Polymorphism in Containerless Crystallization

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Supporting Information

ABSTRACT: The effect of the ambient relative humidity (RH) on the crystallization of malonic and glutaric acid in aqueous microdroplets was investigated using an electrodynamic balance (EDB). Aqueous droplets containing a solute were injected into a chamber maintained at selected RH values, after which the RH was cycled to characterize crystallization and deliquescence behavior. Glutaric acid crystals that were formed at >40% RH deliquesced at 85 ± 2% RH, consistent with the metastable α polymorph. Glutaric acid crystals initially formed at ≤30% RH, however, deliquesced at 90 ± 2% RH, corresponding to the stable β polymorph. These polymorph identifications were confirmed using in situ Raman spectroscopy. In the case of malonic acid, the mass change measured with the EDB at deliquescence revealed the formation of a hydrate, $C_3H_4O_4 \cdot 1.5H_2O$, when the crystals were initially formed at ≥20% RH. The EDB response for malonic acid crystals initially formed at ~5% RH, however, corresponded to an anhydrous form. These observations suggest that, contrary to expectation, metastable crystals were favored at lower supersaturation levels compared with their respective stable forms. This unanticipated behavior is attributed to nucleation within the droplet at the higher RH value but at the air—solution interface of the droplet for lower RH values.

INTRODUCTION

The prediction and control of solid-state structure in molecular crystals remains one of the foremost challenges in solid-state chemistry. This is illustrated by polymorphism, the ability of a molecule to crystallize into more than one form, which has considerable technological significance owing to the dependence of crystal properties on solid-state structure.1–3 Regulating polymorphism is essential to key technological sectors such as food, pharmaceuticals, explosives, and dyes, for which regulatory compliance and functional performance depend on polymorph identity.4–12 Only one polymorph is thermodynamically stable at a specific pressure and temperature, and the observation of other forms necessarily implicates them as kinetically favored. Under nonequilibrium conditions (i.e., supersaturation), kinetically favored polymorphs will crystallize more rapidly, and the initial polymorphs formed during crystallization will be metastable forms, as described by Ostwald’s Rule of Stages.13–15 This rule, first stated by Wilhelm Ostwald in 1897 to account for numerous experiments in which an unstable solid was first formed from a metastable solution or melt, later to be replaced by more stable forms, posits that in such crystallization processes, the system prefers to form the solid phase that involves the least change in free energy of the system, rather than the lowest energy form.1

The selectivity toward a particular polymorph can be influenced by the substrate on which nucleation occurs,16 confinement of crystal growth to nanoscale dimensions,17,18 electric field,19 laser polarization in nonphotochemical laser-induced nucleation (NPLIN),20,21 addition of tailor-made auxiliaries,2 choice of solvent,3 and solution pH.8,22 Given that only one polymorph corresponds to the global thermodynamic minimum at one temperature at a specific pressure, strategies for controlling polymorphism must rely on the careful manipulation of nucleation and crystal growth kinetics.

One of the many properties that can distinguish polymorphs of a given compound is the deliquescence point, which corresponds to the relative humidity (RH) value at which the solid spontaneously transforms into an aqueous solution (denoted as the deliquescence relative humidity, DRH). Conversely, the efflorescence relative humidity (ERH) is the RH value at which a solid is formed as the RH is decreased. The ERH typically is lower than the deliquescence point owing the inherent and unpredictable kinetic factors associated with nucleation of a crystalline phase, resulting in a hysteresis of the deliquescence and efflorescence behavior as would be expected for a nonequilibrium process. If each polymorph has a characteristic signature corresponding to its DRH, these features can be used to identify polymorphs, where the most stable polymorph has the highest DRH.

A particularly useful approach for determining the DRH and ERH values of a crystalline compound relies on the levitation of a charged droplet or a solid particle in an electrodynamic balance (EDB).23,24,25 If the EDB is equipped with a laser, the diffraction image of the levitated particle can be used to corroborate the transition from the liquid and crystalline states (Figure 1). Moreover, the EDB permits the investigation of crystallization outcomes in
Crystal Growth & Design

Figure 1. (A) The dependence of the mass growth factor (MGF), $M/M_{\text{solid}}$, for ammonium sulfate during an RH cycle in the electrodynamic balance (EDB). Points S and F denote the start (solid only) and the finish of a typical cycle, respectively. Points D and E denote the deliquescence and efflorescence points, respectively. The arrows denote the direction of the cycle. The lower panels depict diﬀusion behavior of organic materials and inorganic salts (defocused mode), acquired using a solid-state diode laser ($\lambda = 473$ nm), of a glutaric acid particle levitated in the EDB as (B) a solid particle at RH values prior to deliquescence and (C) an aqueous droplet after deliquescence and before efflorescence. The number of fringes in (C) decreases with declining RH following deliquescence. The particle diameter is approximately 25 $\mu$m.

very small solution volumes and in a containerless vessel, both features mitigating contributions from ill-deﬁned nucleation events that otherwise may occur on the solid surfaces of a container vessel.

The EDB has been used to determine the deliquescence and eﬄorescence behavior of organic materials and inorganic salts such as ammonium sulfate, which has a particular importance in atmospheric chemistry. It also has been used to discover and characterize new metastable forms of inorganic salts, the formation of which was attributed to the rapid attainment of highly supersaturated conditions.26,27 To our knowledge, the EDB has not yet been used to control and characterize polymorphic outcomes. We describe herein the crystallization of malonic acid and glutaric acid in picoliter-sized droplets, each reported to form polymorphs. These investigations demonstrate that the EDB is capable of distinguishing polymorphs from their DRH values and that the polymorph initially formed depends on the conditions the solution droplet experiences when ﬁrst injected into the EDB. Surprisingly, these measurements revealed that, contrary to expectation, metastable crystals were favored at lower supersaturation levels compared with their respective stable forms. This unanticipated behavior is attributed to nucleation within the droplet at the higher RH value but at the air−solution interface of the droplet at lower RH values.

**Malonic and Glutaric Acids.** Malonic acid (MA; HOOCC-(CH$_2$)$_2$COOH) has three known polymorphs,28 only the $\beta$ form (triclinic, P1) has been observed at room temperature.29,30,31 The $\beta$ form transforms to $\alpha$ (orthorhombic, Pcmn) at 352 K, and the $\gamma$ phase (triclinic, P1) transforms to $\beta$ at 56 K. A hydrate, suggested to be $\text{C}_3\text{H}_4\text{O}_4\cdot6\text{H}_2\text{O}$, has been reported.$^{32}$ Two polymorphs have been reported for glutaric acid (GA; HOOCC(CH$_2$)$_2$COOH), $\alpha$ (monoclinic, C2/c),$^{33}$ and $\beta$ (monoclinic, C2/c).$^{29,33,34}$ The $\beta$ form transforms to $\alpha$ at 347 K.$^{28}$ At room temperature, $\beta$ is the thermodynamically favored form and $\alpha$ is metastable. This stability ranking, however, is reversed in nanocrystals grown under conﬁnement in nanoporous glass or polymer matrices.17 Although the EDB has been used to measure the DRH of both malonic and glutaric acid (DRH(MA) = 69−74$^{35}$ DRH(GA) = 90%, DRH(\text{HOOC(CH$_2$)$_2$COOH)} = 85−86%)$^{34}$, largely motivated by interest in atmospheric aerosols containing these compounds,$^{36−39}$ it has not been used to characterize polymorphic behavior.

**EXPERIMENTAL SECTION**

**Electrodynamic Balance (EDB).** The EDB balances the weight of a particle/droplet using a DC voltage ($V_{\text{DC}}$),$^{40}$ while an AC voltage ($V_{\text{AC}}$) maintains the position of the particle/droplet in the center of the trap at a null point. If the charge on a levitated particle/droplet remains constant, the particle mass is proportional to $V_{\text{DC}}$. The DRH and ERH can be deduced from the change in the mass growth factor (MGF),$^{31,42}$ which is the ratio between the measured mass ($M$) and the mass of the dry solid particle ($M_{\text{solid}}$). The MGF can be calculated from the change in the $V_{\text{DC}}$, that is, MGF = $M/M_{\text{solid}}$ = $V_{\text{DC}}/V_{\text{DCsolid}}$. This is illustrated in Figure 1, which depicts an RH cycle for ammonium sulfate in an aqueous droplet, introduced to the EDB chamber initially at RH$_{\text{init}}$ = 40%. The DRH is apparent from the sudden increase in the MGF at RH = 83 ± 2% as the RH is increased. Upon reversing of the cycle, the MGF gradually decreases due to evaporation of water from the droplet, eventually reaching the ERH at which the solid particle forms. The hysteresis loop is characteristic of the inherent kinetic factors that influence the solidiﬁcation of the crystalline particle. If the EDB is equipped with a laser, the diﬀraction image of the levitated particle can be used to corroborate the transition from the liquid and crystalline states (Figure 1; see Figure 2 for a schematic of the EDB). The diﬀraction image of a liquid droplet exhibits a fringe pattern arising from the interference of light from two glare spots on the spherical droplet, whereas the crystalline solid phase is characterized by an image without fringes.$^{43,44}$ The number of fringes decreases with declining RH, as water evaporates and the droplet gets smaller, until the image becomes opaque and fringeless when solidiﬁcation is complete. The liquid droplet size can be deduced from the distance between the fringes, $X$, as described by eq 1, in which $\lambda$ is the laser wavelength, and $L$ is the distance between the diﬀraction image and the droplet.$^{44}$

$$R = \frac{\lambda L}{X(1 + \frac{1}{\sqrt{2}})} \quad (1)$$

The EDB was employed herein to examine the deliquescence and eﬄorescence behavior of MA and GA, which were subjected to RH cycling to determine the DRH and ERH values, with the aim of determining the influence of RH$_{\text{init}}$ on polymorphism. Droplets with radii ranging from 5 to 20 $\mu$m and diacid concentration of 50 w/w% were injected into the EDB chamber, which was previously adjusted to a speciﬁc RH$_{\text{init}}$ value in the range 5% ≤ RH$_{\text{init}}$ ≤ 50% (Table 1; the RH$_{\text{init}}$ value is denoted by the number following the diacid descriptor; for example, MAS0 corresponds to malonic acid and RH$_{\text{init}}$ = 50 ± 5% RH; the ±5% RH represents the range of initial RH values within a nominal group of samples). The droplet was levitated to the null position by adjusting $V_{\text{DC}}$ and conﬁned at this position with the AC ﬁeld corresponding to $V_{\text{AC}}$. The droplet was held at RH$_{\text{init}}$ and 1 atm total pressure for 1 h, followed by evacuation of the chamber to a pressure of 1 Torr.

dx.doi.org/10.1021/cg200824h | Cryst. Growth Des. XXXX, XXX, 000–000
The RH was then increased gradually at a rate of \( \sim 3\% \) RH/min through the introduction of water vapor to an upper limit of RH = 95\%. After 30 min at this RH, the RH was slowly reduced at a rate of \( \sim 1.7\% \) RH/min to a lower limit of RH = 5 ± 2\%, thus completing one RH cycle. The DRH and ERH were determined from the abrupt changes in the mass growth factor and the accompanying transformation in the diffraction image of the levitated particle.

**Materials.** Glutaric acid (99\%) and malonic acid (99\%) were purchased from Aldrich (Milwaukee, WI, USA) and Acros Organics (Somerville, NJ, USA), respectively, and used without further purification. Aqueous solutions (1:1 (w/w)) were prepared using 18.0 M \( \text{H}_{2}\text{SO}_{4} \) (Fisher, Fair Lawn, NJ, USA), respectively, and used without further purification. Aqueous solutions (1:1 (w/w)) were prepared using 18.0 M \( \text{H}_{2}\text{SO}_{4} \) (Fisher, Fair Lawn, NJ, USA), respectively, and used without further purification. The solutions were filtered (membrane filter GSWP02500, 0.22 \( \mu \text{m} \) pore size, Millipore, Chicago, IL, USA) before use.

**Methods.** A hyperbolic electrodynamic levitator-trap (ELT),23 constructed inside a stainless steel chamber, was used to levitate the aqueous droplets and solid particles (Figure 2A). The total pressure inside the chamber was regulated with a vacuum pump connected to the chamber (Figure 2B). The liquid microdroplets (10—40 \( \mu \text{m} \) diameter) were generated with a piezoelectric pico-pipet microparticle generator system (model 201, Uniphoton Systems Inc., Brooklyn, NY, USA). A microdroplet was ejected from the tip orifice upon application of a voltage pulse, and the microdroplet was subsequently charged upon passing through a charging ring, to which a voltage of 40—100 V was applied, depending on the particle. The droplet then entered the EDB through an opening in the upper electrode of the ELT. The \( V_{DC} \) was adjusted manually using a DC power supply (HY10010E, Mastech, San Jose, CA, USA) to keep the particle centered at the null point of the trap. The \( V_{DC} \) and the pressure inside the EDB were monitored using a NI USB-6289 high-accuracy multifunction USB data acquisition (DAQ) device (National Instruments, Austin, TX, USA) and recorded using LabView (National Instruments, Austin, TX, USA) software. The \( V_{AC} \) was adjusted between 600 V (when a particle is levitated at atmospheric pressure) and 100 V (when the chamber is nearly evacuated) to avoid electrical breakdown at low pressure, using a variable transformer connected to a Mini Lite 3008 high voltage transformer (Actown, Juarez, Mexico). The temperature inside the EDB chamber was held at 21—23 \( ^\circ \text{C} \) for all experiments, with temperature fluctuations no greater than 1 \( ^\circ \text{C} \). The relative humidity at atmospheric pressure was controlled by introducing a mixture of argon gas and water vapor to the chamber. Argon was chosen because it is inert and has a higher molecular weight than air, which minimized the introduction of room air and humidity into the chamber when it was opened to inject a solution droplet. At ambient pressure the relative humidity was monitored with a polymer-capacitive sensor (HT120, EdgeTech, West Wareham, MA, USA). At low pressure, the relative humidity was increased by introducing water vapor into the chamber and decreased by evacuating the chamber with the vacuum pump; the relative humidity at low pressures was measured in two ways: directly with the HT120 sensor and also calculated from the water vapor pressure. The water vapor pressure \( (p_{w}) \) was measured with a MKS Baratron pressure transducer (222, MKS Instruments,

<Figure 2. Schematic of the electrodynamic levitation system. (A) Cross-sectional view of electrodynamic levitator-trap (ELT); (B) view from above.>

<table>
<thead>
<tr>
<th>Table 1. DRH and the Corresponding MGF for Malonic Acid (MA) and Glutaric Acid (GA)(^d)</th>
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</thead>
<tbody>
<tr>
<td>sample name</td>
</tr>
<tr>
<td>GA50 (RH(^{init}) = 50%)</td>
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<tr>
<td>GA40 (RH(^{init}) = 40%)</td>
</tr>
<tr>
<td>GA30 (RH(^{init}) = 30%)</td>
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<td>GA20 (RH(^{init}) = 20%)</td>
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<td>MA40 (RH(^{init}) = 40%)</td>
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<tr>
<td>MA30 (RH(^{init}) = 30%)</td>
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<tr>
<td>MA20 (RH(^{init}) = 20%)</td>
</tr>
<tr>
<td>MA10 (RH(^{init}) = 10%)</td>
</tr>
</tbody>
</table>

\(^d\) The samples were injected as solution droplets into the EDB chamber at an initial RH (RH\(^{init}\)). After 1 h, the pressure in the EDB chamber was reduced to 1 Torr. The RH\(^{init}\) values of individual runs are within ±5% RH of the nominal value.
Andover, MA, USA). The relative humidity was calculated using eq 2, where \( P_{\text{sv}} \) is the saturated vapor pressure of water at a prescribed temperature.

\[
\text{RH} = \frac{P_{\text{sv}}}{P_{\text{sv}, \text{wv}}} \times 100
\]  

The two methods for obtaining RH at low pressures were in agreement to ±1%. A 50 mW low power blue diode laser with wavelength of 473 nm (LRS-473-TM-50-5, Laserglow Technologies, Toronto, ON, Canada) was used to illuminate the levitated particle. A Toolmakers Vernier Traveling Measuring microscope was used to observe the particle at 90° to the laser beam propagation direction (Figure 2B). The microscope can move in three dimensions to track the levitated particle. A Digital Eyepiece camera (AM423, DinoLite, Naarden, The Netherlands) was mounted on the microscope and used for observation and image recording. A defocused image mode was used to obtain diffraction images of the levitated particles. The size of liquid particles was determined from the distance between the fringes in these images using eq 1.

An in situ Raman spectroscopy system was integrated with the levitator chamber. A 100 mW 785 nm diode laser (Innovative Photonic Solutions, model no. 10785SM0100B-TH, Monmouth Junction, NJ, USA) was used for excitation. A convergent laser beam was sent through the bottom window of the chamber and focused onto the particle. The Raman-scattered light was collected at right angles, collimated, filtered using a long wavelength pass filter to reject most of the Rayleigh-scattered light, and then focused into a 100 μm optical fiber, which acts as the slit of the spectrometer (see Figure 2). A 1200 g/mm grating was used in a QE65000-Raman Scientific-grade spectrometer (Ocean Optics, Dunedin, FL, USA) to collect spectra. The spectrum integration time was 10 min for each spectrum.

Mercury 2.3 software (The Cambridge Crystallographic Data Centre, Cambridge, UK) was used to calculate the length and direction of intermolecular hydrogen bonds in the various crystal structures of glutaric and malonic acids. The surface free energy of a particular crystal plane (the free energy required to create a unit area of solid/vacuum interface, which is approximately equal to the free energy required to create a unit area of solid/air interface) can be approximately calculated as half the sum of the energies of the intermolecular bonds broken when a crystal is cleaved along that plane.45

### RESULTS AND DISCUSSION

#### Influence of Droplet Size, Electric Field, and Charge.

Electric fields and charges on droplets are known to affect droplet stability44 and polymorph formation.19 A particle in an EDB is subjected to a DC electric field from the balancing electrodes as well as from the net electric charge at the particle surface. For a 20-μm diameter particle, a typical balancing voltage in our experiments is 30 V, corresponding to an electric field of \( 6 \times 10^3 \text{ V/m} \) and a surface charge of \( 7 \times 10^{-13} \text{ C} \) (\( 4 \times 10^6 \) electron charges). This field is 2 orders of magnitude below that needed (\( 6 \times 10^5 \text{ V/m} \)) for affecting polymorph formation. Using the results of ref 46, the surface charge is estimated to change the vapor pressure of the droplet by about 0.1%. Although a range of droplet sizes was used in this study, differences in droplet size had no effect on any of the data reported in Table 1. The insignificance of charge and curvature effects on the vapor pressure of droplets with diameters exceeding 10 μm has also been reported in ref 47. Size does have an effect on the ERH, because the rate of nucleation within a droplet is proportional to its volume,27 but that size dependence is not a focus of this report.

#### Solute Mass Loss.

Unlike ammonium sulfate, both GA and MA lose ≈30% of their mass during the RH cycle (Figures 3, 5 and 7). This is consistent with previous reports and can be explained by the difference between the vapor pressure of inorganic salts and low-molecular-weight organic acids near room temperature (\( 10^{-4} - 10^{-3} \) Pa for MA and GA; \( 10^{-6} - 10^{-8} \) Pa for ammonium sulfate).36,37,48

**Glutaric Acid.** All GA50 droplets injected into the EDB remained solution droplets after injection and crystallized only when the total pressure in the chamber was rapidly reduced below \( 200 - 400 \) Torr, corresponding to an RH of 42–48%. (This is not the same as the ERH, because of the rapidly changing conditions.) Upon increasing the RH, GA50 particles deliquesced at DRH = 85 ± 2% RH (Figure 3; Table 1), consistent with the DRH value reported previously for the \( \alpha \) polymorph,34 and with other EDB studies of GA,49–51 suggesting that the \( \alpha \) polymorph is usually formed in such studies. Six of the ten GA40 droplets injected into the EDB crystallized only when the total pressure in the chamber was reduced below 1 atm, deliquescing on reversal at DRH = 85 ± 2%, identical to the GA50 particles and signaling the formation of the \( \alpha \) polymorph. The remaining four GA40 droplets crystallized 10 min after injection into the chamber at 1 atm total pressure and constant humidity. The resulting particles deliquesced at DRH = 90 ± 2% RH, coinciding with the DRH of the \( \beta \) polymorph (Table 1) and in agreement with DRH values reported for bulk studies of GA.49,50–54 and one EDB study.41 Therefore, both polymorphs formed with comparable probabilities for the GA40 samples. GA30, GA20, and GA5 droplets crystallized within 10 min after injection into the chamber at 1 atm total pressure and constant humidity, and they exhibited identical RH cycles with a DRH of 90 ± 2% RH, also signaling the \( \beta \) polymorph (Table 1). The ERH values measured for all samples ranged from 25% RH ≤ ERH ≤ 40% RH, similar to values reported previously in levitated droplets.1,49

Figure 4 shows the in situ Raman spectra of three levitated GA particles: a GA solution droplet, a GA50 particle, and a GA5 particle. Table 2 compares the characteristic Raman frequencies of these three particles with those of the \( \alpha \)- and \( \beta \)-polymorphs of GA.33,34 The GA5 particle can be assigned to \( \beta \)-GA based on the presence of two characteristic \( \beta \) peaks at 1047 and 1464 cm\(^{-1}\), whereas the GA50 particle can be assigned to \( \alpha \)-GA based on the absence of those two characteristic \( \beta \) peaks. These Raman assignments corroborate the polymorph identifications made using DRH values.
The DRH values and Raman spectra suggest that droplets introduced at high RH_{init} (inducing low supersaturation), a condition that would favor slow crystallization, formed the metastable α-polymorph. Conversely, droplets introduced at low RH_{init} values (inducing high supersaturation), a condition that would favor rapid crystallization, formed the stable β-polymorph. This behavior seems contrary to that expected from Ostwald’s Rule of Stages and from previous reports on the behavior of inorganic salts, which would posit that the metastable α-polymorph would be favored when crystallization was rapid, that is, at the low RH_{init} values.26,27

**Malonic Acid.** All MA5 droplets crystallized within 5 min after injection into the chamber, which was held at 1 atm total pressure. MA5 particles exhibited conventional RH cycling behavior, wherein the MGF along the increasing and decreasing branches coincided above the DRH (Figure 5A; Table 1; DRH = 75 ± 2% RH; MGF at the DRH = 1.65 ± 0.05). The DRH value is consistent with those reported previously for MA particles35,39 and bulk MA.35,54 All Raman peaks observed in the MA5 particle spectra (Figure 6C) are consistent with either the α or β-polymorph of malonic acid. To distinguish between α-MA and β-MA, one must be able to resolve small frequency differences between their common peaks,55 and the reported differences (∼20 cm⁻¹) are near the resolution limit of our Raman spectrometer.

In contrast to the MA5 droplets, all MA40, MA30, and MA20 droplets crystallized only when the total pressure in the chamber was reduced to ∼400 Torr. Furthermore, their RH cycling features (Figure 5B; Table 1; Figure 7; DRH = 70 ± 2% RH; MGF = 1.40 ± 0.05) differed from that of MA5, exhibiting a small “knee” on the increasing RH branch just above the DRH that was not present on the decreasing branch, indicating a difference in mass between the two branches, which can only be attributed to a loss of water (through evaporation under equilibrium conditions) from a solid phase above the DRH. Moreover, the change in the MGF at the DRH for MA20 (as well as MA30 and MA40) is much less than that of MA5, which can be explained by the formation of a hydrate when RH_{init} ≥ 20%. The change in MGF is smaller for MA20 than MA5 because the molar mass of the MA20 hydrate prior to the DRH is greater than the anhydrous form, whereas after deliquescence the molar masses must be equal.

**Table 2. Characteristic Raman Frequencies of Glutaric Acid Polymorphs and Levitated Particles (cm⁻¹)**

<table>
<thead>
<tr>
<th>α-GA peaks</th>
<th>β-GA peaks</th>
<th>GA50 peaks</th>
<th>GA5 peaks</th>
<th>GA solution peaks</th>
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<tbody>
<tr>
<td>939</td>
<td>945</td>
<td>923 ± 10</td>
<td>944 ± 10</td>
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<tr>
<td>1047</td>
<td>1047</td>
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<td>1417 ± 10</td>
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<td>1663 ± 10</td>
<td>1647 ± 10</td>
<td>1667 ± 10</td>
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<tr>
<td>1728 ± 10</td>
<td>1704 ± 10</td>
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</table>
The weight fraction and MGF of the hydrate can be normalized using a correction factor that accounts for the different masses of the hydrate and anhydrous MA, as shown in eq 3, in which $M_{MA}$ is the molar mass of MA and $x$ corresponds to a stoichiometric number of water molecules in the presumed hydrate. The composition of the hydrate can be deduced from eq 3 by determining the multiplicative factor that makes the hydrate MGF vs RH curve overlap the anhydrous curve. The best fit for MA20, MA30, and MA40 curves is achieved with the factor 1.26, corresponding to $x = 3/2$ (Figure 5C), indicating a sesquihydrate, $C_{3}H_{4}O_{4} \cdot 1.5$ 

$$\text{MGF}_{\text{anhydrous}} = \frac{M_{MA} + 18x}{M_{MA}} \times \text{MGF}_{\text{hydrate}}$$

For a solution droplet, the MGF is simply the reciprocal of the droplet concentration, in units of weight fraction. During the portion of the RH cycle in which a solution droplet is subjected to decreasing RH, the droplet is approximately in equilibrium with the water vapor throughout, so at a given RH, the hydrate and the anhydrous solutions should have the same concentration, although their weight fractions are different because the hydrate weight fraction is calculated in terms of the mass of the hydrate. The weight fraction and MGF of the hydrate can be normalized using a correction factor that accounts for the different masses of the hydrate and anhydrous MA, as shown in eq 3, in which $M_{MA}$ is the molar mass of MA and $x$ corresponds to a stoichiometric number of water molecules in the presumed hydrate. The composition of the hydrate can be deduced from eq 3 by determining the multiplicative factor that makes the hydrate MGF vs RH curve overlap the anhydrous curve. The best fit for MA20, MA30, and MA40 curves is achieved with the factor 1.26, corresponding to $x = 3/2$ (Figure 5C), indicating a sesquihydrate, $C_{3}H_{4}O_{4} \cdot 1.5$

Figure 8. Schematic representation of site-dependent nucleation in evaporation-driven crystallization in a levitated aqueous droplet, illustrated here for $\beta$-glutaric and $\alpha$-glutaric acid. For a rapidly evaporating droplet (at low initial relative humidity), the droplet will be more supersaturated in the vicinity of the air—solution interface than in the bulk volume of the droplet. Although $\beta$-glutaric acid is the thermodynamically stable form, the lower surface energy of its lowest energy crystal plane favors its nucleation at the air—solution interface. Conversely, crystallization of $\alpha$-glutaric acid is more likely at high relative humidity because nucleation at the air—solution interface becomes less competitive and the predominant crystal plane in $\alpha$-glutaric acid has a higher surface energy, such that its emergence in a surrounding aqueous medium is less unfavorable.

The Raman spectrum of an MA40 particle is shown in Figure 6, along with an anhydrous MA5 particle spectrum and an MA solution droplet spectrum. The MA40 particle spectrum bears a closer resemblance to the solution droplet spectrum than the MA5 anhydrous particle spectrum, providing further support that the MA40 particle is a hydrate.

On first inspection, the RH cycling features of both GA and MA appear counterintuitive, in conflict with behavior expected for crystallization of polymorphs induced by evaporation of the aqueous solvent. The observed DRH values reveal that the metastable forms ($\alpha$ for GA and hydrate for MA) crystallize initially at the higher RH values, closer to thermodynamic equilibrium, whereas the thermodynamically preferred forms ($\beta$ for GA and anhydrous for MA) crystallize initially at the lower RH values, further from equilibrium conditions where crystallization would be more rapid. One plausible explanation for this behavior relies on the site of nucleation within the droplet when crystallization is provoked by a reduction in the RH from different RH values. When an aqueous solution droplet is injected into the EDB chamber, evaporation occurs rapidly as the droplet achieves equilibrium with the RH value established in the EDB prior to introduction of the droplet (Figure 8). Evaporation creates a concentration gradient inside the droplet between the higher solute concentration emerging at the droplet surface, $C_{\text{eq}}$, and the lower concentration in the center, $C_{\text{o}}$. As the aqueous solution evaporates and approaches equilibrium with

$H_{2}O$. The only other reported hydrate of malonic acid is a hexahydrate ($x = 6$), observed using thermal analysis and infrared spectroscopy, but it thermally decomposes at 256.5 K. It is therefore likely that the sesquihydrate is a different, unknown hydrate of MA. Notably, in 90% of the experiments the deliquesced solution did not recrystallize when the RH was reduced. This is consistent with previous studies that reported no efflorescence for MA at any RH at room temperature or reported an ERH of $<6\%$ RH.

$$\text{MGF}_{\text{anhydrous}} = \frac{M_{MA} + 18x}{M_{MA}} \times \text{MGF}_{\text{hydrate}}$$
the surrounding water vapor, the gradient will gradually disappear. The time required for disappearance of the gradient through solute diffusion \( (t_{eq}) \) once the droplet has achieved equilibrium with RH\textsuperscript{init} can be estimated from eq 4, where \( D \) is the diffusion coefficient of the solute, and \( R \) is the droplet radius. Using reported values of \( D \) for dicarboxylic acids \((1 \times 10^{-6} < D < 1 \times 10^{-5} \text{ cm}^2 \text{ s}^{-1})\)\(^{59,60} \) and \( R = 10 \mu\text{m} \), \( 0.015 < t_{eq} < 0.15 \text{ s} \).

\[
\frac{t_{eq}}{6D} \approx R^2
\] (4)

Because a charged droplet is trapped using an AC electric field, the trapped droplet can oscillate around the null point on account of its interaction with this field, and this motion could potentially cause mixing within the droplet. In our experiments, however, a particle is continually rebalanced so that it is always positioned at the null point of the field where it is stationary. Thus, diffusion is the sole mechanism by which solute transport can occur within a levitated droplet.

When a 20-μm-diameter droplet is introduced into the EDB at RH\textsuperscript{init} > ERH, evaporation will be relatively slow, and \( C_{s} \) will not rise to a level corresponding to a highly supersaturated state, so that nucleation will be slow, and the droplet will achieve a uniform concentration before nucleation occurs. As a result, nucleation will occur at sites throughout the droplet with equal probability. Such behavior has been reported for aqueous droplets of lithium carbonate at 60% RH.\(^{56} \) When an aqueous solution droplet is introduced into the EDB at RH\textsuperscript{init} < ERH, however, \( C_{s} \) will quickly rise to a highly supersaturated condition, exceeding the critical concentration for nucleation (the concentration at the ERH) before evaporation is complete, and a concentration gradient will develop within the droplet.\(^{57} \) Consequently, nucleation will be localized at the air-solution interface. The observation of the metastable polymorphs under this condition, wherein crystallization would be expected to be rapid and favor the kinetic forms, suggests an important role of the air-solution interface itself.

The \( \alpha \)- and \( \beta \)-GA polymorphs belong to the same space group (C2/c). Inspection of the crystal structure of \( \beta \)-GA reveals one-dimensional chains of GA molecules assembled end-to-end along the [101] axis by carboxylic acid dimers, with \( \text{O} \cdots \text{H} \cdots \text{O} \) distances of 2.67 Å, characteristic of strong hydrogen bonds (Figure S1, Supporting Information). The chains are assembled further by interchain hydrogen bonds with \( \text{O} \cdots \text{H} \cdots \text{O} \) distances of 3.02 Å, resulting in molecularly flat two-dimensional (101) layers. The (101) layers stack on each other through dispersive interactions (Figure 9). The solid-state structure of \( \alpha \)-GA reveals distinct (201) layers that contain one-dimensional hydrogen-bonded chains assembled end-to-end along the [112] direction by hydrogen-bonded carboxylic acid dimers with \( \text{O} \cdots \text{H} \cdots \text{O} \) distances of 2.65 Å, but interchain hydrogen bonds are absent (Figure S1, Supporting Information). Moreover, the GA carboxylic acid groups are rotated out of the (201) plane and the layers are more corrugated, to the extent that the chains are rotated substantially out of the plane (Figure 9).\(^{61} \) The structure also reveals unusually short interlayer \( \text{C} \cdots \text{H} \cdots \text{C(carboxylate)} \) and (carboxylate)\( \text{O} \cdots \text{C(carboxylate)} \) that appear to be a consequence of interdigitated packing of the corrugated planes. Collectively, these features — weaker in-plane hydrogen bonding, out-of-plane interlayer interactions, greater corrugation — suggest that the surface free energy of the \( \alpha \)-GA (201) plane is greater than that of the \( \beta \)-GA (101) plane. As such, one would expect that the formation of a \( \beta \)-GA nucleus at the air-solution interface, with the (101) plane parallel to the interface, would be favored over the formation of \( \alpha \)-GA nucleus oriented with the (201) plane parallel to the interface. Further growth of \( \beta \)-GA would then proceed by stacking of the low surface energy (101) planes, which is thought to be the primary growth mode in aqueous solutions.\(^{20} \) Consequently, \( \beta \)-GA would be preferred at low RH\textsuperscript{init}, the condition at which crystallization is more likely at the air-solution interface than in the bulk volume of the droplet because of the rapid onset of supersaturation near the surface. Conversely, high RH\textsuperscript{init} the concentration remains relatively uniform throughout the droplet such that the difference in the driving force for nucleation at the air-solution interface is not as large and nucleation at the air-solution interface becomes less competitive. Moreover, the predominant crystal plane in \( \alpha \)-GA has a higher surface free energy, such that its emergence in a surrounding aqueous medium would be less unfavorable. Under these conditions, nucleation of \( \alpha \)-GA, which is kinetically favored, can predominate.

The crystal structure of \( \beta \)-MA (Figure S2, Supporting Information) reveals one-dimensional chains assembled end-to-end along the [021] direction by hydrogen-bonded carboxylic acid dimers \((d_{\text{O} \cdots \text{H} \cdots \text{O}} \approx 2.69 \text{ Å})\), with strong hydrogen bonds between chains in the (0T2) plane \((d_{\text{O} \cdots \text{H} \cdots \text{O}} = 2.76 \text{ Å})\) and weaker hydrogen bonds in the (100) plane \((d_{\text{O} \cdots \text{H} \cdots \text{O}} = 2.96 \text{ Å})\). Consequently, \( \beta \)-MA can be described as two-dimensional (0T2) layers held together by weaker, although significant, hydrogen bonds. Although this hydrogen-bonding motif suggests a 3D network, it is reasonable to suggest that the surface free energy of a \( \beta \)-MA nucleus, dominated by the (0T2) layers with strong in-plane hydrogen bonding, would be less than that of the sesquihydrate form. Although the structure of malonic acid sesquihydrate has not been reported, the crystal structure of the homologous oxalic acid dihydrate reveals a three-dimensional network of carboxylic acid groups and water molecules that impart a hydrophilic character to its crystal faces. This suggests that nucleation of the sesquihydrate form would not be favored at the air-solution interface, thus favoring nucleation of \( \beta \)-MA at the air-solution interface at low RH\textsuperscript{init} conditions, possibly with the (0T2) plane parallel to the interface. Conversely, the presumed
hydrophilic character of the MA sesquihydrate would make nucleation within the levitated droplet more likely at higher RH init values, at which nucleation at the air–solution interface becomes less competitive.

### SUMMARY

The effect of ambient humidity on the polymorphic outcome of evaporation-driven crystallization in levitated aqueous solution droplets of malonic acid and glutaric acid revealed the formation of metastable α-GA and a sesquihydrate of MA at higher initial relative humidity, whereas the thermodynamically preferred β-GA polymorph and anhydrous MA formed at lower initial relative humidity. The stable forms appear under conditions wherein high supersaturation develops near the air–solution interface due to a lower surface free energy of their predominant crystal planes than their polymorph and hydrate forms, respectively. The ability to observe this behavior can be attributed to the unique “containerless” environment of the levitated droplet, which obviates spurious nucleation events that can compromise reproducibility, as well as to the microscopic size of the droplet, which permits the creation of large concentration gradients during evaporation.

### ASSOCIATED CONTENT

- **Supporting Information.** Crystal structures of GA and MA. This material is available free of charge via the Internet at http://pubs.acs.org.

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### ACKNOWLEDGMENT

The authors acknowledge the generous support of the National Science Foundation through Award Number CBET-0932810, the MRSEC Program of the National Science Foundation under Award Number DMR-0820341, New York University and the Polytechnic Institute of New York University, and the NYU Molecular Design Institute. Any opinions, findings, and conclusions or recommendations expressed in this article are those of the authors and do not necessarily reflect the views of the National Science Foundation.

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