Supporting Information

Crystal Regeneration – A Unique Growth Phenomenon observed in Organic Crystals Post Breakage

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1. Experimental Methodology

1.1 Materials

Acetaminophen (Paracetamol) meeting USP testing specifications (CAS no. 103-90-2) was sourced from Sigma Aldrich and Ethanol of analytical standard (CAS no. 64-17-5) from VWR chemicals. Both the chemicals were used as received and had a purity of 98%.

1.2 Seed preparation

A saturated solution of Paracetamol and Ethanol at 25°C was prepared by mixing an excess of the solid in a 500 ml glass bottle filled with Ethanol. Contents were stirred for 48 hours using a magnetic stirrer and further settled for 48 hours while controlling the temperature at 25°C using a water bath. Once excess paracetamol settles at the base of the bottle, the saturated supernatant was carefully extracted using a pipette and stored in a separate glass bottle in a 25°C incubator. The concentration of this solution was found to be 0.173 $g_{paracetamol}/ml_{Ethanol}$ using gravimetric analysis. All the experimental procedures from seed preparation, growth of macroscopic crystals to breakage and growth studies were carried out using the same stock solution prepared above.

To prepare the seeds, supernatant was pipetted into a crystallisation dish and covered using parafilm. 1 mm perforations were made for slow and controlled evaporation of the solvent at room temperature for 4-5 days. *Room temperature throughout the experimental procedure refers to 25°C ± 1°C*. Seeds of approximately 1-2mm diameter were carefully extracted from the base of the dish and pat dried with a soft tissue to avoid further crystallization.

In order to grow the seeds into 4-5mm (small) sized crystals they were placed in a well plate with each well containing the supersaturated solution and a single seed. Plate was covered with a lid and placed in the fumehood for 1 week to enable evaporative crystallisation – the solution was refilled as required.

In order to grow 7-8mm (large) sized crystals, small crystals were looped on a string made of Kevlar fibres and consequently immersed into in a 30ml glass vial of the saturated solution (Fig. 5.(A)). Parafilm with a 1mm perforation was used to seal the vial for controlled solvent evaporation over a period of 1 week. Crystals were hung in solution in order to minimise contact with the walls and base of the vial to prevent growth inhibition of facets.

The size of the crystal refers to an approximation of the longest edge to edge distance of a crystal.

1.3 Cutting AAP crystals

A scalpel was used to achieve all the cuts illustrated in the main text. To achieve a 50% cut, slight point pressure was applied pressure on the center of the surface (001) – this produced an indentation resulting in a self-propagating crack that split the crystal into two clean halves and exposed the facet (010). To achieve the 25% and 10% cuts, approximately 25% and 10% of the crystal respectively was broken from the edge, parallel to the cleavage plane by applying point pressure on facet (011). In the final scenario the crystal was 'chipped' where a fracture perpendicular to the cleavage plane was made. The original crystal after breakage is referred to as the 'mother' while the fragmented piece is termed as the 'daughter'. Overall the growth of 6 types of broken crystals was studied; 50% Cleaved, 25% Mother, 10% Mother, 25% Daughter, Chipped Daughter and Chipped Mother. All growth experiments were carried out using evaporative crystallization of saturated solution at room temperature, 25°C ±1°C.

1.4 Macro Photography set-up

The medium crystals were placed in a petri dish filled with the supersaturated solution, covered with a lid and stored in a fumehood for 1-1.5 weeks for constant evaporation at room temperature. Crystal images were taken every 5 hours using a Leica stereomicroscope and crystal dimensions a and b were measured using the Leica Application Suite (LAS) 4 software.

The large crystals were cut and hung in solution as described in sections 1.2 and allowed to grow via slow evaporation. A novel camera set up was developed (Figure 1(b) and (c)) using Nikon D90 DSLR cameras, Nikon AF-S 18-105mm lenses and Neewer extension tubes to achieve macro photography of AAP crystals. This enabled procuring high resolution, close-up images of crystals that highlighted the major facets during growth. To minimise reflection from the crystals, solution and the glass vial,

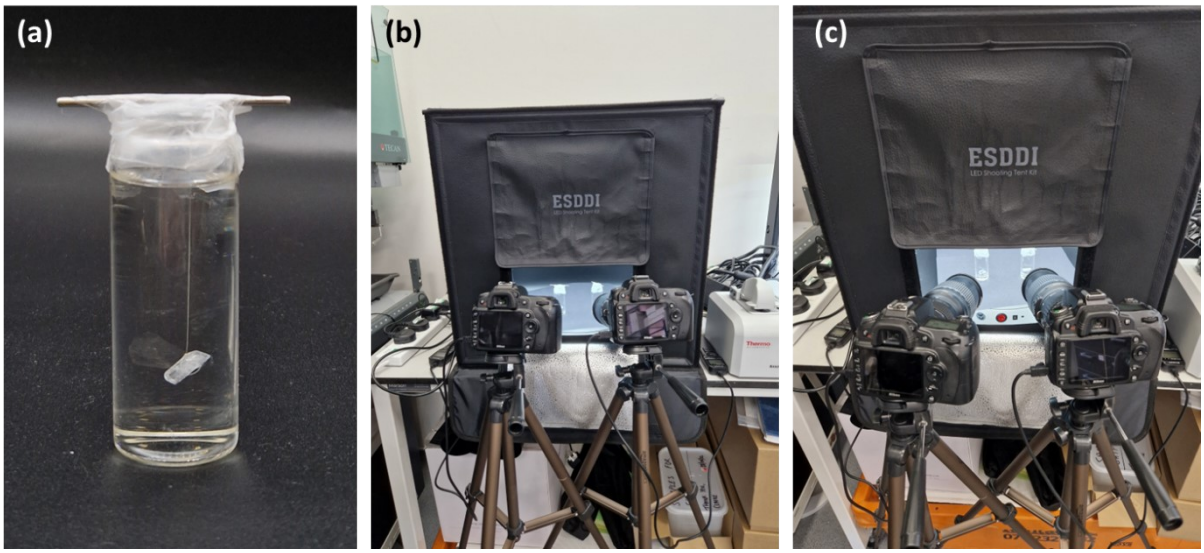

Figure S1 (a) Large crystal dangled in solution using kevlar fibres (b) and (c) Macro photography set-up used for crystal imaging

lighting was controlled using a photography light box and light diffuser. A camera was pointed directly at the vial being placed inside the light box against a black background and pictures were taken every half an hour over a period of 2-2.5 weeks using a timer. A side view of facet (010) was put in focus in order to observe the normal facet propagation. Using the images obtained a time-lapse video was created to show the behavior of crystals (50%, 25% Mother and 10% Mother) which can be found in the supporting documents folder.

1.5 Growth rate measurements

Optical micrographs of the small crystals and the macro-photographs of the large crystals, analysed manually using (LAS) 4 and ImageJ software respectively were used to obtain growth kinetics. Overall growth rates for the cleaved and whole crystals were found by measuring the change in two characteristic dimensions, a and b, over time using the following equation:

$$G = \frac{dL}{dt} \qquad \textit{Equation S1}$$

Where G is the growth rate, L is the length of the characteristic dimension in mm and t is the time in hours.

1.6 Powder X-Ray Diffraction Analysis

Powder X-ray diffraction (PXRD) patterns of AAP crystals before and after regeneration were collected by a PANalytical X'Pert PRO X-ray diffractometer. The X-ray diffraction measurement was performed using Cu Ka radiation (1.5405 Å) at 40 kV and 20 mA. All samples were conducted at a scanning rate of 50 seconds per step over a diffraction angle range from 5° to 35°.

The BFDH morphology and PXRD pattern for form I and II paracetamol was calculated in Mercury (version 2021.3.0, CCDC, Cambridge, U.K.) using crystal structure data deposited in the Cambridge Structural Database (CSD), reference: HXACAN35 [1] and HXACAN21 [2] respectively. The unit cell parameters of the stable monoclinic form are as follows: a=7.0661A, b=9.3367 A and c=11.6508 A of *P*21/n space group. While the unit cell parameters of the metastable orthorhombic form II are: a=7.1986A, b=11.782 A and c=17.183 A of *P*cab space group.

1.7 Scanning Electron Microscopy (SEM)

For SEM analysis, AAP crystals were mounted on carbon conductive adhesive tape and coated with gold in a Emitech K575X pumped coating system. Coating is done at sputter current of 20mA for 30 second to obtain Au coating of thickness approximately 15-20 nm. The gold-coated sample was analysed with a JEOL 6010LA scanning electron microscope with an accelerating voltage of 10 kV at a working distance between 13-14mm.

2. Results

2.1 Effect of size

Figure 2 shows the growth kinetics of large cleaved crystal where a trend similar to that of small crystals was observed – regeneration takes place in 2 phases followed by slow overall growth. However, the difference in size results in the larger crystals to regenerate over a longer period of time – phase 1 from 0-75 hours corresponding to rapid regeneration and phase 2 from 75-150 hours corresponding to slow regeneration. As can be seen from Table S1 the average growth rates for a small and large cleaved crystal for the characteristic lengths a and b are relatively similar. Although some

rates e.g. In phase 2, are considerably different for small and large crystals, the large standard deviation in the growth rates of larger crystals is substantial enough to be taken into consideration. This highlights the crystal to crystal variability during growth of large crystals mainly due to external factors such as impurities or unprecedented temperature fluctuations. Hence, further repeat experiments as well as the effect of external factors on regeneration need to be carried out to validate the growth rates of the large crystals.

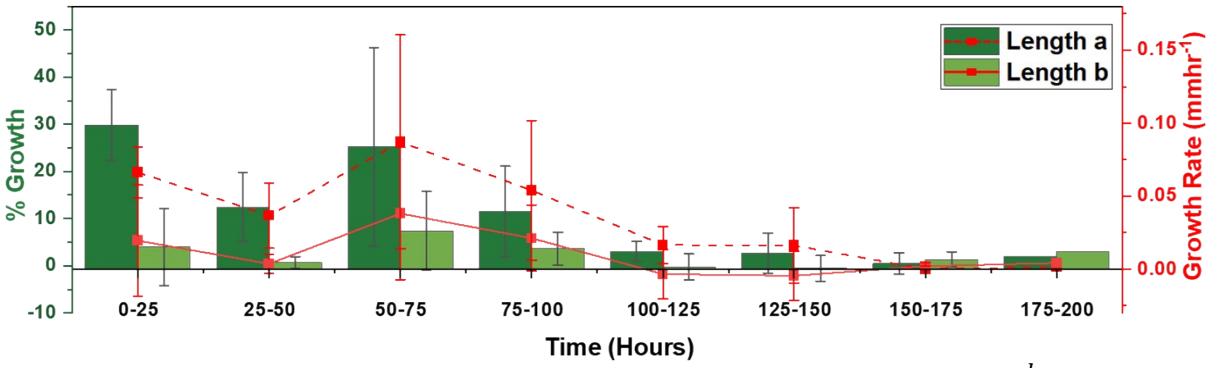

Figure S2 Growth of length and for a large cleaved paracetamol crystal a b

Table S1 Comparison of growth rates in the different phases of regrowth for a cleaved crystal in 2 different size ranges.

		Length a		Length b	
	Initial Crystal Size	**Small**	**Large**	**Small**	**Large**
Growth rate ($mmhr^{-1}$)	**Phase 1**	0.060 ± 0.009	0.063 ± 0.038	0.023 ± 0.005	0.020 ± 0.029
	Phase 2	0.025 ± 0.006	0.029 ± 0.009	0.030 ± 0.005	0.004 ± 0.018
	Overall Growth	0.017 ± 0.008	0.002 ± 0.003	0.017 ± 0.008	0.003 ± 0.002

2.2 Effect of extent and orientation of breakage

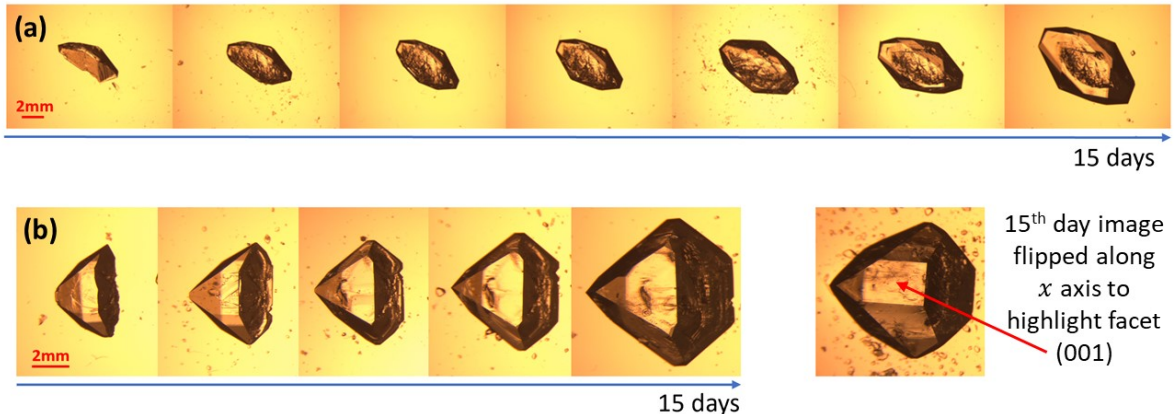

Figure S3 General trend of post breakage growth as observed for (a) 25% and 10% daughter and (b) chipped mother and daughter

2.3 P-XRD

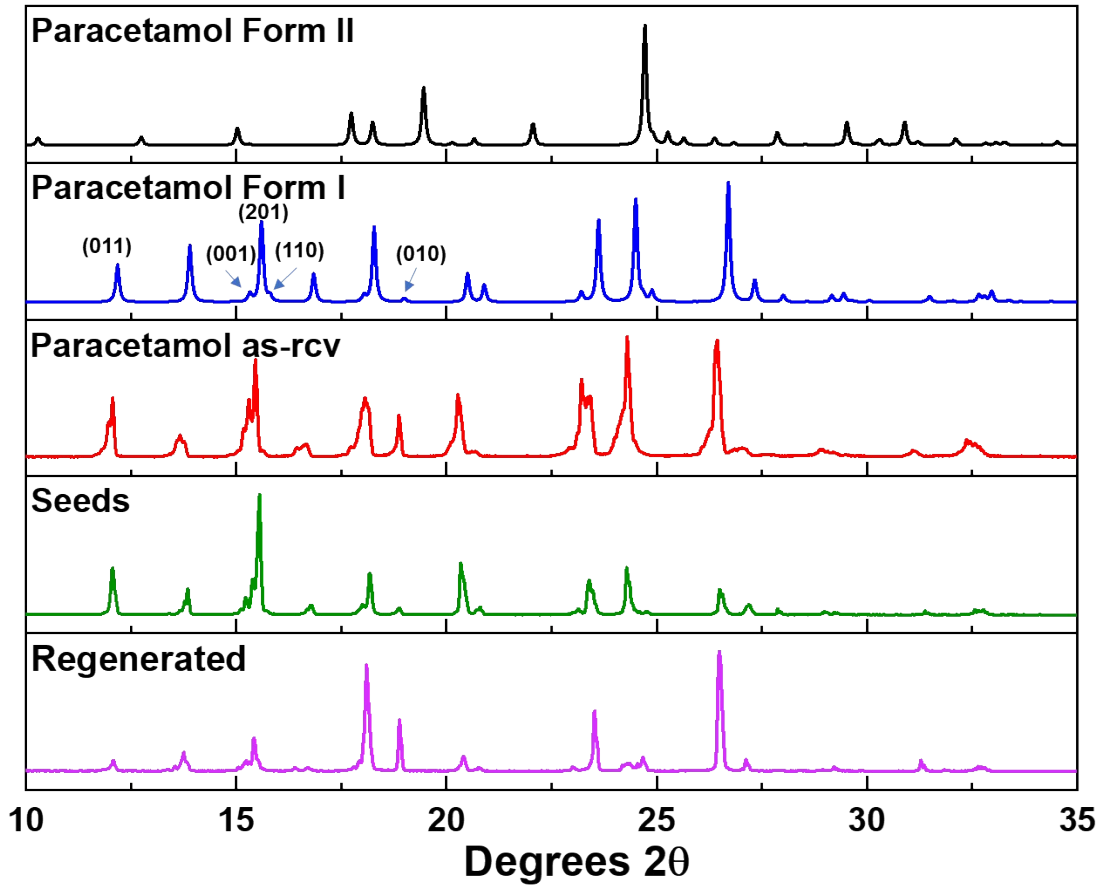

Figure S4 Powder XRD patterns of paracetamol top to bottom – form II (HXACAN21) and form I (HXCAN35) predicted via Mercury, paracetamol powder as received from Sigma Aldrich, seeds obtained via slow evaporation and the regenerated paracetamol crystals.

3. Regeneration in Carbamazepine crystals

Carbamazepine (CBZ) crystals were grown, broken and regrown using the same procedure as paracetamol but using methanol as the solvent. PXRD results confirmed the CBZ seeds to be of the most stable form III as can be seen from Figure S5. The preliminary results showing signs of regeneration in CBZ in Figure S6.

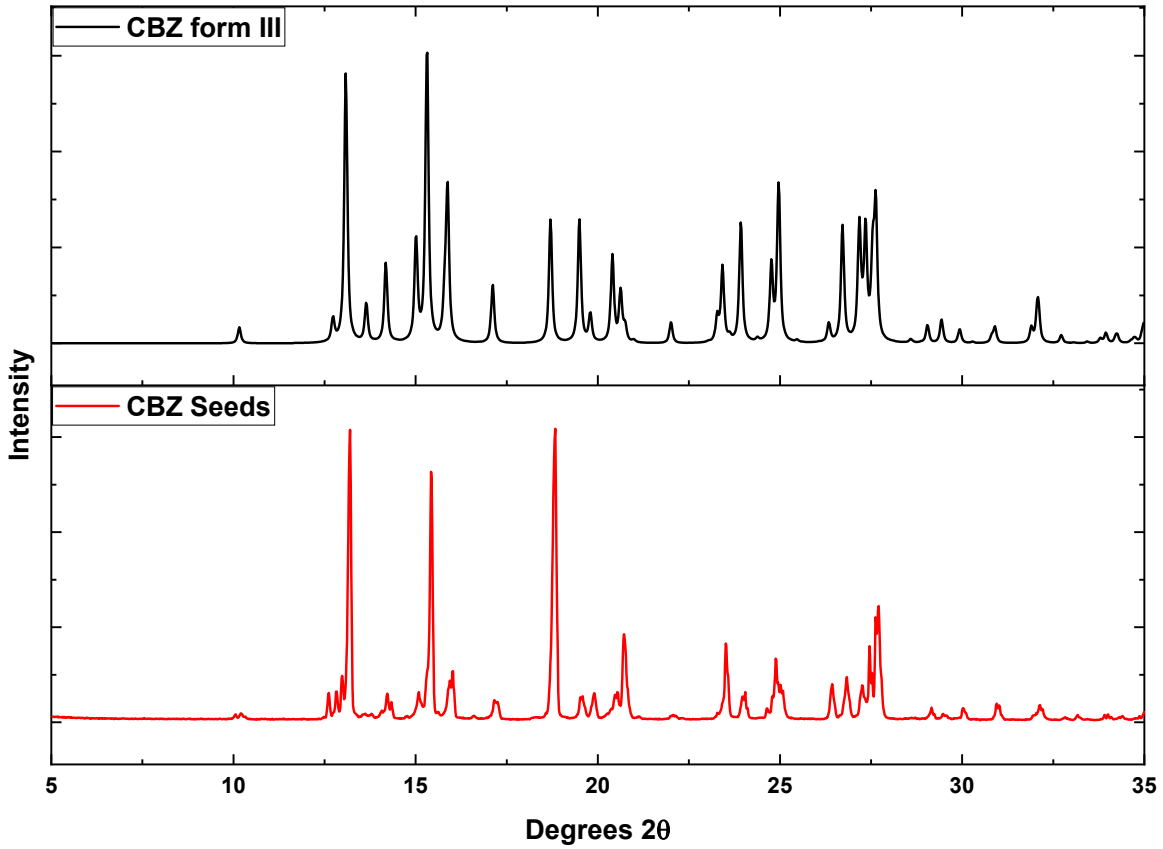

Figure S5 Powder XRD patterns of Carbamazepine top – form III (CBMZPN10)[3] predicted via Mercury, bottom - seeds obtained via slow evaporation.

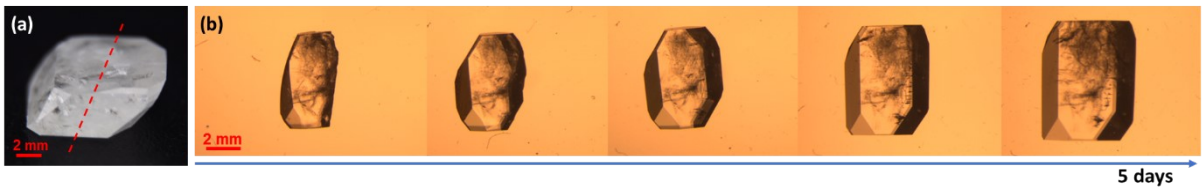

Figure S6 (a) Macroscopic crystal of carbamazepine (~6 mm) grown via slow evaporation of methanol (b) Regeneration behavior observed in small carbamazepine crystal

References

[1] R. Anitha, M. Gunasekaran, S. S. Kumar, S. Athimoolam and B. Sridhar, Spectrochim. Acta A Mol. Biomol., 2015, **150**, 488-498.

[2] T. N. Drebushchak and E. V. Boldyreva, Z. Kristallogr., 2004, **219**, 506-512.

[3] V. L. Himes, A. D. Mighell and W. H. De Camp, Acta Cryst. B, 1981, 37, 2242-2245.