Interactions of Melamine with Physiological Constituents

by

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Abstract

Melamine is found in many items belonging to households, schools, canteens, paints, and hospitals as well as fertilizers, and seed coatings. Food contaminated with melamine can potentially cause renal problems or formation of bladder and kidney stones since melamine-cyanuric complexes have been reported to cause renal tubule blockage. In this study, Raman microscopy and thermogravimetric analysis were used to determine the composition of melamine-oxalate crystals formed in the presence of physiological components such as uric acid, L-cystine, urea, and creatinine. Crystals were made in water and artificial urine with melamine and oxalic acid at molar ratio of 1:10 together with a physiological component. The formation of melamine-oxalate crystals in water and artificial urine suggests that melamine oxalate can interact with physiological components to form three component crystals.

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Terms and Definitions

1. Melamine: Is a nitrogen-based chemical used in many industries including production of household and hospital items.

2. Raman Microscopy: Raman Spectroscopy is a non-destructive chemical analysis technique which provides detailed information about chemical structure, phase and polymorph, crystallinity and molecular interactions.

3. Thermogravimetric Analysis: A technique in which the mass of the sample is monitored against time or temperature while the temperature of the sample, in a specified atmosphere, is programmed.

Abbreviations

1. Melamine: mel
2. Cystine: Cys
3. Creatinine: Crea
4. Oxalic Acid/Oxalate: Oxa
5. Thermogravimetric analysis: TGA

CHAPTER I

Introduction

Melamine is found in many households, schools, canteen, and hospital objects such as dishware, countertops, containers, paint, dry erase boards, non-stick pots, and pans. In 2007, melamine was used to falsify and boost the apparent protein levels of grains that were used to produce pet foods. As a result, pet fed melamine-tainted food suffered renal failure (Brown et al., 2007). In 2008, melamine was added to milk formula for the same reason resulting in renal failure and renal stones in babies (Brown et al., 2007). Renal failure in pets and babies was due to the formation and deposition of the melamine-cyanuric acid complex or kidney stones in kidney tubules that led to renal tubule blockage (Gossner et al., 2009).

A typical kidney stone is composed of calcium oxalate, uric acid, struvite, and cystine. Bladder stones can have calcium oxalate, calcium phosphate, ammonium urate, cysteine, and magnesium ammonium phosphate (Khan 2016). The effect of melamine toxicity when mixed with cyanuric acid, a metabolic result of melamine, has been the focus of scientists' investigation.

(Khan 2016).

Other research has investigated the toxicity of melamine and how it affects stone formation at various quantities of melamine (Thanasekaran et al. 2012). Thanasekaran et al. 2012 also found that melamine plays a role in the formation of calcium oxalate stones even at low concentrations. Dong et al. 2019, found evidence of melamine-uric acid stone formation in rats. Although Takazawa et al. reported that cyanuric acid leaching in

tableware is negligible, many studies used cyanuric acid in conjunction with melamine as starting materials for crystallization. Fertilizers containing melamine cause the transfer of melamine from pasture to cow milk (Botha, 2010). Scientists have studied dual-component melamine stone formations such as cyanuric acid: melamine (Khan 2016), uric: melamine (Dong 2019), and calcium oxalic: melamine (Thanasekaran et al. 2012), but no one has looked into triple-component melamine crystal formation induced by melamine and oxalic acid.

The focus of this research is to investigate melamine and oxalic interactions with physiological constituents such as L-cystine, uric acid, urea, and creatinine. Crystals are formed in this study using melamine and oxalic acid in molar ratios of 1:10. Melamine concentrations are held constant at 4 mM for this study, while the other components required to form the crystals are varied. The constituents are mixed in water and artificial urine as the medium. The crystals are prepared in water as well as artificial urine as the medium. Raman microscopy coupled with Raman spectroscopy, and Thermogravimetric analysis (TGA) were used for the characterization of crystals.

Raman spectroscopy technique is used in a wide variety of applications. Raman spectroscopy uses a monochromatic laser beam on a sample. This light can be scattered, absorbed, or reflected. Scattering can occur due to Rayleigh scattering or Raman scattering of photons. Raman scattering is due to inelastic collisions of photons with the molecules of the sample. The photons can be used to determine the vibrational modes of the bonds in the sample molecules.

Thermogravimetric analysis (TGA) can also be useful for characterization of sample by determining a sample's thermal stability, oxidative stability, decomposition

temperatures, as well as percent composition. The technique measures weight loss as a function of temperature in a controlled atmosphere. The weight changes over increase in temperature in the plot indicates transitions in the decomposition of the substance and this information is specific to the compound analyzed.

Thesis Statement

Melamine can readily complex with oxalic acid, a common physiological component, to form crystals. This study investigates whether such interactions between melamine and oxalic acid can induce a third component to be included into the crystalline complex. The molecular interactions of melamine with physiological constituents such as oxalic acid, uric acid, L-cystine, urea, and creatinine have been interpreted using Raman spectroscopy and thermogravimetric analysis of melamine crystals formed in water and in artificial urine.

CHAPTER II

Materials and Methods

All chemicals for research are available in Dr. Beng Ooi's research laboratory at Middle Tennessee State University's Department of Chemistry.

Materials for crystal preparation

The melamine (99+ % purity), uric acid (99+%), and creatinine (98%) were purchased from Aldrich (St. Louis, Missouri), whereas oxalic acid (100.2 %), L-cystine, urea(99%) were purchased from Baker Chemical Co (Phillipsburg, New Jersey), Alfa Aeser (Ward Hill, Massachusetts), and Fisher BioReagents respectively (Fair Lawn, New Jersey) respectively. Stock solutions of 3,240 ppm melamine, 143,000 ppm oxalic acid, 60 ppm uric acid, 112 ppm L-cystine, 539,339 ppm urea, and 200.22 ppm creatinine were prepared in deionized water. These were used for making crystals in deionized water.

Methods for crystal preparation

Crystals were formed in deionized water with varying concentrations of oxalic, uric, L-cystine, urea, and creatinine. Throughout the experiment, melamine was kept at a constant concentration of 4 mM.

The melamine oxalate crystals were prepared by mixing melamine and oxalic acid solutions at molar ratios of 1:10 and left at room temperature to crystalize slowly. Varying

concentrations of L-cystine, urea, uric acid, or creatinine mixture was added to synthesize melamine: oxalic acid plus a third component crystal. If the addition of a third component with the 1:5 molar ratio melamine: oxalic acid mixture did not result in crystal formation the experiment was repeated using melamine: oxalic acid at molar ration of 1:10.

Crystals of melamine-oxalate and melamine-oxalate plus a third component were synthesized using control/artificial urine purchased from Innovating Science (Avon, New York). The control artificial urine was warmed in the 37 °C incubators. Melamine-oxalate crystals in urine were prepared using melamine: oxalate 1:10 molar ratio.

All crystals that were prepared in deionized water were also made using artificial urine in place of water. In order to prepare the three-component crystals, L-cystine, uric acid, urea, and creatinine were dissolved in prewarmed artificial urine at concentrations of 91.39 ppm, 49.1 ppm, 41742 ppm, and 200.107 ppm respectively before combining with melamine in synthetic urine and oxalic acid. To reduce the chelating effect of the components in synthetic urine, only the oxalic acid stock solution is in deionized water rather than urine. The final concentration of each component are the same that is used for making crystals in deionized water (see table 1).

Table 1: Concentration in Molarity of Crystal components made in dH2O and Artificial Urine

Crystal	Melamine	Oxalic	Third Component
Mel-Oxa	4 mM	40 mM	
Mel-Uric-Oxa	4 mM	40 mM	0.292 mM
Mel-Cyst-Oxa	4 mM	40 mM	0.38 mM
Mel-Urea-Oxa	4 mM	40 mM	695 mM
Mel-Crea-Oxa	4 mM	40 mM	1.77 mM

Melamine is abbreviated as "mel", oxalate: "oxa", cystine: "cyst", and creatinine: "crea".

Raman Microscopy:

Crystals are laid out on copper foil that has been cleaned with 2mM HCl and rinsed with deionized water. Before Raman analysis, these crystals are dried in a desiccator. The Enwave Raman Spectroscopy (ProRaman-L, model uSense-I-785) was initially used for the acquisition of crystal vibrational modes and the OMNIC software for data processing. Images and spectral signals were collected using the Horiba HR Evolution Confocal Raman Microscope (Piscataway Township, New Jersey). Data from Raman spectroscopy was not used due to lack of accuracy during analysis compared to Horiba Raman Microscope. Horiba Raman Microscopy (Piscataway Township, New Jersey) spectra was used to identify trends in the spectra associated with neat crystal components as well as components interacting with one another in the crystal lattice.

Three component crystals in dH2O and artificial controlled urine were compared with neat components, melamine-oxalate crystals in dH2O and artificial urine.

Thermogravimetric analysis

Thermogravimetric analysis (TGA) is utilized for crystal characterization through the analysis of the characteristic decomposition pattern. For selected crystals, single-crystal X-ray diffraction analysis was performed to determine the precise dimensions of unit cells and the position of atoms within a crystal lattice. The TGA samples were run in a nitrogen atmosphere. The first experiments were run under standard conditions: 10 °C/minute. The experimental conditions were changed to increase the sensitivity of TGA to weight changes of samples to improve the detection of third component in the crystal. Mass Flow Control settings are 60 mL/minute of nitrogen gas. The heating rate was set at 50.0 °C/minute with the final temperature at 1000.00 °C. As weight loss is detected the heating rate is decreased to 1-2 °C/minute (which is machine controlled). The heating rate was set to high resolution-dynamic sensitivity (4.00) setting to detect changes in weight loss and decrease the mass flow. High resolution-dynamic sensitivity distinguishes overlapping decomposition events by continuously altering the heating rate in response to weight loss.

CHAPTER III

Results and Discussion:

Analysis of crystals made in deionized water using Raman spectroscopy and TGA:

Crystals made in deionized water were analyzed using Raman microscopy to detect changes in vibrational modes in the three components melamine crystals spectra compared to melamine-oxalate crystals spectra. The figures below are spectra comparison of the three components crystals and melamine-oxalate crystals.

Melamine-uric-oxalate crystals in water have the fibrous-like morphologies with a mixture of thickness (Figure 1) compared to the compact rods/stem-like mel-oxa crystals. The detection of the unique peaks is attributed to the differences in vibrational modes due to uric acid interaction with both melamine and oxalate. Peaks at 624 cm-1, 651 cm-1, 1038 cm-1, and 1127 cm-1 are present in the spectra of uric acid neat and melamine-uric-oxalate indicating those are due to uric acid (Figure 2). The other labeled peaks are probably due to uric acid interactions with melamine and oxalate in the crystal structure.

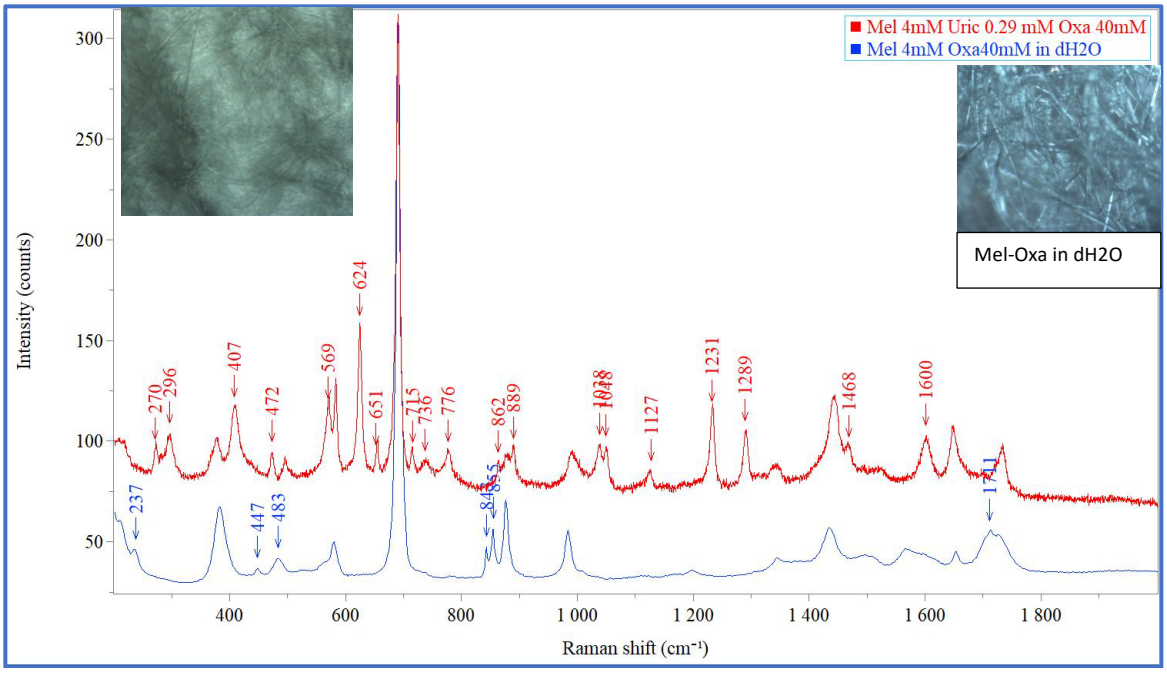

Figure 1. Characterization of Melamine-Uric-Oxalate Crystals in dH2O Using Raman Microscopy

Raman microscopy detected one morphological crystal "stem" form that is different from melamine-oxalate. Peak at 270, 296, 407, 472, 569, 624, 651, 715, 736, 776, 1028, 1048, 1231, 1289, and 1600 cm-1 are unique to mel-uric-oxalate crystal. Inset images of melamine-uric-oxalate (left) and melamine-oxalate (right) were taken at 50x magnification. Peaks labeled on the melamine-oxalate spectra are not detected in the melamine-uric-oxalate spectra.

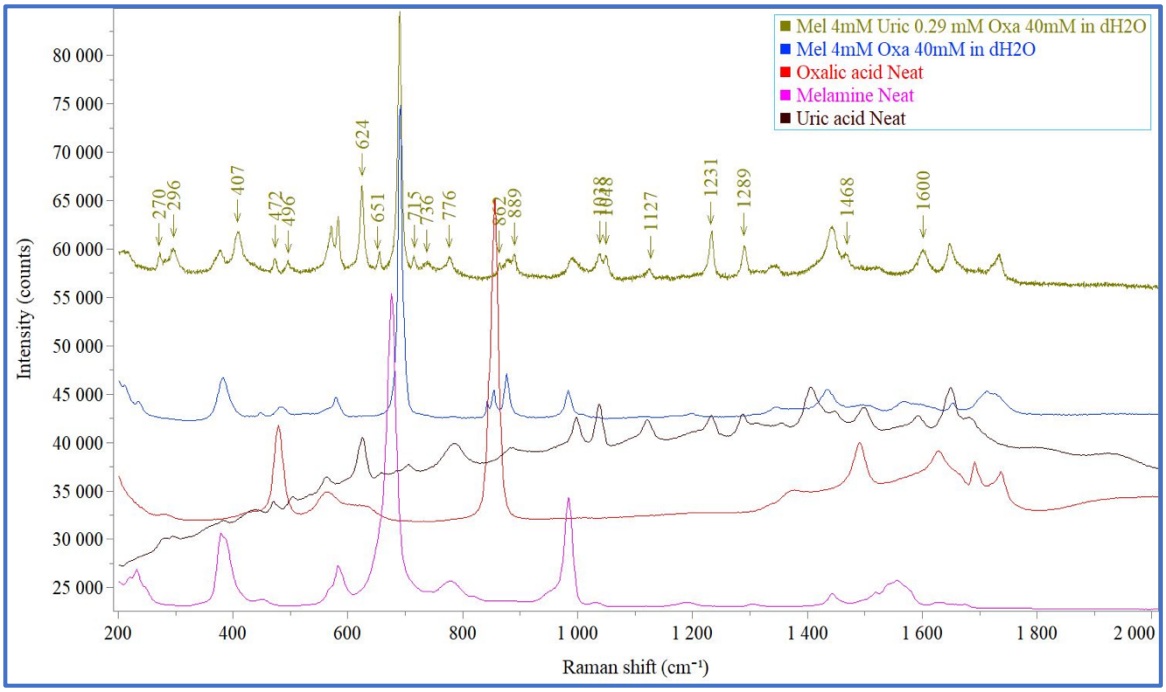

Figure 2. Characterization of Melamine-Uric-Oxalate Crystals in dH2O Using Raman Microscopy

Peaks labeled in the melamine-uric-oxalate spectra are not found in mel-oxa crystal or in the neat melamine compound. Peaks at 624 cm-1, 651 cm-1, 1038 cm-1, and 1127 cm-1 are only observed in the spectra of uric acid neat and mel-uric-oxa crystal.

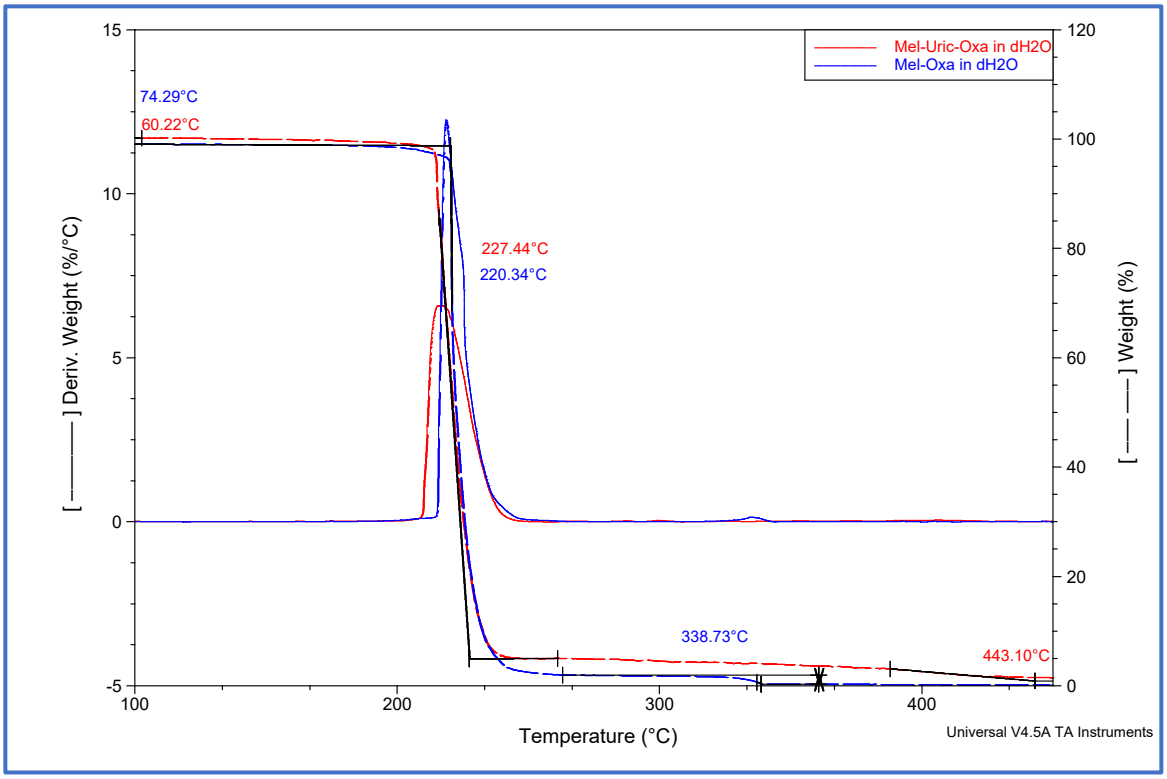

Figure 3. Characterization of Melamine-Uric-Oxalate Crystals in dH2O Using Thermogravimetric Analysis

Thermogravimetric Analysis of melamine-uric-oxalate crystal made in deionized water (red) shows three unique degradation steps at 60.22 °C, 227.44 °C, and 443.10 °C. Solid lines are the % weight loss versus temperature paths of each crystal. Dashed lines are the derivatives of the solid lines showing weight change per °C.

Thermogravimetric analysis (TGA) of mel-uric-oxa crystals in deionized water shows three thermal decomposition onset temperatures of 60.22 °C, 227.44 °C, and 443.10 °C (Figure 3). The initial degradation step in the melamine-uric-oxalate crystal and melamine-oxalate are due to the loss of small amount of moisture in the crystals (see Table 2). Mel-uric-oxa initial onset decomposition of 60.22 °C which is lower than mel-

oxa at 74.29 °C indicating that water is bound stronger in melamine-oxalate than mel-uric-oxa crystal. On the other hand, the second and third degradation steps had an onset temperature that is significantly higher in mel-uric-oxa crystal than melamine-oxalate indicating that uric acid has strong interaction with melamine and oxalate. Differences in percent decomposition of components and maximum temperatures attained at each of the phases are observed (see Table 2). These differences in the TGA data of mel-uric-oxa crystals compared to mel-oxa crystals suggest the differences are due to uric acid interactions with melamine and oxalate in the crystals. In addition, Raman spectra peaks of mel-uric-oxa crystals that matches with peak in uric acid neat spectra are not observed in the mel-oxa crystal spectra are further evidence that the mel-uric-oxa does contain uric acid.

Table 2: Thermogravimetric Analysis of Crystals made in dH2O

Crystal	Onset Decomp. Temp	Max. Temp.	Percent Decomposition	Residues
Mel-Oxa in dH2O	74.29 °C 220.34 °C 338.73 °C	83.34 °C 218.95 °C 335.57 °C	0.972 % 97.11 % 1.694 %	982.27°C 0.7077%
Mel-Uric-Oxa in dH2O	60.22 °C 227.44°C 443.10 °C	216.48 °C	0.8504% 94.87 % 4.758 %	956.78 °C 0.4858 %
Mel-Cyst-Oxa in dH2O	209.95 °C 258.66 °C 320.28 °C	211.49 °C 261.67 °C 324.05 °C	98.39% 0.31% 1.69%	982.52 °C 0.6562%
Mel-Urea-Oxa in dH2O	106.52 °C 114.37 °C 218.84 °C	113.85 °C 114.66 °C 215.56 °C	2.214% 19.40% 78.57 %	983.43 °C -0.257%
Mel-Crea-Oxa in dH2O	205.15 °C 209.52 °C 222.07°C	207.90 °C 213.84 °C 215.73 °C	2.522% 28.52% 70.44%	982.11°C 0.019%

Melamine-cystine-oxalate (mel-cys-oxa) crystals in deionized water had two morphological shapes i.e the cluster and stem-like/ rod shaped (Figure 4). The melamine-oxalate crystals also formed “stem-like” crystal structures but, the crystals are slender and more compact (Figure 1). Melamine-oxalate crystals lack the “cluster” shape morphology seen in mel-cys-oxa crystals. These differences were detected in the Raman spectra as unique peaks, 772 cm-1 in the “cluster” and 1103 cm-1 in the “stem” spectra (Figure 4). Peak found at 1470 cm-1 in both “cluster” and “stem” is due to interaction of cystine with melamine and oxalate. The Raman spectra of mel-cys-oxa crystals does not have the peaks at 237 cm-1 and 449 cm-1 that are present in the spectra of melamine-oxalate crystals (Figure 4). Such differences suggest that mel-cys-oxa and the mel-oxa are different crystals. The differences can be attributed to the differences in vibrational modes because of cystine being part of crystals and is interacting with both melamine and oxalate. Thermogravimetric analysis of melamine-oxalate in water shows three thermal decomposition onset temperature at 74.29 °C, 220.34 °C, and 338.73 °C (Figure 5). On the other hand, the thermal decomposition onset temperatures at 209.95 °C, 258.66 °C, and 320.28 °C for mel-cys-oxa crystal formed in water are different (Figure 5). Other differences are percent decomposition of components and maximum temperatures attained at each of the phases (see Table 2). Initial onset temperatures of 74.29 °C and 0.97% weight loss in the mel-oxa crystals indicate the presence of a very small amount of water vaporization/desorption not seen in mel-cys-oxa crystal. The higher onset temperature for the mel-oxa crystal at the second and third degradation phase suggest weaker hydrogen bonding in the three components crystals as well as difference in composition between the two crystals.

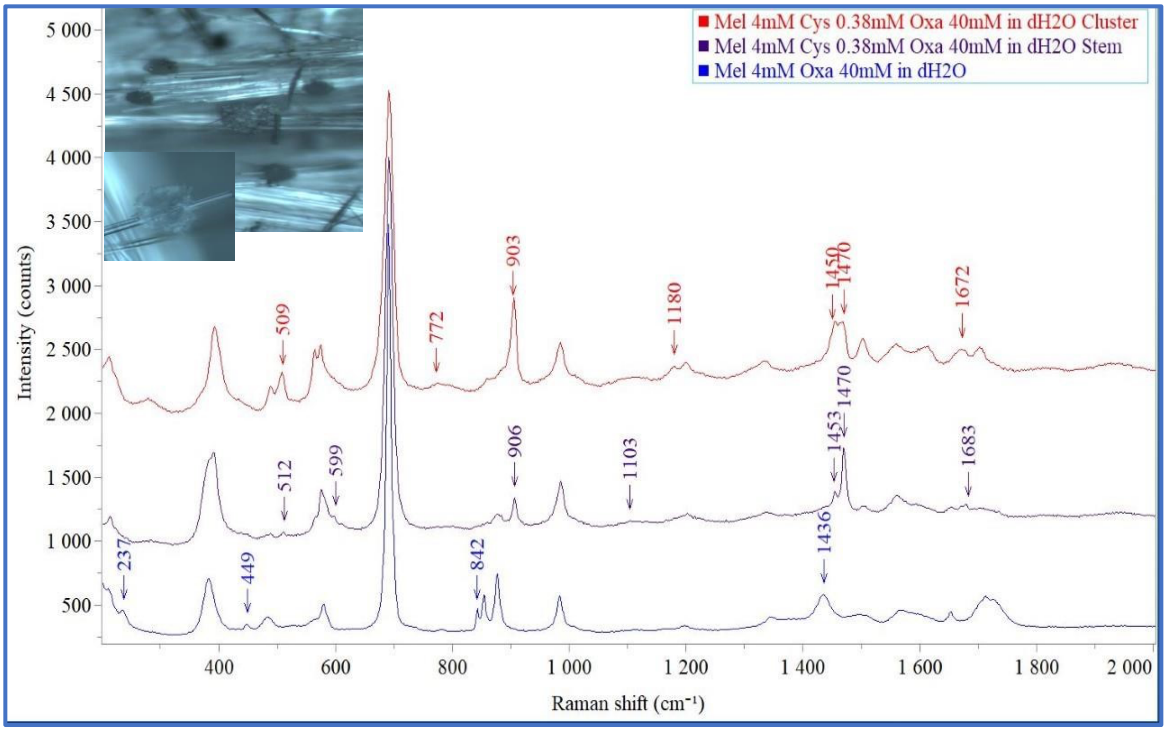

Figure 4. Characterization of Melamine-Cystine-Oxalate Crystals in dH2O Using Raman Microscopy.

Raman microscopy detected two different morphological crystal forms ("cluster and "stem") each with slightly different spectra. Peaks at 772 cm-1 is unique to the "cluster" spectra. The 1103 cm-1 peak in the "stem" spectra is shifted to 1180 cm-1 in the "cluster" spectra. In addition, those peaks as well as the 509/512 cm-1, 903/906 cm-1, 1450/1453 cm-1, and 1470 cm-1 peaks are not present in the spectra of melamine-oxalate crystal. Peaks at 237 cm-1, 449 cm-1, 842 cm-1, and 1436 cm-1 in the melamine-oxalate crystal are not present in the mel-cys-oxa spectra. Images inset were taken at 50x and 10x magnification.

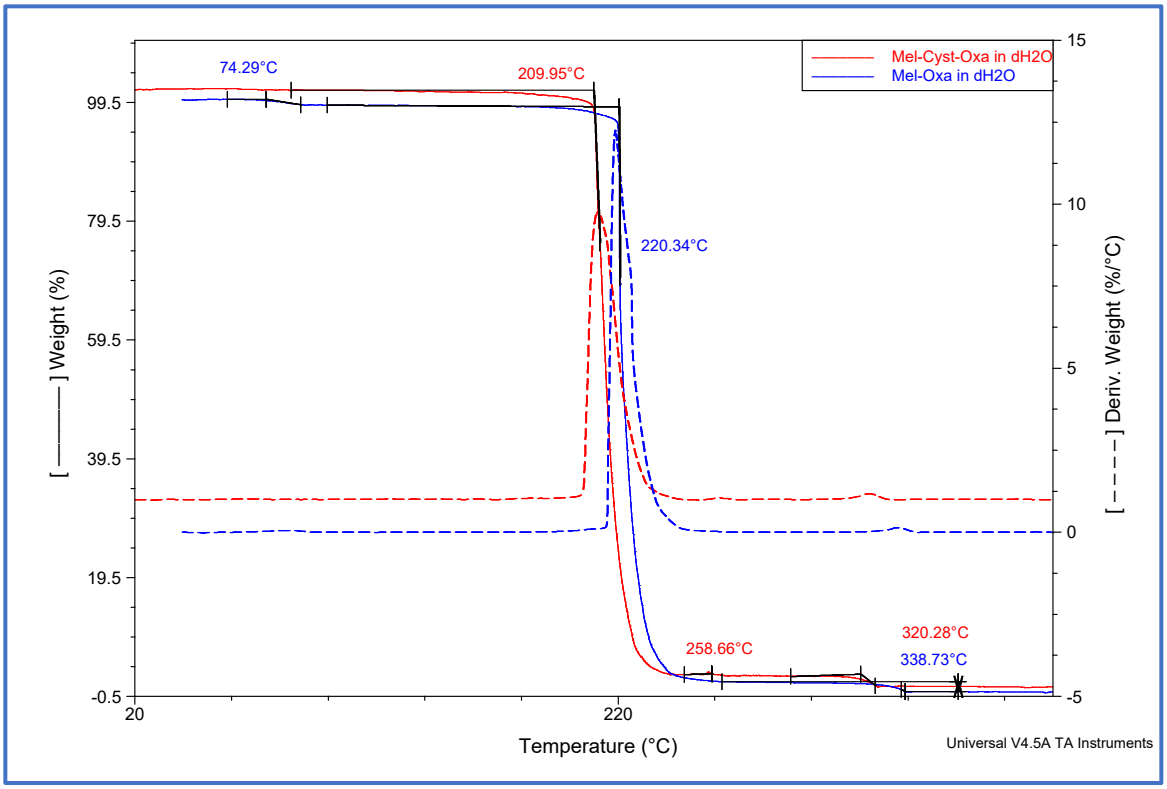

Figure 5. Characterization of Melamine-Cystine-Oxalate Crystals in dH2O Using Thermogravimetric Analysis

The thermal decomposition onset temperatures for the crystals are labeled with their corresponding colors. Solid lines are the % weight loss versus temperature paths of each crystal. Dashed lines are the derivatives of the solid lines showing weight change per °C. The onset temperature for the thermal degradation of melamine-cystine-oxalate crystal are at 209.95 °C, 258.66 °C, and 320.28 °C, whereas the melamine-oxalate decomposition onset temperatures occurred at 74.29 °C, 220.34 °C, and 338.73 °C (see table 2).

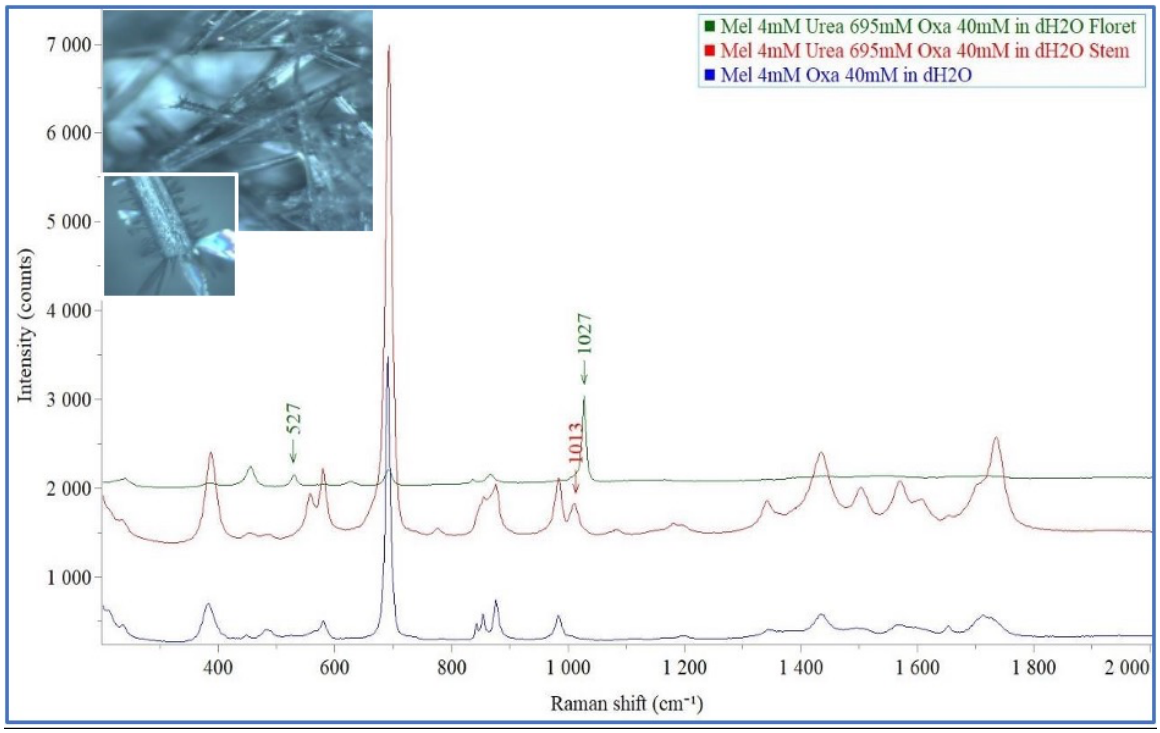

Figure 6. Characterization of Melamine-Urea-Oxalate Crystals in dH2O Using Raman Microscopy

Raman microscopy detected a “floret” and “stem” morphological form each with slightly different spectra. Peak at 527cm-1 is unique to the “floret” spectra. The 1013 cm-1 peak in the “stem” spectra is shifted to 1027 cm-1 in the “floret” spectra. The inset images were taken at 50x and 10x magnification.

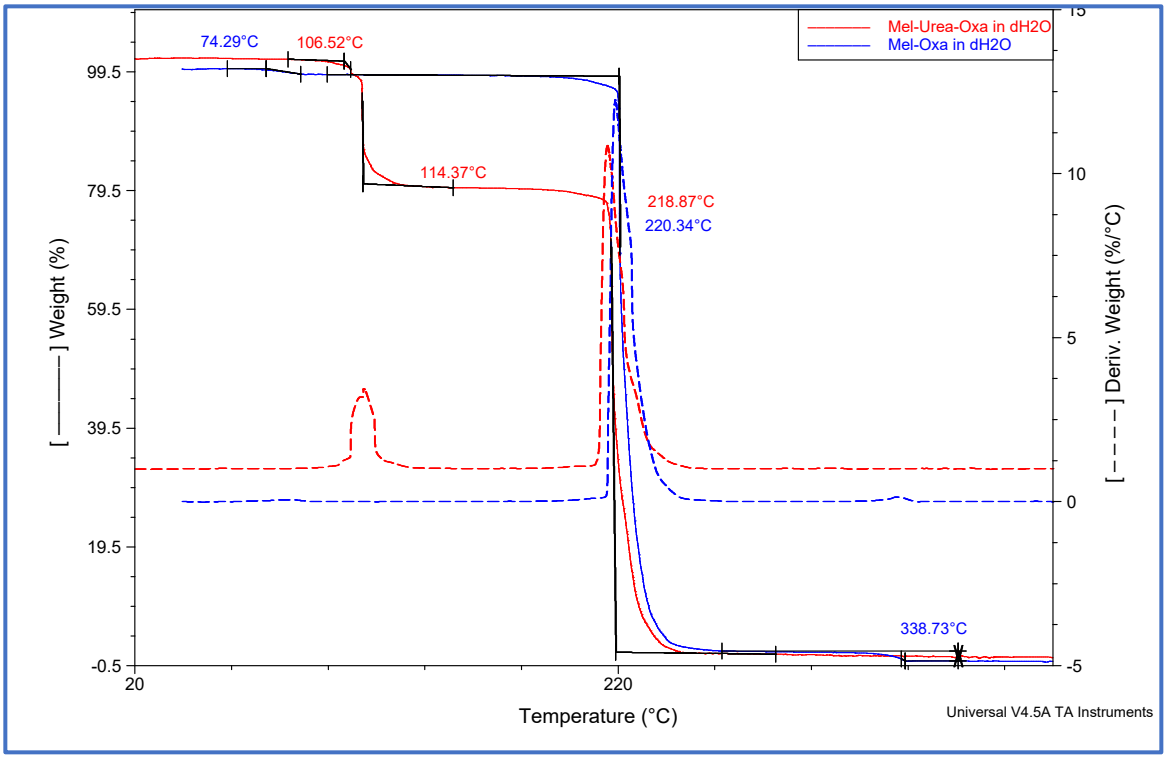

Figure 7. Characterization of Melamine-Urea-Oxalate Crystals in dH2O Using Thermogravimetric Analysis

Thermal onset decomposition temperatures for the crystals are labeled in their corresponding colors. The melamine-urea-oxalate crystal has three unique thermal decomposition onset temperatures of 106.52 °C, 114.37 °C, and 218.87 °C, which is different from melamine-oxalate crystal (see Table 2).

Melamine-urea-oxalate crystals in deionized had two morphologies, “floret” and “stem-like/rod” shape (Figure 6). Although melamine-oxalate crystals are also“stem-like” in appearance, the crystal structures are finer and more compact (Figure 1). Mel-oxa crystals lack the “floret” shape morphology seen in mel-urea-oxa crystals. Raman microscopy identified these differences as unique peaks in the “floret” spectra at 527 cm-

1 and 1027 cm-1 and at 1013 cm-1 in the “stem-like” spectra (Figure 6). These differences indicate changes in vibrational modes due to the presence of urea in the crystal and its interaction with melamine and oxalate. Thermogravimetric analysis of mel-urea-oxa crystal in deionized water shows three thermal decomposition onset temperatures at 106.52 °C, 114.37 °C, and 218.84 °C that are different from that of mel-oxa crystals (Figure 7). Other differences are percent decomposition of components and maximum temperatures attained at each of the phases (see Table 2). The initial onset temperature of 106.52 °C in the melamine-urea-oxalate crystal is at higher temperature compared to 74.29 °C in mel-oxa indicating stronger interactions of water with the components in the mel-urea-oxalate crystal. On the other hand, the second and third onset degradation temperatures of mel-urea-oxa crystal is lower than melamine-oxalate indicating the crystals compositions are different and to the presence of urea causes a weaker interaction in the crystals. Hence, the TGA confirmed the Raman spectroscopy data that the two crystals are not the same.

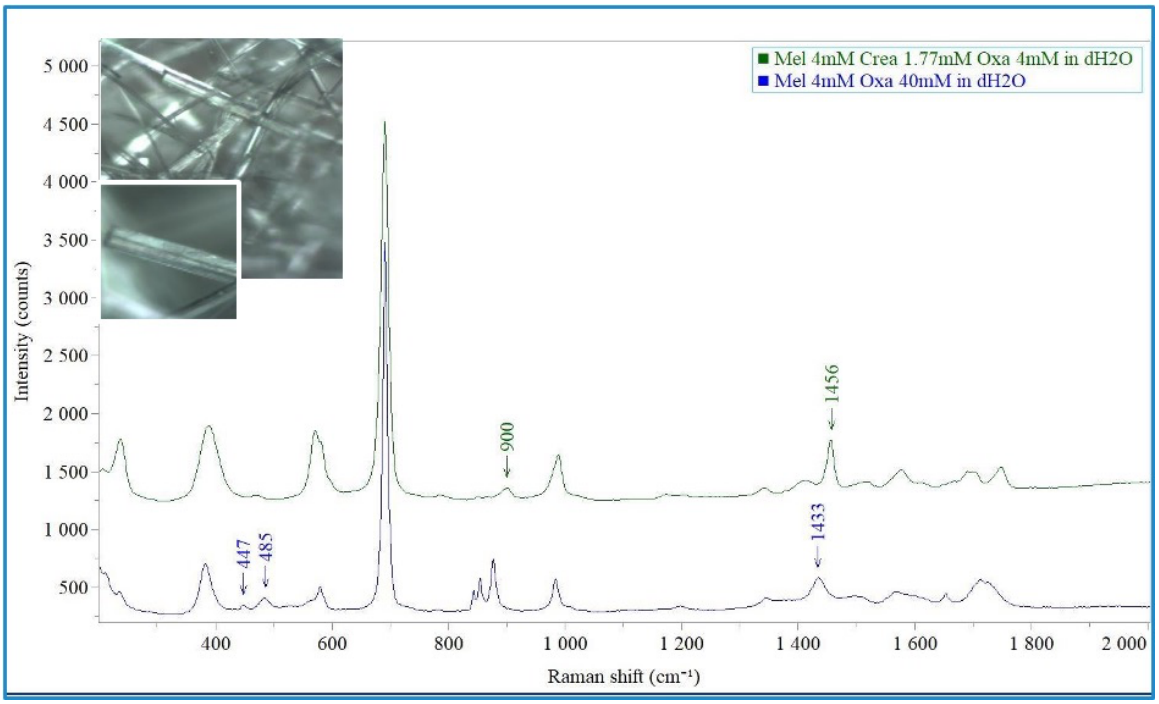

Figure 8. Characterization of Melamine-Creatinine-Oxalate Crystals in dH2O Using Raman Microscopy.

Raman microscopy detected one morphological structure of varying thickness in the melamine-creatinine-oxalate crystal. Peaks at 1456 cm-1 is unique to this crystal spectra, while peak at 900 cm-1 is a creatinine peak. Peaks at 447 cm-1, 485 cm-1, and 1433 cm-1 in the melamine-oxalate spectra was not observed in melamine-creatinine-oxalate crystals. Inset images were taken at 50x and 10x magnification.

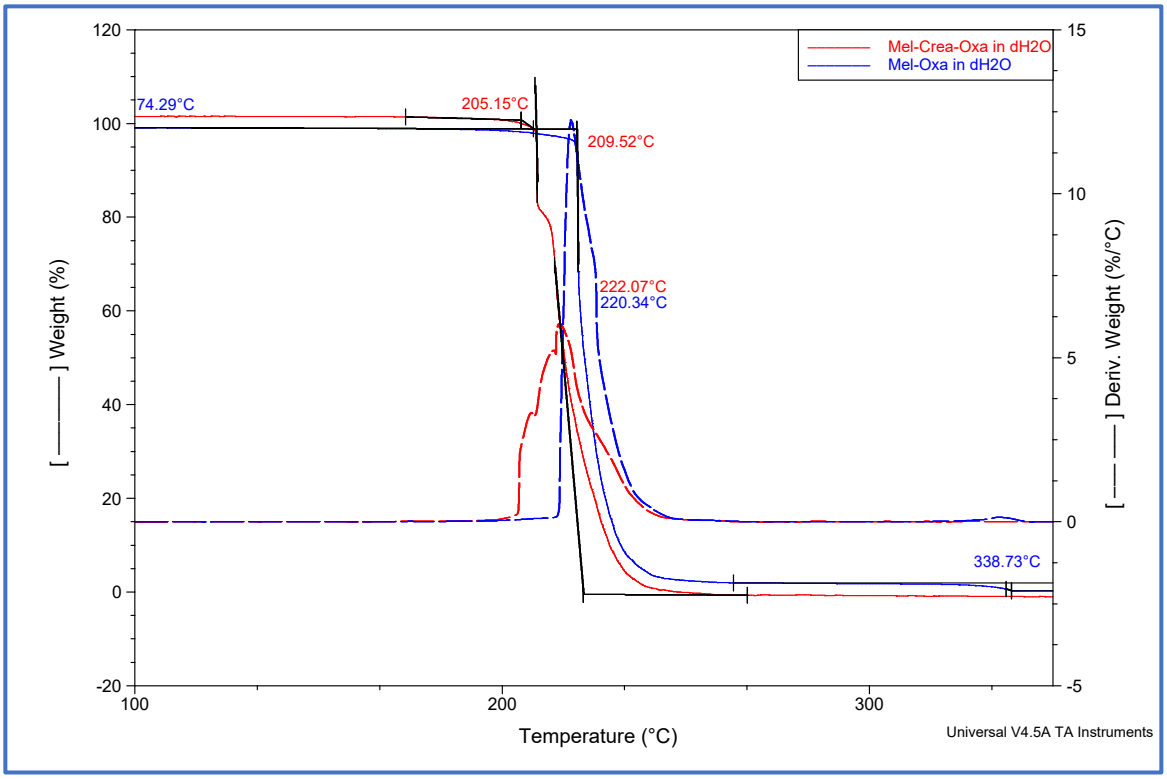

Figure 9. Characterization of Melamine-Creatinine-Oxalate Crystals in dH2O Using Thermogravimetric Analysis

Melamine-creatinine-oxalate crystal has three unique degradation steps that are different from melamine-oxalate with onset decomposition temperatures at 205.15°C, 209.52 °C, and 222.07 °C (see Table 2).

Melamine-creatinine-oxalate (mel-crea-oxa) and melamine-oxalate crystals in water both have the "stem-like/rod" morphologies but courser and the mel-crea-oxa crystals are less compact compared to the mel-oxa crystal. Raman microscopy identified between differences in vibrational mode between the mel-crea-oxa crystals and mel-oxa crystals spectra observed in figures. The 900 cm-1 peak in the mel-crea-oxa spectra is also found in creatinine neat spectra confirming these peak is due to creatinine. However,

the mel-crea-oxa spectra lack peaks at 447 cm-1, 485 cm-1, and 1433 cm-1 that are in the melamine-oxalate spectra (Figure 8). These differences in vibrational modes indicate changes in vibrational modes due to the presence of creatinine in the crystal and its interaction with melamine and oxalate components. Thermogravimetric analysis of melamine-creatinine-oxalate crystal made in deionized water shows three thermal decomposition onset temperature that are different from mel-oxa crystal (Figure 9). The first onset degradation temperature in melamine-oxalate is due to moisture desorption/vaporization from the crystal, which is not found in mel-crea-oxa crystal. The onset temperature for thermal degradation of mel-crea-oxa crystal is at a lower temperature of 205.15 °C compared to melamine-oxalate onset temperature of 220.34°C suggesting that the three components' crystals have weaker hydrogen bonding between the components (Figure 9). The differences in the TGA data of melamine-creatinine-oxalate crystals compared to melamine-oxalate crystals suggest the differences are due to creatinine's interactions with the crystals matrix.

Raman microscopy of all crystals revealed that the spectra of these crystals are distinguishable although there are overlapping peaks (Figure 10). The peaks at 900 cm-1, 906 cm-1, and 889 cm-1 in the mel-crea-oxa, mel-urea-oxa, and mel-uric-oxa, respectively are shifted from the 877 cm-1 peak of the mel-oxa spectra suggesting changes in the interaction as a result of the third component in the crystals. There is also the presence of unique peaks in each crystal spectra due to interaction of the third component that changes in vibrational modes of melamine-oxalate interactions significantly. These are proof that all these crystals are different from one another and also different from the melamine-oxalate crystals.

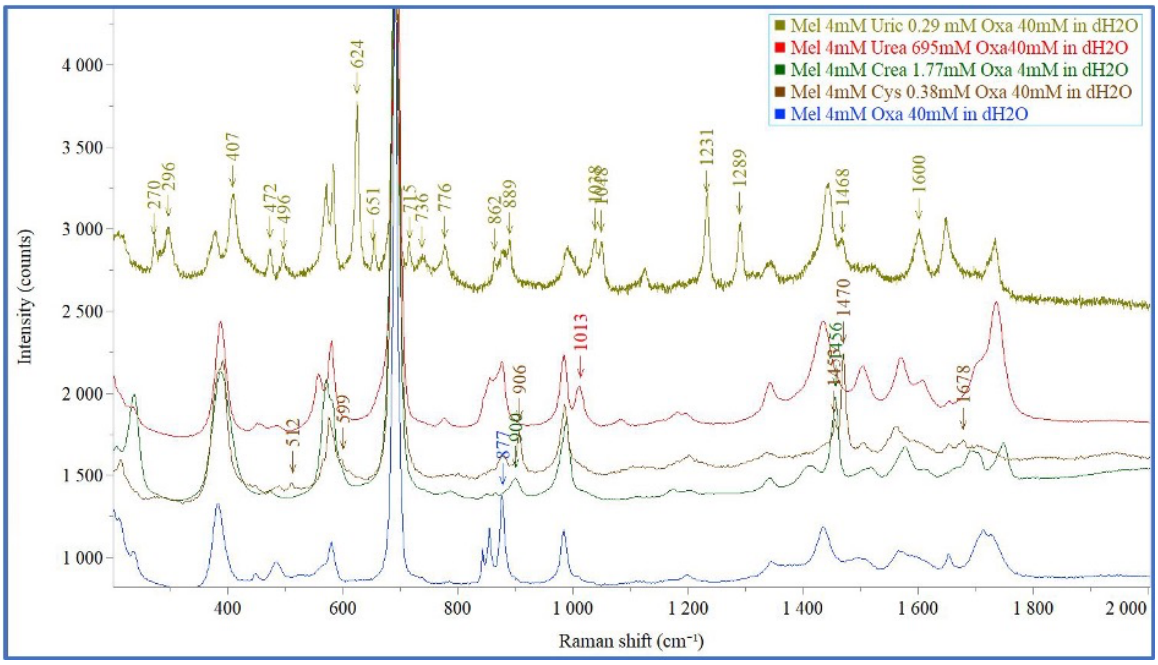

Figure 10. Comparison of Raman Spectra of Crystals Made in Deionized Water

Analysis of crystals made in artificial urine using Raman spectroscopy and TGA:

Despite the fact that the artificial urine media is unusual since this contained detergents and preservatives, melamine was able to form crystals with oxalate. This is proof that the interactions between melamine and oxalate must be highly favored such that the unusual media did not prevent the crystals formation. Similarly, the melamine together with oxalic acid was able to promote the incorporation of a third component such as urea and cystine forming the three component crystals in the artificial urine. This further suggest that the interactions between the components must be strong and stable enough to form under such adverse conditions. However, even though these crystals can form in both types of media, the molecular interactions may vary depending on whether the crystals were formed in deionized water or in artificial urine. The same analysis

technique and data interpretation used previously for crystals made in deionized water were also applied to crystals made in artificial urine.

Figure 11 show that the Raman spectra of the three components mel-urea-oxa and mel-cyst-oxa crystals are unique and distinguished because they have variations in molecular interactions. Spectral differences in the mel-urea-oxa and mel-cyst-oxa in the Raman signals suggest that urea and creatinine are respectively present and are interacting with melamine and oxalate in the crystals made in artificial urine. However, Raman analysis of mel-crea-oxa and mel-uric-oxa crystal samples did not detect noticeable spectral differences when compared to the spectra of mel-oxa. This could be attributed to the difficulty of selecting the crystals in the sample for analysis since all samples contain a mixture of the three component crystals and the melamine-oxalate crystals.

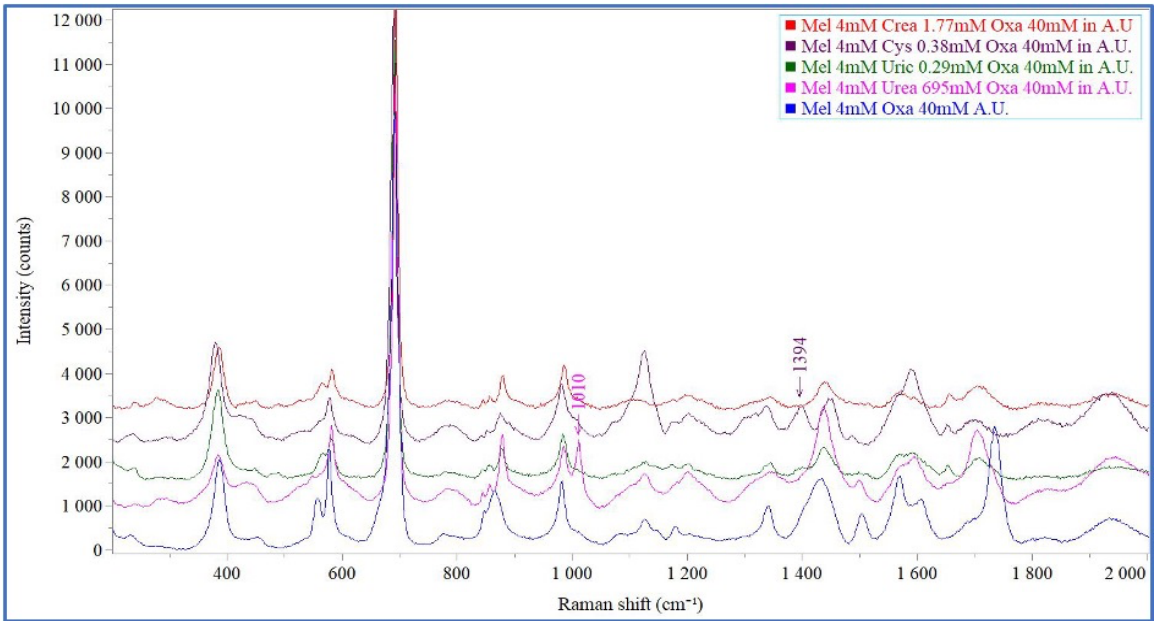

Figure 11. Comparison of Raman Spectra of the Three Components Crystals to Melamine-Oxalate in Artificial Urine

Melamine-uric-oxalate crystals in artificial urine has the stem/rod morphologies with a uniform thickness like in mel-oxa (Figure 12). The onset degradation temperatures of mel-uric-oxa crystals at 167.84 °C, 206.64 °C, and 236.60 °C are different from the onset temperatures of 204.22°C and 214.42 °C for melamine-oxalate suggesting that the two crystals are not the same in composition or the molecular interactions of the components. (Figure 13). Differences in percent decomposition of components and maximum temperatures attained at each of the phases are observed (see Table 3). The differences in the TGA data of mel-uric-oxa crystals compared to mel-oxa crystals suggest the differences are due to uric acid interactions with melamine-oxalate. TGA analysis indicate that mel-uric-oxa crystals are present along with mel-oxa crystals in the sample. The Raman microscopy technique of probing individual regions must have missed the crystals containing uric acid.

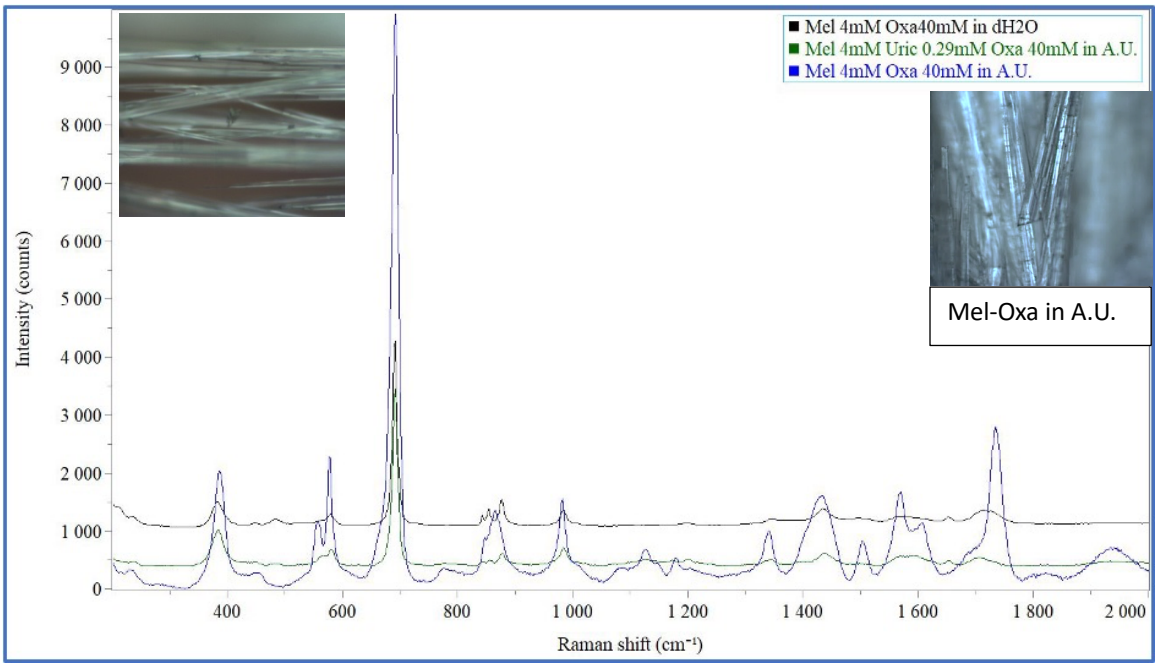

Figure 12. Characterization of Melamine-Uric-Oxalate Crystals in Artificial Urine Using Raman Microscopy

Raman Microscopy did not detect any differences between the melamine-uric-oxalate crystal in artificial urine and melamine-oxalate. The image was taken at 50x magnification.

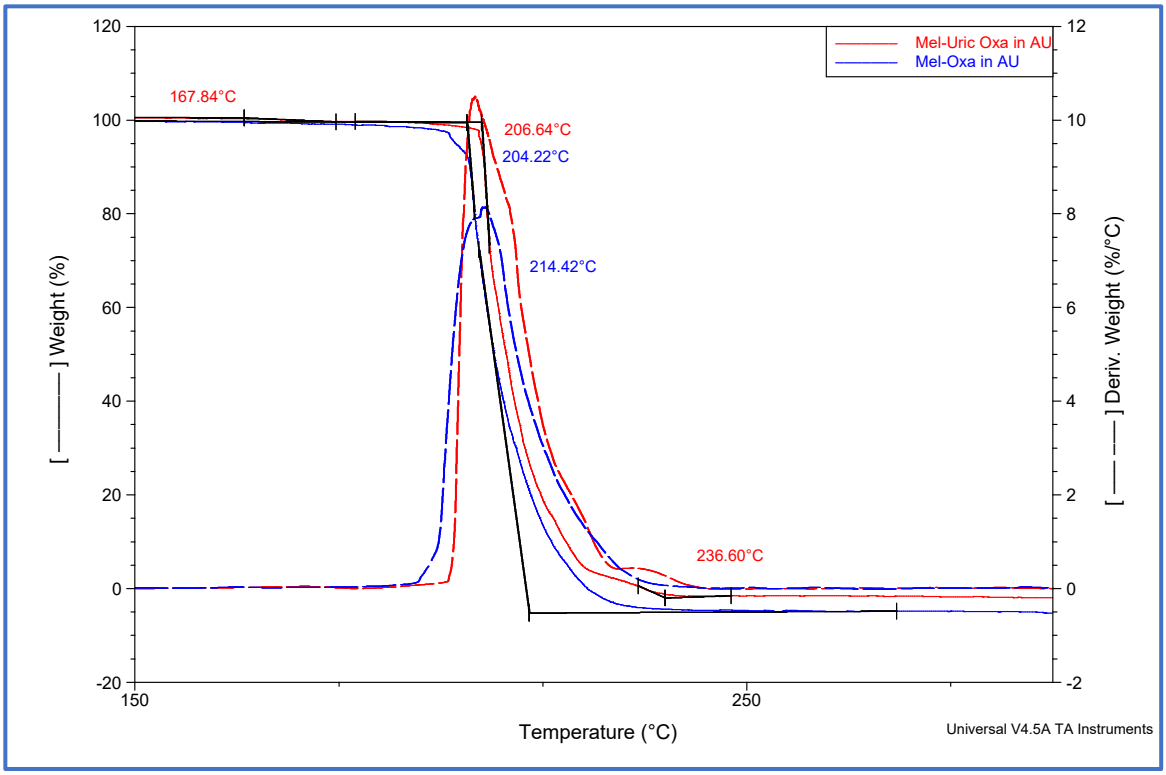

Figure 13. Characterization of Melamine-Uric Oxalate Crystals in Artificial Urine Using Thermogravimetric Analysis

Melamine-Uric-Oxalate has three onset degradation temperatures at 167.84 °C, 206.64 °C, and 236.60 °C, whereas melamine-oxalate has two onset degradation temperatures at 204.22°C and 214.42 °C (see Table 3).

Table 3: Thermogravimetric Analysis of crystals made in Artificial Urine

Crystal:	Onset Decomp. Temp	Max. Temp.	Percent Decomposition	Residues
Mel-Oxa in A.U.	204.22°C 214.42 °C	205.40°C 206.82 °C	27.71% 77.16%	981.93°C 0%
Mel-Uric-Oxa in A.U.	167.84 °C 206.54 °C 236.6 °C	176.57 °C 205.39 °C 231.15 °C	1.158 % 97.22% 3.883%	981.76°C 0%
Mel-Cyst-Oxa in A.U.	211.22 °C 219.93°C	212.22 °C 213.37 °C	25.37 % 75.23 %	981.09 °C 0.978%
Mel-Urea-Oxa in A.U.	94.18 °C 124.46 °C 217.53 °C 227.80 °C	88.76 °C 123.35°C 218.15 °C 218.95 °C	0.97% 26.91% 26.74% 46.18 %	975.68 °C 0.4377 %
Mel-Crea-Oxa in A.U.	170.75 °C 210.49 °C	207.46 °C	1.611 % 99.6%	982.41 °C 2.035 %

Melamine-cystine-oxalate (mel-cys-oxa) crystal in artificial urine showed a rod shape in the crystal structure slightly different from the highly packed slender rods seen in melamine-oxalate crystals (Figure 12). The mel-cys-oxa spectra have peaks at 1394 cm-1 and 1593 cm-1 that are associated with cystine since these are found in the cystine neat spectra. However, the mel-cyst-oxa crystals does not have the 1505 cm-1 peak of mel-oxa spectra (Figure 14). The differences can be attributed to the differences in vibrational modes as a result of cystine being part of the crystals and interacting with both melamine and oxalate in artificial urine. TGA of mel-cyst-oxa in artificial urine has the higher decomposition onset temperatures that (204.22°C and 214.42 °C) than mel-oxa in artificial urine (204.22 °C and 214.42 °C) (Figure 15). Other differences are percent decomposition of components and maximum temperatures attained at each of the phases

(see Table 3). The TGA data of melamine-cystine-oxalate crystals formed in artificial urine showed thermal decomposition onset temperatures that are different from melamine-oxalate crystal suggesting the two crystals are not the same. Raman spectroscopy and TGA analysis of the mel-cyst-oxa crystal indicate that cystine is likely present in the crystals and interacting with melamine and oxalate as indicated by the differences in Raman spectra and decomposition characteristic.

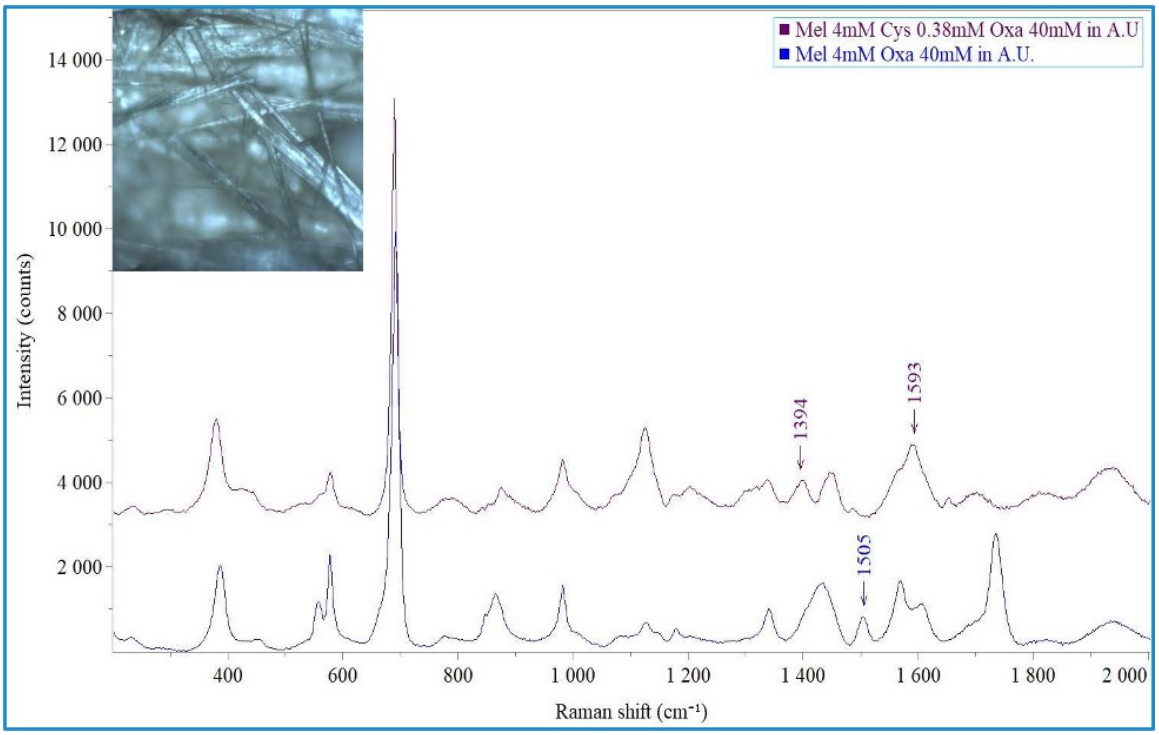

Figure 14. Characterization of Melamine-Cystine-Oxalate Crystals in Artificial Urine Using Raman Microscopy

Raman microscopy detected just the “stem” morphological form of varying thickness. Peaks at 1394 cm-1 and 1593 cm-1 in melamine-cystine-oxalic crystal rare found in the cystine neat. The peak at 1505 cm-1 in the melamine-oxalate crystal is not seen in the

melamine-cystine-oxalate crystal. Inset images were captured at 50x and 10x magnification.

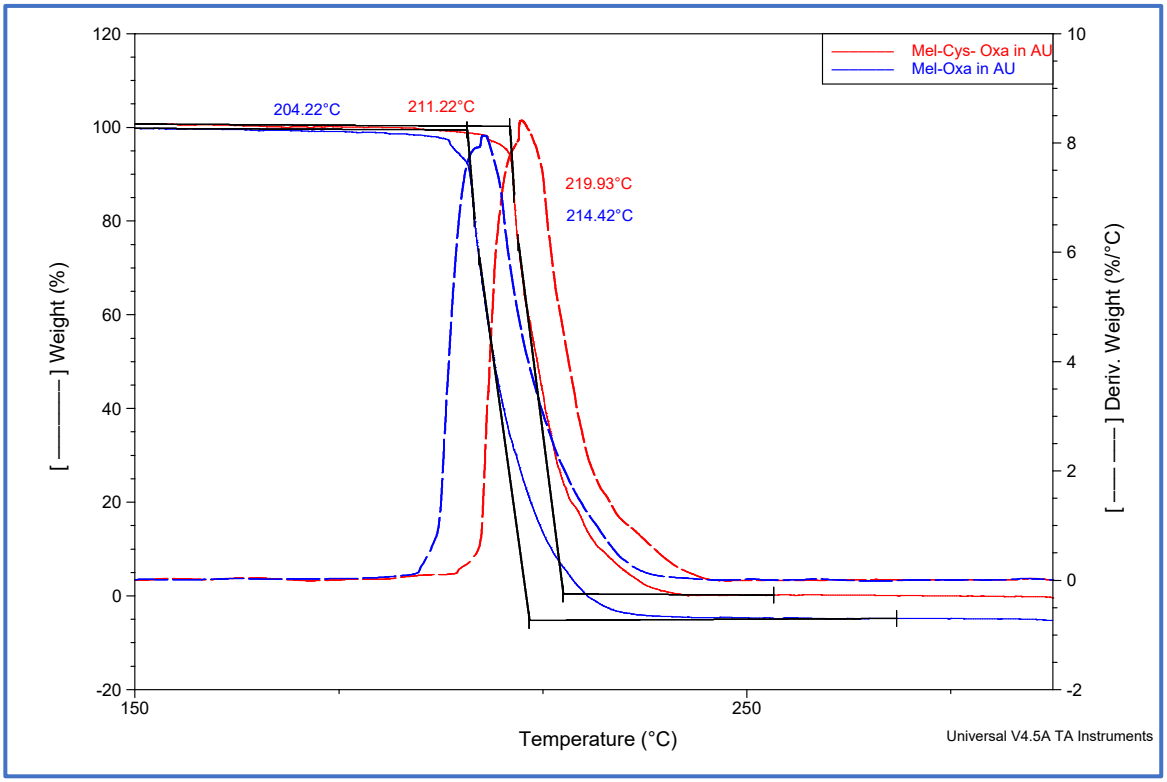

Figure 15. Characterization of Melamine-Cystine-Oxalate Crystals in Artificial Urine Using Thermogravimetric Analysis

TGA of melamine-cystine-oxalate in artificial urine has the onset thermal decomposition temperature at 211.22 °C and 219.93 °C. Whereas the melamine-oxalate in artificial urine have onset decomposition temperatures that occurred at 204.22 °C and 214.42 °C.

Like the melamine-urea-oxalate crystals formed in water, melamine-urea-oxalate crystals in artificial urine have two morphological shapes i.e. both the “floret”

and “stem-like” crystal forms in artificial urine (Figure 16). Melamine-oxalate crystals have only “stem-like” structure and are slenderer and more compact but lacking the “floret” morphology. Raman microscopy identified these differences as unique peak in the “floret” spectra at 1579 cm-1 is detected only in the crystals formed in artificial urine. The peak at 543 cm-1 is observed in melamine-oxalate crystal but shifted due to changes in the interactions between the components. The peak at 1007 cm-1 is found in both the “stem” and “floret” spectra but it is not present in mel-oxa crystal in artificial urine (Figure 16). In addition this peak is also found in mel-urea-oxa in deionized water at 1013 cm-1 (Figure 6). These differences indicate changes in vibrational modes due to the presence of urea in the crystal and its interaction with melamine and oxalate. Thermogravimetric analysis of mel-urea-oxa crystal in artificial urine shows four onset decomposition temperature at 94.18°C, 124.47 °C, 217.53 °C, and 227.80 °C that are different from mel-oxa crystals formed in artificial urine (Figure 17). Neat urea has onset temperatures at 170.06 °C, 216.90 °C, and 290.36 °C. The initial onset temperature in the mel-urea-oxa crystal (94.18 °C) is due to water vaporization/ desorption as it is not characteristic of the mel-oxa crystals formed in artificial urine. Onset temperature at 124.47 °C is lower than onset temperatures of 204.22 and 214.42 °C for melamine-oxalate in formed in artificial urine. This is due to weaker interactions between the three components in the crystals due to urea. Other differences such as percent decomposition of components and maximum temperatures attained at each of the phases further confirmed that the mel-urea oxa crystals does not have the same thermal degradation characteristics as melamine-oxalate formed in artificial urine (see Table 3).

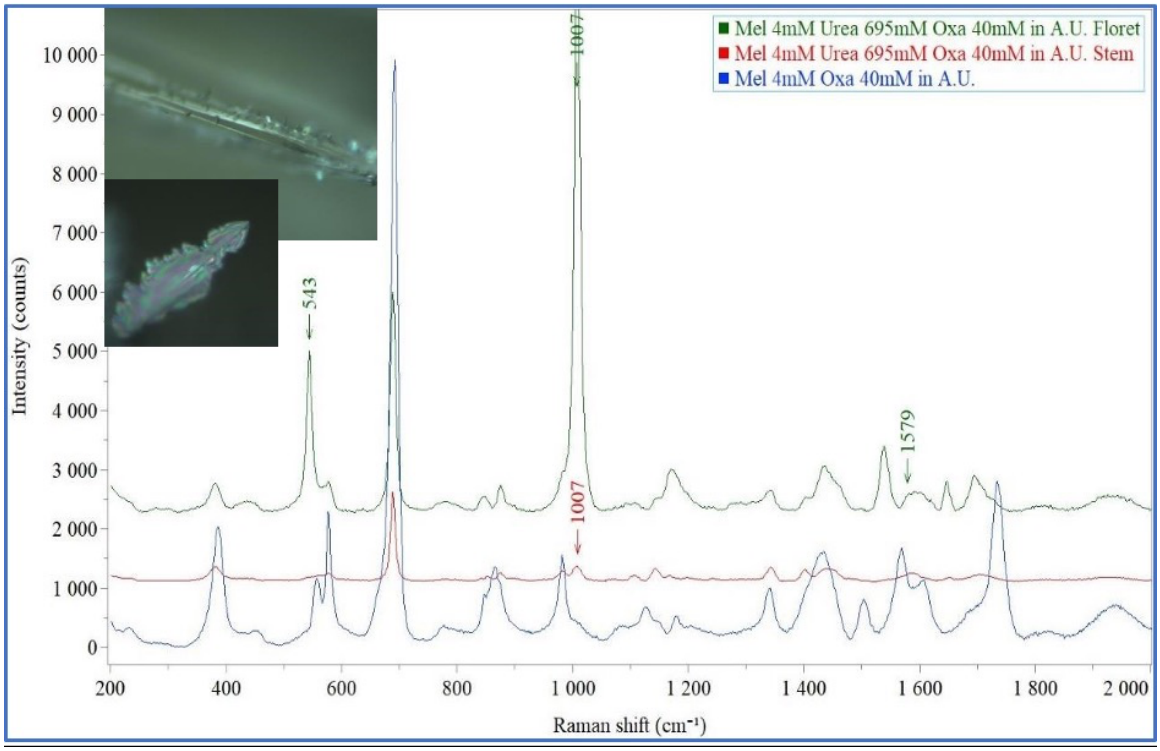

Figure 16. Characterization of Melamine-Urea-Oxalate Crystals in Artificial Urine Using Raman Microscopy

Both the “floret” and “stem” morphological crystal forms were also. Peak at 1579 cm-1 is unique to the “floret” spectra formed in artificial urine. However, the Raman peaks at 1007 cm-1 peak present in both the “stem” and “floret” spectra is unique to the melamine-urea-oxalate crystal formed in artificial urine. The peak at 543 cm-1 is shifted mel-oxa crystal in artificial urine. The inset images were taken at 50x and 10x magnification.

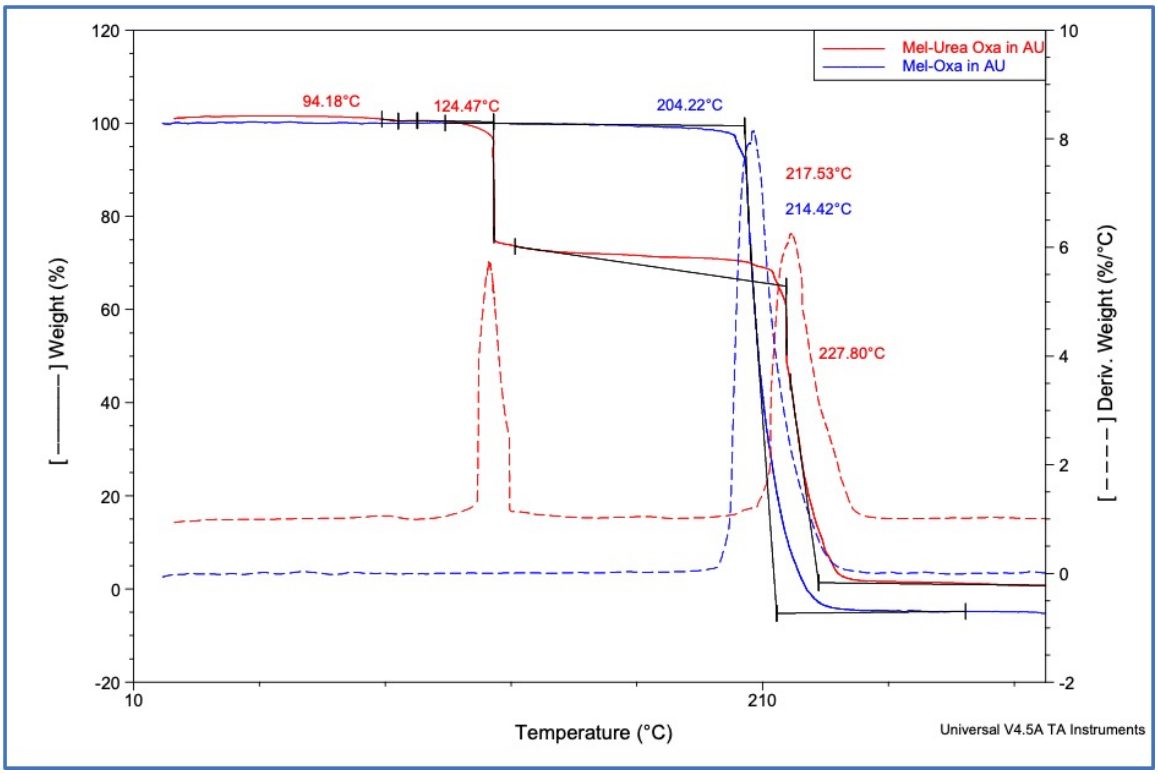

Figure 17. Characterization of Melamine-Urea-Oxalate Crystals in Artificial Urine Using Thermogravimetric Analysis

Melamine-urea-oxalate in artificial urine has three thermal decomposition onset temperature of 94.18°C, 124.47 °C, 217.53 °C, and 227.80 °C (see Table3). Whereas melamine-oxalate has two onset depredation temperatures at 204.22°C and 214.42 °C.

Melamine-creatinine-oxalate (mel-crea-oxa) and melamine-oxalate crystals in artificial urine both produced rod-shaped crystals with slight differences in thickness and compactness. (Figure 18). Raman analysis did not detect noticeable differences in the spectra of the mel-crea-oxa sample compared to mel-oxa crystal. However, Thermogravimetric analysis of melamine-creatinine-oxalate crystal in artificial urine shows thermal decomposition onset temperature that are different from mel-oxa crystal

(Figure 19). The onset temperature in mel-crea-oxa crystal is at a lower temperature (170.75 °C and 210.49 °C) indicating weaker interaction between the three components compared to melamine-oxalate (204.22 °C and 214.42 °C) (Table 3). The differences in the TGA data of melamine-creatinine-oxalate crystals compared to melamine-oxalate crystals suggest the differences are due to creatinine's interactions in the crystal matrix that changes the crystal's properties. This shows that mel-crea-oxa crystals were amongst the mel-oxa crystals in the sample. The Raman microscopy technique of probing individual regions must have missed those crystals containing creatinine.

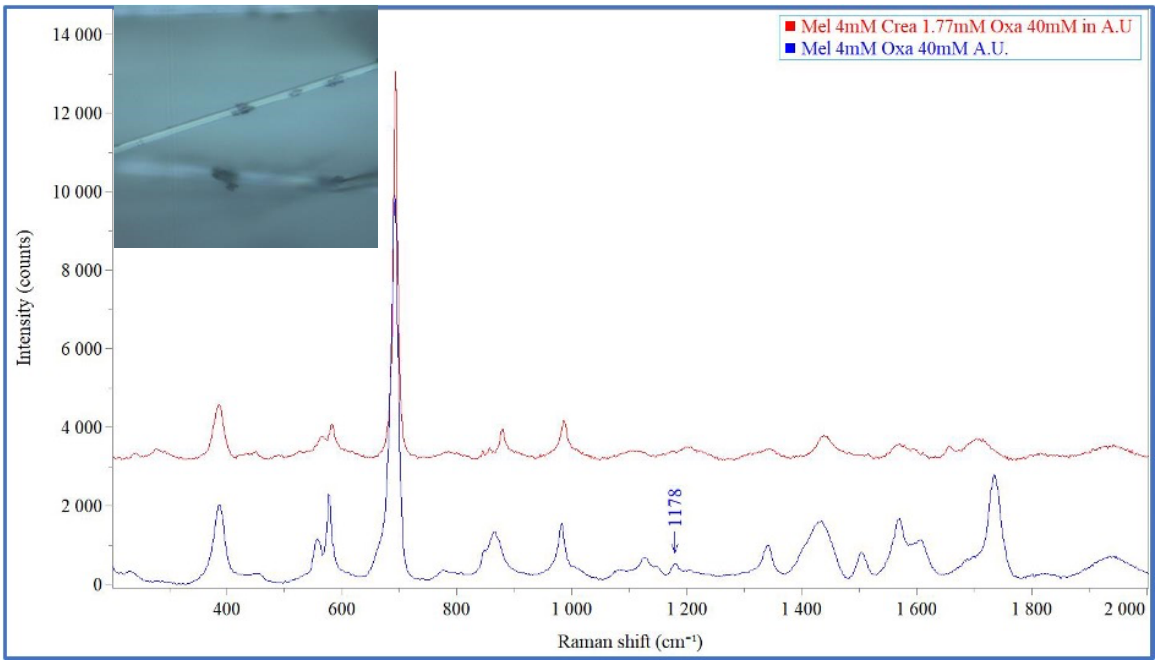

Figure 18. Characterization of Melamine-Creatinine-Oxalate Crystals in Artificial Urine Using Raman Microscopy

Raman microscopy detected one morphological crystal form ("stem"). Peak at 1178 in mel-oxa crystal is stronger than mel-crea-oxa. Image inset is at 50x magnification.

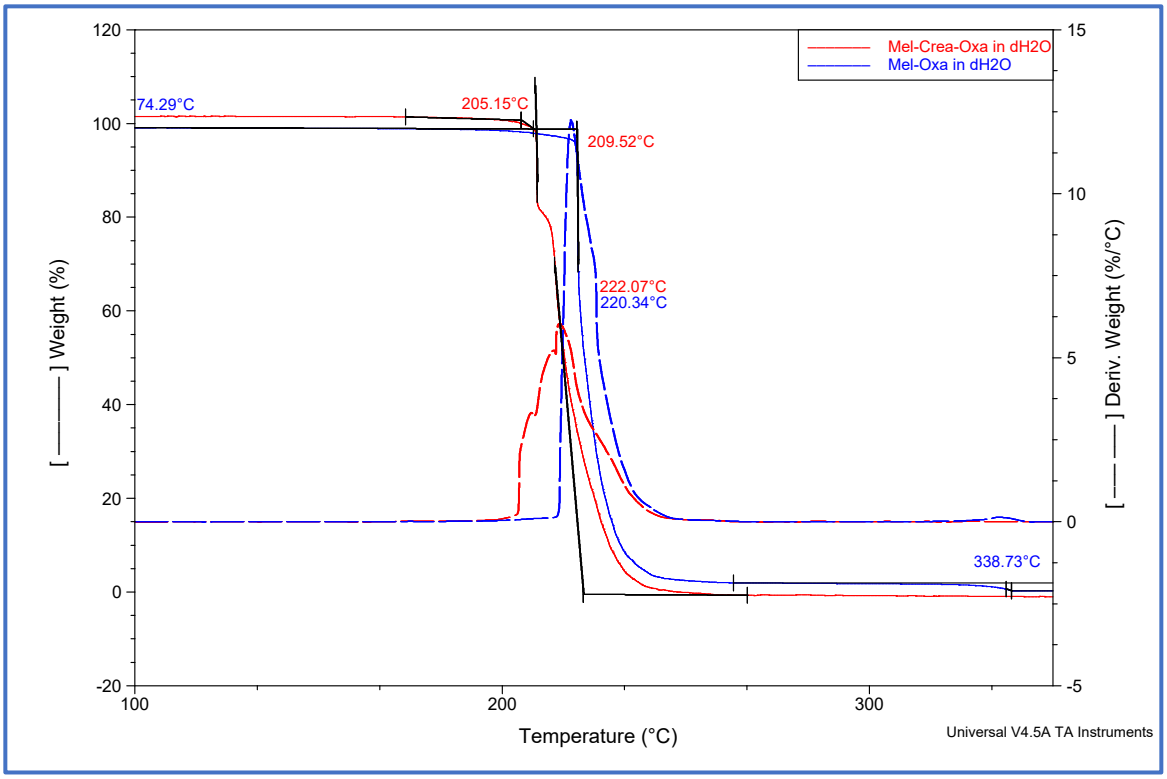

Figure 19. Characterization of Melamine-Creatinine-Oxalate Crystals in Artificial Urine Using Thermogravimetric Analysis

Melamine-creatinine-oxalate in artificial urine has two thermal decomposition onset temperature of 170.75 °C, and 210.49 °C (see Table3), as well as melamine-oxalate with onset degradation temperatures of 204.22°C and 214.42 °C.

Comparisons of TGA Data of Crystals Made in Deionized Water and in Artificial Urine

Thermogravimetric analysis can be used to show differences crystal compositions as well as the strength of interactions of components within the crystals matix. Comparison of TGA data of three-component crystals versus mel-oxa made in deionized water indicates that the intermolecular interaction strength for the crystals in

increasing order are: mel-urea-oxa < mel-crea-oxa < mel-cyst-oxa < mel-oxa < mel-uric-oxa. However, comparisons of TGA results of crystals in made in water versus artificial urine indicates that changes in the strength of interactions of components in crystals may be due to the media.

Analysis of TGA data of crystals formed in artificial urine suggest that all crystals are different from the mel-oxa crystal and that the melamine-creatinine-oxalate and the melamine-uric-oxalate crystal samples contained three components crystals although Raman microscopy did not detect those crystals in the sample.

CHAPTER IV

Conclusion

Melamine has been proven to complex well with cyanuric acid to form crystals in water as well as in the body (Khan 2016). This study was a preliminary test to see whether melamine will also form complexes with physiological components such as oxalic acid, uric acid, L-cystine, urea, and creatinine showed that melamine readily formed crystals with oxalic acid. This study also addresses the role of melamine and oxalate in aiding the formation of the three component crystals as well as the strength of the molecular interactions between the components in the crystals in contrast with melamine-oxalate complex. Thermogravimetric analysis and Raman microscopy was used to characterize crystals made in deionized water and in the harsher conditions of the artificial urine to see whether melamine and oxalate can induce a third component to form a three-component complex. This was accomplished by identifying changes in vibrational modes such as loss or gain on peaks, and peak shifts in the Raman spectra. The decomposition characteristics such as onset decomposition temperatures, percent decomposition, as well as maximum temperatures at each decomposition phase of the crystals were used to confirm and support the Raman spectral data.

Raman spectroscopy and TGA analysis of melamine-urea-oxalate, melamine-creatinine-oxalate, melamine-cystine-oxalate, and melamine-uric oxalate crystals indicated that melamine, oxalate can complex with either uric acid, urea, cystine, or creatinine to form crystals. The strength of interactions between components in the crystals can be interpreted based on the decomposition onset temperatures and maximum

temperatures acquired for decomposition. In addition, since melamine-oxalate crystals could also be formed in artificial urine despite the harsh environment created by colorants, detergents, and preservatives suggest that the melamine interactions with oxalate is strong. The formation of melamine-oxalate plus a third component in the crystals suggests that both melamine and oxalate can induce the third components to form complex regardless of whether the medium is water or artificial urine. Although Raman microscopy indicated that only crystals containing urea and cystine formed three component crystals in artificial urine, TGA analysis showed that all of the crystals formed three component crystals. In conclusion, melamine readily interacts with oxalic acid to for crystals and that such interaction can further promote the interaction of an additional physiological constituents such as uric acid, urea, cystine, or creatinine. These interactions that contribute to the physical and chemical characteristics of the crystals can be detected by Raman spectroscopy and TGA techniques.

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