate and grow off the UAD (001) surface.
Though the UA crystals by necessity grow in fixed orientations with respect to the underlying
UAD substrate, the point(s) of nucleation on the UAD plate face tend to be somewhat random.
Also, one or several UA crystals can nucleate on a single UAD crystal surface. As the UA crystals
grow (and UAD dissolves), they eventually overhang the UAD (011) and (102) side faces. Distinguishing UAD and UA crystals in partially transformed samples is possible with hot stage microscopy, since UAD crystals turn opaque when they dehydrate at elevated temperatures (<100°C) while UA crystals remain transparent.

UAD crystals grown from 4:1 solutions of UA:(7) also appear to transform to UA in solution over time, albeit in a highly unusual way. Figure 5.6 shows UAD crystals after 48 hours in the 4:1 growth solution. Pairs of new UA crystals appear to grow from the $a$-axis ends of the

Figure 5.5. Schematic of (a) pure UAD and a 4:1 UA:(7) single crystal with views down the (b) c-axis (c) a-axis and (d) b-axis. Images generated using WinXMorph.\textsuperscript{241,242}

Figure 5.6. Optical micrographs of 4:1 UA:(7) after (left) 1 and (right) 2 days of growth. Scale bar = 100 µm.
UAD crystal. The relative orientation of the two phases was consistent with UA-UAD epitaxial matching, but the very specific growth direction and pairwise appearance of the new crystals was quite different than what was observed in pure aqueous solution. Over even longer times, the two end crystals increase in size while the central UAD crystal dissolves. Samples maintained in the 4:1 growth solution for 9 days appear as barbells. Hot stage microscopy images (Figure 5.7) show the central UAD crystal dehydrates while the end crystals remained transparent. Face indexing confirmed that the end crystals were UA. Although our observations were consistent with epitaxial growth, the very regular pair-wise growth of UA crystals from the ± \( a \)-axis ends of UAD remained unexplainable.

![Figure 5.7. Optical micrograph of crystals after 9 days during HSM of UA:(7) 4:1 at ~100°C. Scale bar = 100\(\mu\)m.](image)

Scanning electron microscopy proved to be illuminating. SEM images of UAD crystals after two days in solution revealed unambiguously that the end crystals were in fact emerging from the center of the UAD crystal rather than on the surface (Figure 5.8). (The resolution of optical
microscopy images was not high enough to discern this, and growth from the center of the UAD crystal was not at all expected. SEM of samples that continued to age for longer time periods in solution illustrate how the transformation to a pair of fused UA single crystals occurs (Figure 5.9). The UA crystal that ultimately forms diffracts as a single crystal and does not appear to be twinned.

It was thought possible that a small crystal of 24-DAP may have been present in the initial UAD crystal, acting as a nucleation site for UA growth. To test for this, the mapping feature of Raman microscopy was used to sample various depths and positions within the merging site of the two UA crystals. Figure 5.10 displays the Raman spectra recorded from 600 to 825 cm\(^{-1}\). Raman lines associated with 24-DAP in this region appear at 600, 684, 715 and 791 cm\(^{-1}\). From experimental data, we know UA has two prominent Raman lines around 625 and 780 cm\(^{-1}\). The

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**Figure 5.8.** Packing diagram, unit cell parameters and schematic morphology with labeled Miller Indices of (left) UAD and (middle) UA. (right) SEM image of day 2 4:1 UA:(7) crystal with labeled Miller Indices. Scale bar = 10 µm.

---
two peaks observed in all 20 spectra correspond to UA, with no traces of (7). However, the change in shape of the initial UAD crystals from rectangular to hexagonal does indicate inclusion of

Figure 5.9. (left) SEM image of 4:1 UA:(7) crystal after ~20 days. Scale bar = 20 µm. (right) Raman image after >30 days in growth solution.

Figure 5.10. Raman microscopy spectra of merging UA crystal (Figure 5.9, right) at four different depths (Z=0, 5, 10, 15 µm) and five different points along the surface (green, yellow, grey, orange, and blue lines correspond to middle, right middle, right, edge, and merge point of the top UA crystal).
impurity. The UA crystals that grew on the ends had similar morphologies to those grown from water.

24-DAP causes very small crystals of UA about 1 µm thick to nucleate in the center of UAD crystals. However, the high concentration of uric acid in the growth solution eventually causes UAD to precipitate. A schematic of this transition is shown in Figure 5.11. Although UAD is a kinetic phase and the transformation of UAD to UA is irreversible, it should be possible for UAD to nucleate on UA surfaces.

5.3.2 In situ AFM Studies on UA (100) – Previous Work

In order to get a better understanding of how the 24-DAP molecule was directly affecting the molecular-level growth of uric acid crystals, in situ atomic force microscopy experiments were performed. In previous work by Sours et al, crystal growth on UA (100) faces of single crystals was monitored via in situ AFM as a function of supersaturation and pH. Growth was found to

Figure 5.11. Schematic of transition from UAD to UA.
proceed by a layer-by-layer mechanism, initiated at screw dislocation sites that were apparent across the surface. The in-plane step kinetics were highly anisotropic, with growth along the $\pm c$-axis nearly 10X faster than along the $\pm b$-axis. Due to this anisotropy, topographical images typically present many (010) steps and relatively fewer step edges moving in the [001] direction. The (010) steps typically had measured heights consistent with unit cells (2 molecular layers) or multiples thereof. The fast moving $\pm c$-steps sometimes showed a splitting into monolayers at the very ends. While it is most conventional to track the position of individual steps or features in sequential topographical images, the density of steps and very rapid growth along $\pm c$ made this impossible. Slow scan axis disabled (SSAD) was needed to quantify the in-plane step velocities. The $b$-step velocity was linear over a pH range of 4.4-5.2.

5.3.3 Growth of UA from Solutions Containing 24-DAP

A preliminary study on UA (100) growth in acetate buffer was done to reproduce prior results. While an area dense with growth hillocks was found on one occasion (Figure 5.12), the majority of topographical images appeared relatively smooth with step sizes measuring <10 nm (Figure 5.13). In light of the highly stepped nature of the surface, instead of tracking the in-plane kinetics we focused instead on quantifying the growth normal to the (100) plane. The growth rate

Figure 5.12. Tip of a growth hillock observed on the (100) face of UA in 0.1M acetate/acetic acid buffer saturated with uric acid. (Flow rate = 1.0 mL/hr, vertical scale = 10V, scan rate = 1.97 Hz, 512 lines/image, scan down).
normal to the plate face is determined by changes in the average Z position of the cantilever tip. The ΔZ varied within 1 µm from image to image within one given sample and was similar for all three samples. On average, ΔZ was ~370 nm per image, which correlates to 1.42 ± 1 nm/sec. Roughness measurements calculated for each image in the series (RMS average = 39 nm) did not show any observable trend which might skew the growth rate measurements.

AFM growth experiments were next repeated in buffered solutions containing uric acid and 24-DAP in a 25:1 molar ratio. Three crystals were studied under these conditions. The topographs collected (Figure 5.14) appeared drastically different than those in the absence of 24-
DAP. The vertical scale in Figures 5.13 and 5.14 are identical. Qualitatively the surfaces in the 24-DAP solutions are much rougher (RMS average = 53 nm) with large pits which measure > 250 nm in depth. However, the growth rate normal to the (100) surface under these conditions, ~1.27 ± 0.7 nm/sec, was not dramatically lower than that measured in the absence of 24-DAP. Again, the measured roughness values in sequential images did not show any obvious trend.

5.3.4 Monitoring UA Growth in Model Urine

To better model the growth of uric acid in vivo, in situ AFM experiments were next performed in model urine. The experimental set-up was exactly as described previously, substituting model urine for acetate buffer. Figure 5.15 is a representative topograph obtained
after a (100) UA surface was exposed to urine solution containing uric acid for several minutes. At first the orientation of the long steps was surprising, since they were perpendicular to the $\pm c$-axis of UA (in water the surface was dominated by (010) steps). Previous research in the lab had shown that while UA was favored from 37°C aqueous solutions, and UAD from room temperature aqueous solutions, UAD typically grew from model urine at both temperatures. We suspected that the material depositing on the (100) UA surface was actually a layer of (001) UAD, since this would account for the new orientation of the surface steps.

Prior to this date, members of the lab had only ever seen epitaxy in the form of UA on UAD and not vice versa, so the viability of UAD growth on UA in urine needed to be confirmed.

UA seed crystals with dye inclusions were grown from aqueous solutions containing methylene

Figure 5.15. Typical (100) face of UA in model urine saturated with uric acid. (Flow rate = 1.0 mL/hr, vertical scale = 10V, scan rate = 1.97 Hz, 512 lines/image, scan down, 3x3 µm image).

Prior to this date, members of the lab had only ever seen epitaxy in the form of UA on UAD and not vice versa, so the viability of UAD growth on UA in urine needed to be confirmed. UA seed crystals with dye inclusions were grown from aqueous solutions containing methylene
blue as has been described elsewhere.\textsuperscript{21} Once added to model urine solutions supersaturated with uric acid, the blue color of the crystals would allow them to be visually identified as the seeds. Supersaturated urine solutions were maintained at 37°C in order to mimic the AFM conditions. Optical micrographs of samples harvested from solution appear in Figure 5.16. The UA seed crystals appear to have new crystals attached to their surfaces, though their orientations are highly variable and not consistent with epitaxial growth. Under hot stage microscopy, the new crystals do turn opaque, suggesting dehydration occurred.

\textbf{Figure 5.16.} Optical micrographs of methylene blue doped UA seed crystal with crystals grown from model urine solution when heated to (left) 50.8°C and (right) 150.0°C.

PXRD analysis of precipitates pulled from the model urine solution (Figure 5.17) showed a weak diffraction line near $2\theta = 10$, though it does not align perfectly with the expected $2\theta$ for UAD. DSC of the precipitate (2 mg samples in closed pans, heating rate = 10°C/min) showed several transitions (Figure 5.18). While the endotherm below 100°C may correspond to UAD, the transitions at 160°C and 243°C do not. Single crystal XRD was attempted on these
small crystals, but their small sizes and weak diffraction prohibited determination of even a unit cell. These experiments should be repeated in the hopes that the results will be less ambiguous.

Figure 5.17. Simulated PXRD of (a) MSU, (b) UA, (c) UAD, (d) UAM, and (e) experimental PXRD from precipitate of model urine solutions grown at 37°C.

Figure 5.18. DSC of precipitate from model urine solution.
5.3.5 Growth of UA in Urine with 24-DAP

The growth of UA in the presence of 24-DAP in model urine solution was attempted. Preliminary results are shown in Figure 5.19. The growth of the (100) surface with 24-DAP looks much different than in pure urine solutions, however this was only captured once and therefore cannot be used to represent crystal growth in this environment as a whole. Steps seem to appear at a 135° angle from normal starting from the bottom right and projecting towards the top left. The growth fronts appear to be pointed and continuously appear over time. More trials of these growth conditions need to be performed in order to determine if this observation is consistent.

![Figure 5.19. (100) face of UA in model urine saturated with 25:1 UA:(7).](image)

5.4 Conclusions

The effects of 24-DAP, originally identified from a uric acid cocrystal study, on the microscopic and nanoscopic growth of uric acid crystals was studied. Single crystal XRD and SEM revealed unconventional growth of uric acid in bulk solutions where UA grew from the middle of UAD crystals. AFM revealed a completely different growth pathway of UA in acetate buffer when in the presence of the 24-DAP molecule, which was shown from PXRD not to be due to the growth
of any new phases. Growth of UA in model urine solutions most likely (but yet to be confirmed) led to the formation of UAD on the (100) surface, indicated by a switch from $b$-steps to $c$-steps. Results of growth in urine solutions containing 24-DAP should be considered preliminary, but look interesting and show a potential for future study.

The 24-DAP molecule was clearly shown to have an impact on the growth of UA crystals, even in ratios as small as 25:1 UA:24-DAP. These results open the possibility of using compounds like this to target host crystals in conditions like kidney stones to alter the properties of the deposits in vivo, without using intrusive methods. While this particular molecule may be specific to uric acid, the potential of using targeted compounds to interfere with and possibly prevent the growth of many different types of organic and inorganic stones is recognized. More research needs to be conducted on the effects of this additive and possibly others.
Chapter 6. Water Dynamics in Uric Acid Lattices

6.1 Introduction

Uric acid crystal growth from various media have been examined in many previous studies from our lab.21,117,118,120,164 The dihydrate form (UAD) is less stable than anhydrous uric acid (UA), and the transformation of UAD to UA can occur both via dehydration in air8 and by recrystallization in water.244 The transformation rate of UAD to UA was found to be strongly dependent on the initial UAD growth solution conditions. When UAD is grown from aqueous solutions containing dye (UAD-dye), small concentrations of dye include within their matrixes (<1 mol%).21,117,119,121 Some UAD-dye crystals dehydrate in air at temperatures up to 10°C higher than UAD grown from pure aqueous solution (UAD-W). UAD-dye crystals also transform in solution to UA more slowly than UAD-W.

Similarly, when UAD crystals are grown from model urine solution (UAD-U), very low concentrations of some alkali salts can be detected in the crystals. UAD-U and UAD-W appear identical, both physically (Figure 6.1) and by most characterization techniques including synchrotron x-ray diffraction. Though UAD-U and UAD-W dehydrate in air at the same temperature, UAD-U transforms to UA in solution at a much slower rate than UAD-W. Figure 6.2 reproduced from Presores245 compares the solution transformation rates in urine of UAD-W

![Figure 6.1. Optical images of UAD-W (left) and UAD-U (right) by Frank Liu. Scale bar = 200 µm.](image-url)
and UAD-U when the UAD-U was initially grown at pH = 4 or 5 and temperatures = 37 or 25°C. While UAD-W fully transforms to UA in 30 hours, the UAD-U grown at pH 4 and 25°C is only 10% converted after 48 hours. The concentrations of included ions in the UAD-U crystals failed to explain this dramatic change in transformation rate. *In situ* atomic force microscopy images obtained by Frank Liu (Figure 6.3) suggest that UAD-W and UAD-U in fact have different microstructures. Dissolution of the UAD-W (001) face showed a smooth surface with randomly oriented steps throughout. In contrast, UAD-U (001) surfaces showed granular regions and a much rougher overall texture. Differences in granular vs smooth microstructures have been observed in the biomineralization of calcite, and rationalized as a consequence of different assembly mechanisms. Smooth UAD-W surfaces would result from assembly via a classic layer-by-layer growth mechanism, while UAD-U would result from assembly via amorphous nanospheres, or a non-classical growth mechanism, which lead to more granular textures. While we are not aware of other reports which demonstrate classical and non-classical assembly in the same organic system, it is true that higher uric acid supersaturations are achievable in urine (4.46 mM) compared to water (1.19 mM) and that these conditions may favor different growth mechanisms.

![Figure 6.2. Transformation of UAD-U in model urine. Reproduced with permission from reference 245.](image-url)
It is difficult to quantify any differences in the domain sizes in UAD-W and UAD-U using X-ray diffraction techniques because UAD single crystals grown under all conditions exhibit both molecular disorder\textsuperscript{12} and significant twinning (Figure 6.4). In Parkin’s UAD structure model\textsuperscript{12} the uric acid molecule sits in two positions with refined occupancies of 83 and 17%. The position of

![Figure 6.3. In situ atomic force microscopy image of the (001) face of (left) UAD-W crystal and (right) UAD-U crystal grown at ambient conditions, pH = 4 after partial dissolution in water. Images courtesy of Frank Liu.](image)

![Figure 6.4. Disorder/twinning in molecular structure of uric acid. Reproduced with permission of the International Union of Crystallography from reference 12.](image)
the oxygen atom in the water molecules is well-ordered, however the water molecules’ hydrogen atom positions are assumed. The UAD structure consists of densely packed layers of uric acid molecules in the (001) plane with parallel 1-D water channels running between them (Figure 6.5). We hypothesize that differences in UAD to UA transformation rates are due to underlying differences in the domain coherence length in UAD-W and UAD-U crystals, with the latter exhibiting a greater degree of molecular disorder. Such differences are likely to affect the ability of UA to nucleate epitaxially on UAD surfaces, a key step in the solution-mediated transformation process.

Figure 6.5. Packing diagrams of UAD (refcode ZZZPPI02) with different levels of molecular order/disorder. (left) Major orientation (in red) and minor orientation (in blue) were refined by Parkin as having occupancies of 83 and 17%. (middle) Hypothetical structure where all uric acid molecules adopt the major orientation. (right) Hypothetical structure of disorder.
Based on the AFM data, we hypothesize that the disorder seen in the X-ray diffraction data of UAD-W is in large part due to the superposition of differently oriented domains, but more likely reflects true molecular disorder in UAD-U. The local environment of water molecules is expected to be different if it is surrounded by uric acid neighbors all in the major orientation vs when the neighbors adopt a mix of major and minor orientations more reflective of true molecular disorder. The large incoherent scattering cross section of the hydrogen atom makes neutron scattering an excellent method to probe the water motions in these two environments. Here, we identify quantitative differences in the localized diffusion rate of water in polycrystalline samples of UAD-W and UAD-U using quasi-elastic neutron scattering (QENS). This work would not have been possible without the considerable assistance of Dr. Timothy Prisk at the NIST Center for Neutron Research (NCNR).

6.2 Experimental

6.2.1 Materials

Water was collected from a Milli-Q Integral Water Purification System at 18.2 mΩ. Uric acid (99+%), Aldrich) was used as received. Model urine was synthesized from an established recipe which contained the following compounds: sodium sulfate anhydrous (Na$_2$SO$_4$, 99%), magnesium sulfate heptahydrate (MgSO$_4$·7H$_2$O, 98%), potassium chloride (KCl, 99%), ammonium chloride (NH$_4$Cl, 99.5+%), sodium phosphate monobasic monohydrate (NaH$_2$PO$_4$·H$_2$O, ≥99.0%), sodium phosphate dibasic (Na$_2$HPO$_4$, ≥99%), sodium chloride (NaCl, 100.5%), sodium citrate dihydrate (Na$_3$C$_6$H$_5$O$_7$·2H$_2$O, certified), and urea (certified ACS). Compounds were purchased from EMD Millipore, Sigma-Aldrich and Fisher Scientific and used as received.
6.2.2 UAD Samples

UAD-W crystal samples were prepared by dissolving 0.8 grams of uric acid into four liters of water. UAD-U crystal samples were prepared by adding 1.5 grams of uric acid into two liters of model urine solution, pH = 4.9. Each solution was heated to boiling to effect dissolution, then poured into crystallizing dishes to cool and covered with aluminum foil. After two days at 20°C, crystals from these solutions were vacuum filtered and stored in vials at -20°C until ready for use. Samples sizes for EWS and QENS measurements were 0.5 g and 1.2 g for UAD-W and UAD-U, respectively. The phase purity of UAD samples was confirmed with powder x-ray diffraction (PXRD) performed on a Rigaku Ultima IV X-ray diffractometer (Cu Kα radiation, 40 kV, 44 mA current). PXRD data was collected at room temperature with a 2θ range of 5-40° and analyzed with Jade v9.0 software.

6.2.3 Instrumentation

Neutron experiments were performed at the National Institute of Standards and Technology Center for Neutron Research (NIST-NCNR) using the High-Flux Backscattering Spectrometer (HFBS). The Elastic Window Scans (EWS) were performed by cooling the UAD-W and UAD-U samples from 250 and 300K, respectively to 5K, followed by heating from 5 to 295K at a ramp rate of 1K/min. Quasi-Elastic Neutron Scattering (QENS) measurements were performed at 270 and 230K for the UAD-W sample for 15 and 6 hours each. UAD-U was measured at 290, 270, 250, and 230K for 6, 12, 10, and 3 hours each.

6.3 Neutron Backscattering Theory and Results

The Center for High Resolution Neutron Scattering (CHRNS) High-Flux Backscattering Spectrometer (HFBS) at the NIST-NCNR site is shown in Figure 6.6. This instrument is a reactor-
based, indirect geometry spectrometer that uses cold neutrons with an energy of 2.08 meV. The
doppler monochromater and single-crystal analyzer make use of Si (111). The range of incident

![Figure 6.6](image.png)

**Figure 6.6. The Center for High Resolution Neutron Scattering (CHRNS) High Flux Backscattering Spectrometer (HFBS) at the National Institute of Standards and Technology Center for Neutron Research (NIST-NCNR). Image courtesy of Yiming Qiu.**

neutron energies is determined by the frequency of the doppler drive. In the fixed window scan
mode, the doppler is set to zero frequency and the instrument measures only elastic scattering (i.e.
scattered neutrons having identical initial and final energies). In the quasi-elastic mode, the
doppler drive is in motion creating a spread in incident neutron energies of +/- 15 µeV. In an
Elastic Window Scan (EWS), the sample temperature is ramped over a large range while the elastic
scattering of the sample is measured. Changes in the slope indicate conditions under which
transitions in the sample occur that are visible to the spectrometer. Once identified, specific
temperatures can then be probed with Quasi-Elastic Neutron Scattering (QENS). The small
change in energy of the scattering results in a broadening of the elastic signal, allowing for
calculation of the atomic motions that give rise to the broadening. Figure 6.7 shows the range of energy transfers that can occur over a broad scattering range.\textsuperscript{248} Although not measured in our studies, Figure 6.7 additionally shows inelastic scattering. This scattering is the result of energy exchange due to vibrational or stretching modes that occur with discrete energy steps. Any broadening observed in UAD-W and UAD-U samples can then be fit to Lorentzian functions, where localized diffusion rates can be extracted for each sample.

\begin{figure}[h]
\centering
\includegraphics[width=0.5\textwidth]{energy_diagram.png}
\caption{Energy diagram displaying elastic, quasi-elastic and inelastic scattering energies. Reproduced with permission from reference 248.}
\end{figure}

6.3.1 Elastic Window Scan

EWSs were performed on both UAD-W and UAD-U samples, shown in Figure 6.8. The total elastic intensity is a measure of the non-diffusive fraction of atoms in the sample, where non-diffusive here means that these atoms relax on time-scales longer than \(~5\) ns. The intrinsic differences between samples are apparent by the difference in the slope of each pair of curves. The differences between the heating and cooling curves for each sample are only due to the speed of the temperature ramp rate and do not indicate hysteresis in the sample. The more subtle curves of
UAD-W changed by about 0.10 intensity units between 5 and 300K, which makes up about 33% of the maximum intensity. The UAD-U curves are steeper and changed by about 0.23 intensity units over this same temperature range, making up about 50% of the maximum intensity. A second change in slope can also be observed in UAD-U around 50K, which was absent from the UAD-W sample. Due to the contribution of coherent Bragg reflections to the measured elastic intensity from the crystalline samples, meaningful values of the mean square displacement were not able to be obtained from these EWSs. However, qualitative analysis of these EWSs reveals differences in the water dynamics of each system, which gave reason to investigate these systems further with QENS.

Figure 6.8. EWS of UAD-W and UAD-U samples upon cooling and heating at 60 seconds per point. Red/green and black/blue markers indicate heating/cooling of UAD-W and UAD-U samples, respectively.
6.3.2 Quasi-Elastic Neutron Scattering

QENS measurements were collected on the UAD-W sample at 230 and 270K (Figure 6.9). By zooming in to the ±10 μeV region (Figure 6.10), a small amount of quasi-elastic broadening is observed as compared to vanadium, which is a resolution-limited peak located at E = 0. The UAD-U sample was measured at 230, 250, 270, and 290K (Figure 6.11). The zoomed in region between ±10 μeV (Figure 6.12) shows an increase in broadening as the temperature of the sample is increased. Diffusion is a thermally activated process. An increase in temperature should result in shorter relaxation times and accordingly a broader quasi-elastic signal. The observed broadening in both of these systems was found to be independent of Q. This indicates that the relaxation is due to a localized or rotational diffusion, rather than long-range transport of water molecules. To improve statistics, we have summed the data from the individual detectors with no loss of information.
Figure 6.9. QENS of UAD-W at 230K (red) and 270K (blue) compared to the vanadium standard (black).

Figure 6.10. QENS of UAD-W at 230K (red) and 270K (blue) zoomed in to ±10 µeV.
Figure 6.11. QENS of UAD-U at 230K (teal), 250K (green), 270K (red), and 290K (blue) compared to the vanadium standard (black).

Figure 6.12. QENS of UAD-U at 230K (teal), 250K (green), 270K (red), and 290K (blue) zoomed in to ±10 µeV.
By comparing UAD-W and UAD-U samples at 230K with the y-axis plotted on a log scale (Figure 6.13), a difference in the broadening is observed. At 230 K, the intrinsic width of the quasi-elastic signal is too narrow to permit a reliable estimation of the relaxation times. Nevertheless, the clear differences in the scattering between the UAD-W and UAD-U show that the water molecules are more mobile in the latter material than the former. However, the data at 270K (Figure 6.14) shows much more substantial broadening in both samples. Starting with the UAD-W sample at 270K, the data was plotted in the software package developed by NIST called DAVE (Figure 6.15). After a linear function was fit to the background, a delta function was fit to the large elastic signal, and a Lorentzian function to the quasi-elastic broadening. This resulted in a Lorentzian Full Width Half Max (FWHM) of 1.67\(\mu\)eV. After applying the same functions to the UAD-U data collected at the same temperature (Figure 6.16), a FWHM of 1.794\(\mu\)eV was obtained. These widths are resolution-corrected values. After dividing these numbers in

![Figure 6.13. QENS data of UAD-W (black) and UAD-U (blue) samples at 230K plotted on a log y scale.](image)
half to obtain the Half Width Half Max (HFHM), they were applied to Equation 6.1 shown below to generate $\tau$. This represents the time it takes for a hop or jump of the water molecule to occur.

$$\tau = \frac{\hbar}{\text{HFHM}}$$ \hspace{1cm} \text{Equation 6.1}

From this equation, $\tau = 788 \text{ps} \pm 37$ in UAD-W, which is longer than in UAD-U where $\tau = 734 \text{ps} \pm 17$. To calculate the localized diffusion rate of each system, Equation 6.2 was used.

$$\text{Rate} = \frac{1}{\tau}$$ \hspace{1cm} \text{Equation 6.2}

The localized diffusion rate of UAD-W was $1.3 \text{ ns}^{-1} \pm 0.06$, which is slower than for UAD-U which was $1.4 \text{ ns}^{-1} \pm 0.03$. Although these diffusion rates seem extremely similar, it is clear that the localized diffusion rate of UAD-U is faster than UAD-W by a statistically significant margin.

Equations 6.1 and 6.2 were also used to calculate $\tau$ and rate of diffusion for UAD-U at 230 and 290K. It was found that for every 20K jump in sample temperature (250 to 270K and 270 to 290K).
290K), the value of $\tau$ doubled. This therefore resulted in a doubling of the diffusion rate by this same temperature step. All data values can be found in Table 6.1.

**Figure 6.15.** Screenshot of UAD-W data at 270K plotted in DAVE software with elastic signal fit to delta function, quasi-elastic broadening fit to lorentzian function and background corrected.

**Figure 6.16.** Screenshot of UAD-U data at 270K plotted in DAVE software with elastic signal fit to delta function, quasi-elastic broadening fit to lorentzian function and background corrected.
Table 6.1. FWHM, $\tau$, and $1/\tau$ (rate) values from QENS data for UAD-W and UAD-U samples.

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<th>$\tau$ (ps)</th>
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6.4 Conclusions

Uric acid was used as a model compound to study the dynamics of water within the 1-D channels that may be affected by different degrees of disorder. An EWS of UAD-W and UAD-U revealed intrinsic differences between the water dynamics of these samples that were confirmed with QENS measurements. Calculations of $\tau$ at 270K for each sample were 788ps ± 37 and 734ps ± 17 respectively, leading to a faster localized diffusion rate of water in UAD-U vs. UAD-W (1.4 ns$^{-1}$ ± 0.03 vs 1.3 ns$^{-1}$ ± 0.06). A comparison of the UAD-U samples showed a doubling of $\tau$ and therefore of the diffusion rate every 20K jump in sample temperature. For the first time, we demonstrate quantitative differences in the water mobility in an organic hydrate with different degrees of molecular disorder.
Chapter 7. Summary of Work

Through the work detailed in this thesis, a better understanding of the crystallization and properties of uric acid in its many solid state forms was found. The main component of 10% of kidney stones\textsuperscript{42,44} and the sole phase identified in gout, uric acid primarily exists as anhydrous uric acid (UA), uric acid dihydrate (UAD), or deprotonated as urate with sodium as the counter ion (monosodium urate monohydrate, MSU). The observed phase is dictated by pH, which is typically low in the urine of stone formers (< 5.5)\textsuperscript{39} yielding UA or UAD, and closer to neutral in the interstitial fluid of those affected by gout, resulting in MSU. Its formation \textit{in vivo} is the result of purine metabolism in humans, though in many other species uric acid is oxidized further to the soluble molecule allantoin by the uricase enzyme.\textsuperscript{50} However, this enzyme was silenced by mutation in humans, and therefore leaves uric acid as the end product. Uric acid crystallization can be attributed mainly to its poor aqueous solubility, which under \textit{in vivo} conditions is 2.5 mM (37°C, pH = 6). This decreases drastically with pH, explaining its crystallization in those with low urinary pH (0.32 mM at 37°C, pH = 4).\textsuperscript{64}

The conventional picture of a uric acid kidney stone can be described as small crystalline fragments held together by an amorphous organic matrix. The matrix is thought to contain various impurities including biological pigments, which give rise to the stones color. After reproducing the synthesis of a urochrome pigment urorosein (Ur), recrystallization conditions were optimized to allow for the growth of single crystals. For the first time, the single crystal structure of this pigment was obtained from synchrotron x-ray diffraction data. Ur was found to degrade in methanol solutions but was stabilized by the presence of uric acid. Inclusion of the pigment was observed in both UA and UAD from aqueous growth solutions.
This work has demonstrated the ability of biological impurities to include within the crystalline lattice of uric acid, expanding the view of where “matrix” resides in these heterogeneous deposits. This expands the conventional picture of kidney stones and leads to question other impurities that may reside here as well. If it is possible in the future to obtain uric acid kidney stones samples, x-ray micro-tomography would be a valuable tool to use to examine these composites and identify other minor components. They could then be individually incorporated back into lab grown crystals to study their effects.

In an attempt to generate more soluble uric acid phases, cocrystals were synthesized with various pyridine and pyrimidine derivatives. Cocrystallization is a technique used often in pharmaceuticals to increase the solubility of active ingredients by combining them with an inert coformer.\(^{72-74,250}\) To identify compatible coformers for uric acid, hydrogen bonds that UA forms to its neighbors were examined. Molecules with the ability to form bi- and tridentate bonds were sought out, since they would bind more strongly to uric acid than the mono- and bidentate bonds it forms to itself.\(^8\) Six cocrystals were synthesized, of which one with 2-aminopyridine showed an increase in solubility over pure uric acid by \(>100\%\).

Through the success and failure of a group of coformers, the preferred characteristics of a good coformer were realized. These characteristics include rigid aromatic molecules with amino groups as hydrogen donors. Going forward, additional coformer molecules that fit this description should be attempted in order to find more cocrystals, creating the potential for more soluble uric acid phases. Coformers known for their solubility may have an advantage, however the characteristics of a cocrystal are not ever able to be predicted from those of their individual components. Once a great coformer potential is realized from preliminary methods of testing, it

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should be subjected to clinical testing to identify how it holds up in vivo and to identify any other obstacles that may present themselves.

Coformers that failed to form cocrystals sometimes resulted in other interesting results. 2-amino-5,6-dimethyl-4-hydroxypyrimidine (ADP) formed a (1\textit{H}) pyrimidin-4-one tautomer when recrystallized from aqueous solutions of 1:1 UA:ADP molar ratio powder. 1:4 ratios resulted in a 50/50 ratio of (1\textit{H}) and (3\textit{H}) pyrimidin-4-one tautomers under the same conditions. While UA and 2,6-diaminopyridine successfully formed a cocrystal from a 1:1 molar ratio, incidental sublimation of the coformer generated crystals of a new polymorph.

Non-crystalline material was generated in the cases of melamine and triamterene, which formed a film and a gel when recrystallized from aqueous solutions, respectively. Recrystallization of UA and 2,4-diaminopyrimidine in a 1:1 molar ratio formed a cocrystal, but 4:1, 10:1, 16:1, and 20:1 ratios yielded UAD with new morphologies. Specifically in the case of 4:1, a consistently unusual “inside out” epitaxial growth of UA was observed growing from the inside out of pyramidal UAD crystals. Eventually, the UAD crystals dissolved and the two UA crystals on either end merged to form one single UA crystal. Atomic force microscopy (AFM) was used to study the growth of UA in the presence of this coformer. The (100) surface of UA in the doped growth solution showed pits with depths >250 nm, which were completely absent from the pure growth solution where the surface appeared relatively smooth with randomly oriented steps.

Results from these failed cocrystal attempts remind us that unexpected results can be just as interesting as those expected. While uric acid crystallizes in humans, it is also the end product in avian and reptile species where it is excreted as an amorphous solid.\textsuperscript{57-59} If some of these coformers are able to change the properties of uric acid to remain as a non-crystalline material, this could have outcomes just as successful as cocrystals with increased solubility. While the two
non-crystalline products described here were found by chance, studying the excrement of these avian and reptile species may give insight into how their systems are able to prevent uric acid crystallization.

The final section of my work is presented as a stand-alone piece investigating the water dynamics of UAD lattices, but was really sparked by the work of past and present group members who have also investigated differences in the properties of UAD crystals grown from water (UAD-W) and from model urine (UAD-U). However, the solution transformation rate from UAD to UA of the two systems studied by Dr. Janeth Presores was found to occur with vastly different timescales.\textsuperscript{245} Additionally, monitoring the dissolution of (001) face of UAD from the two growth conditions by AFM showed a smooth surface for UAD-W and granular areas on the (001) surface of UAD-U. This implied that two different growth mechanisms formed each of these crystals. Smaller differences in the microstructure of these systems are hidden most likely by the inherent disorder in the UAD crystal structure, which may occur with varying degrees in the two UAD systems. This led us to focus on the motions of the water mobility, which would vary depending on its local environment. Neutron scattering allowed us to probe these motions, where it was found that water in UAD-U diffused faster than water in UAD-W.

Quantitative differences in these two seemingly identical crystals were calculated for the first time. In order to support these findings, computational calculations should be carried out by modeling ordered and disordered UAD lattices. Preliminary work by Alysia Zevgolis shows that this likely is the case.
Appendix. Permissions for Figures and Tables

Figures 1.3 and 6.2:

Janeth Presores

to me  

Hi Victoria,

Yes, you may include Figures 1.3 and 5.10 from my dissertation in your dissertation.

Figures 1.5 and 5.1:

Glenn Austin

to me  

Dear Ms. Hall:

You may utilize the uric acid images in your dissertation.

Thank you,
Glenn

Glenn W. Austin, President
Louis C. Herring & Co.
1111 S Orange Ave., FL 2
Orlando, FL 32806-1236
Tel: 407-841-6770

Figure 1.7:
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Figures 2.2-2.6 and 2.10-2.13:
Figures 4.1-4.7 and Tables 4.1-4.4:
Figure 5.2:
Figure 6.6:

Prisk, Timothy R. (Fed)
to me ✗

Hi Victoria,

I uploaded pictures of HFS to Google Drive: https://drive.google.com/open?id=1VcP-rfSmC-JlMeUjIMUaTggZQj2-Frhe

Please credit the photographs to Yiming Qiu.

Cheers,

Timothy
Hmm, I don’t remember where I got this one from!

In last year’s lecture I changed it to a more picturesque version, that I definitely made myself (probably worse of course!):

S(Q,\omega)

Quasi-elastic

Elastic

Inelastic

\omega=0

Energy Transfer

I think in both cases you can reference a private communication.

Best

Vicky
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strategies for the preparation of designed solids. an investigation of the 2-amino-4(1H)-
pyrimidone ring system for the molecular self-assembly of hydrogen bonded alpha and beta


