Design of Experiment Study of the Seeding of Magnesium Sulfate Solutions

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ABSTRACT: Statistical Design of Experiment methods have been used to investigate 24 seeding parameters in over a thousand crystallization cycles. Surprisingly, the mass or surface area of seeds were of no consequence. Only the method of stirring and the reuse of vessels were significant. We explain these results in terms of the single event and embryo coagulation mechanisms.
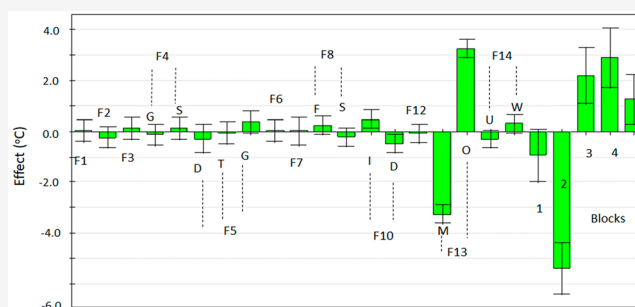
INTRODUCTION

Statistical Design of Experiment[1] (DoE) is widely used in crystallization studies particularly in process development,[2,3] to determine the conditions leading to desired crystal product characteristics, such as crystal size distribution and polymorphic form.[4] The reason for its widespread use is that it is capable of reducing dramatically the number of experiments required. It appears not to have been used to explore the seeding process itself (although it has been used to produce seed batches of appropriate characteristics[5]) despite the vast number of experiments needed for reliable results in such determinations. Nordstrom, Svard, and Rasmusen,[6] for example, ran 2911 crystallization cycles to check the effect of process parameters on the crystallization of salicylamide and the same group recorded 1160 cycles in the investigation of m-hydroxybenzoic acid polymorphs.[7]

We wished to understand the gap both in space and time between the introduction of seed and the first detectable crystallization. For example, if a single crystal of magnesium sulfate heptahydrate is dipped momentarily into a suitable supersaturated solution of magnesium sulfate, then a considerable period of time will elapse, dependent on conditions, before any crystallization is noticeable. The ensuing crystallization may appear to be sudden and takes place throughout the solution, resulting in a crop of fine crystals. This phenomenon was first noted by Miers[8] and then by Strickland-Constable et al.[9] but has not been widely acknowledged during crystallization studies. Since almost nothing is known about this phase of the crystallization process, we wished to explore as many parameters of the seeds, seeding

process, and solution conditions as possible. These are the circumstances under which DoE can make a vast reduction in the experimental effort required[10] in addition to concentrating the mind on the necessary details.

EXPERIMENTAL SECTION

Magnesium sulfate heptahydrate was purchased from Alfa Aesar and also from a local pharmacy. The solution concentrations were made up according to the concentration-solubility data provided by Ting and McCabe[11] and Mydlarz and Jones.[12]

The Cambridge Reactor Design "Polar Bear Plus" crystallizer was used for the two initial DoE fractional factorial sets of six factors in eight triplicated cycles and for some of the subsequent experiments. This apparatus is an integral system with temperature ramps generated by Peltier heating/cooling, controlled by a Eurotherm programmer. Two turbidity sensors were available, a roof prism and an 180° mirror reflection system. The flat-bottomed cylindrical glass crystallization vessel of internal diameter 75 mm and height 85 mm has both overhead and magnetic stirring. The stirring rate used was 250 rpm for both modes. The thermal insulation on the Polar Bear crystallizing vessel was judged as insufficient, so a 2 cm thick polypropylene jacket with a sealable observation window was constructed by the mechanical workshop in the School of Chemistry at the University of Southampton.

The HEL CrystalEyes E1041S software with turbidity and temperature probes was used to monitor solutions in jacketed glass vessels heated and cooled using ThermoHaake C25P, ThermoHaake C35P, and Julabo 25F programmable water baths at various periods of this investigation. The crystallization vessels that the CrystalEyes software monitored were made by the glassblowers in the Chemistry Department at Southampton University. These were cylindrical jacketed vessels of 6 cm diameter and either 8 or 16 cm tall, with detachable lids with three ports. Several lids were available including one with a noncentral hole, to improve the mixing by minimizing bulk rotation, and one with two additional small ports to enable filtration of the solution via a peristaltic pump (Welco, Japan, model WL10) and a filter. The temperature probe and turbidity probe of the HEL CrystalEyes model E10415 occupied two of the ports in the three-port lids. The third port was used for the introduction of seeds and to alter the concentration of the solution when needed. A further vessel with baffles was constructed in which three glass strips 5 cm long by 2 mm wide were fused at their ends to the vessel with a gap of 1 mm away to the side of the vessel to maximize mixing and minimize vortexing. The normal cylindrical magnetic stirrers were incapable of stirring the whole volume of the solution in the taller vessel, so triangular stirrer bars of 5 cm length and 1 cm side, which have greater lift, were used for consistency in all vessels. A 50 mm stirrer bar of traditional cylindrical design was employed for the two initial DoE sets of experiments reported here. The rotation rate was 250 rpm in all the experiments. Photographs of the crystallizer vessels are in the Supporting Information.

A rate of cooling or heating of 1 °C per hour or 3 °C per hour was used for the crystallization and dissolution events. In all cases, the same heating and cooling ramp was used, and crystallization comparisons were only made between experiments using the same rate of heating. The volume of solutions was around 150 mL in the vessels used in conjunction with the CrystalEyes system, except for that in the taller vessel, where around 300 mL was used. The methods of determining crystallization, proliferation, and dissolution temperatures have been discussed in detail in a previous paper.[13] For all the results reported here, visual assessment of the turbidity charts was adequate. Details of the factors are in the tables in the Results and further details including the statistics in the Supporting Information.

Four preliminary DoE matrices were investigated, the results of which are in Tables 1, 2, and 3 of the Results. The first two sets

Table 1. Tabulation of Significance of Factors 1−6 from Combined DoE Matrices 1 and 2

factor		effect (°C)	conf int	significance
F1	seeding temperature	−4.188	±1.898	yes
F2	cooling rate	2.179	±1.613	yes
F3	stirring method	2.063	±1.063	yes
F4	concentration	−1.482	±1.411	yes
F5	overheat temperature	0.973	±1.376	no
F6	seed size	0.576	±1.063	no

Table 2. Tabulation of Significance of Factors 1−3 and the Significant Interaction F1 × F2 from DoE Matrix 3

factor		effect (°C)	conf int	significance
F1	seed size	0.167	± 0.193	no
F2	cooling rate	0.38	± 0.193	yes
F3	seeding temperature	−0.067	± 0.193	no
F1*F2	interaction	0.192	± 0.193	yes

explored solution concentration, seeding temperature, overheating temperature above the dissolution temperature, overhead versus magnetic stirring, cooling rate, and seed size in eight runs of three cycles each. The third set was a full-factorial matrix exploring seed size, cooling rate, and temperature of seeding in 8 × 3 cycles. The fourth set was a fractional factorial exploration of seed size, cooling

Table 3. Tabulation of Significance of Factors 1−5 from DoE Matrix 4

factor		effect (°C)	conf. int.	significance
F1(A)	loose seeds	−0.983	± 0.669	yes
F1(B)	dipped and withdrawn seeds	0.222	± 0.710	yes
F1(C)	tethered seeds	0.762	± 0.710	yes
F2	seed size	0.333	± 0.502	no
F3	cooling rate	0.902	± 0.493	yes
F4	washed vs unwashed seeds	−0.298	± 0.502	no
F5	overheat temperature	−0.352	± 0.493	no

rate, seed washing, overheat temperature, and the method of seed presentation at three levels, namely, loose seeds, seeds confined in a tea bag dipped and withdrawn, and seeds in a tea bag left in solution. This required 14 × 3 cycles. In addition, a non-DoE set of crystallization cycles was run to explore different seedings. This comprised the comparison of 62 earlier seeded plus 63 unseeded experiments with the seeding brought about by the replicated addition of one 15 mg of seed, of two seeds of about 50 mg each, of 5 mg of sieved seeds of size <75 μm, and of 50 mg of seeds of size > 200 μm. The reason for these choices was to try to explore the surprising results of Ting and McCabe[14] and others[15] in respect of seed size.

The major matrix was intended to be an exploration of 14 seeding factors in 38 triplicated runs divided into 4 blocks: owing to a mistake in the fourth block, it had to be correctly repeated leading to 14 seeding factors in 48 runs in 5 blocks. Eight of the seeding factors related to the seeds themselves, namely, size, mass, preparation, presentation, storage, confinement, dehydration, and washing, and six to the solution, namely, addition temperature, concentration, overheat, and stirring method (magnetic or overhead) whether stirred and at what rate. This is reported as Table 4 in the Results.

The effects of different cooling rates from the first set of experiments were in agreement with the literature,[16−20] so were considered not worth repeating. A further DoE set investigated seed mass, washing, presentation, and whether stored after grinding in 18 runs of 5 cycles each. This was to confirm the results of the previous DoE set and is reported as Table 5 in the Results.

It was then realized that solution and vessel history could be making an impact and that the 6 °C overheat was inadequate to remove nucleating entities from solution. Two sets of non-DoE experiments on a hot plate were investigated, then one Plackett-Burman[10] design of DoE on the hot plate and one full factorial DoE in the usual crystallizing vessels. Tables 6, 7, 8, and 9 in the Results refer to these experiments. The first set explored the effects of overheat temperature and duration on the seeded crystallization temperature. The second compared mostly unseeded crystallizations in three flasks at a time, variously new, reused, washed at a high temperature, washed with detergent, washed with Decon 90, or silanized. Decon 90 is a detergent used for the removal of trace radioactivity from glass vessels during radiochemical experiments. It was considered that this would clean trace crystallization nuclei from the surfaces of vessels used for previous magnesium sulfate crystallizations. A volume of 20 mL was used, and the solutions were stirred with small cylindrical stirrer bars at 250 rpm. It was not possible to control the temperature so closely as in crystallization vessels, and much faster cooling rates were employed, 10−60 °C per hour. The temperatures were recorded to half a degree. Initial experiments showed that the vessel containing the thermometer usually crystallized first, despite washing with water at 57 °C between runs. So, the thermometer was placed in a fourth otherwise identical vessel. It was important to ensure that the necks of the conical flasks were of similar geometry and diameter; otherwise, differential evaporation, detected by different dissolution temperatures, ruined the comparisons.

The reuse of vessels was investigated in the third set. It would hardly have been possible to set up a DoE experiment in jacketed crystallization vessels with new vessels versus used vessels as a factor,

Table 4. Tabulation of Significance of Factors 1−14 from DoE Matrix 5[a]

factor		(a) on first crystal detection			(b) on elbow of proliferation		
		effect (°C)	conf int	significance	effect (°C)	conf int	significance
FI	seed size	0.04	±0.42	no	−0.05	±0.45	no
F2	seed mass	−0.24	±0.42	no	−0.12	±0.45	no
F3	concentration	0.13	±0.42	no	0.10	±0.45	no
F4(G)	ground seeds	−0.13	±0.42	no	−0.18	±0.45	no
F4(S)	sieved seeds	0.13	±0.42	no	0.18	±0.45	no
F5(D)	dipped and withdrawn seeds	−0.29	±0.43	no	−0.36	±0.45	no
F5(T)	tea-bagged seeds	−0.06	±0.43	no	−0.08	±0.45	no
F5(G)	glued seeds	0.35	±0.43	no	0.44	±0.45	no
F6	seed addition temperature	0.04	±0.42	no	0.03	±0.45	no
F7	stirring rate	0.01	±0.54	no	−0.18	±0.57	no
F8(F)	freshly ground seeds	0.23	±0.36	no	−0.24	±0.37	no
F8(S)	stored seeds	−0.23	±0.36	no	0.24	±0.37	no
F10(I)	intact seeds	0.48	±0.35	yes	0.15	±0.37	no
F10(D)	dehydrated seeds	−0.48	±0.35	yes	−0.15	±0.37	no
F12	overheating temperature	−0.07	±0.36	no	0.03	±0.37	no
F13(M)	magnetic stirring	−3.25	±0.35	yes	−2.53	±0.37	yes
F13(O)	overhead stirring	3.25	±0.35	yes	2.53	±0.37	yes
F14(U)	unwashed seeds	−0.3	±0.36	no	−0.25	±0.37	no
F14(W)	washed seeds	0.3	±0.36	no	0.25	±0.37	no
Block 1		−0.96		yes	−2.01		**yes**
Block 2		−5.37	±1.02	yes	−4.48	±1.09	yes
Block 3		2.18	± 1.10	yes	3.14	± 1.18	yes
Block 4		2.89	±1.18	yes	2.06	±1.23	yes
Block 5		1.26	±0.98	yes	1.29	±1.04	yes

[a]Also shown are the block factors of the five blocks, see text for elucidation.

Table 5. Tabulation of Significance of Factors 1−4 and the Significant Interaction F1 × F3 from DoE Matrix 6

factor		(a) on first crystal detection			(b) on elbow of proliferation		
		effect (°C)	conf int (±)	significance	effect (°C)	conf int (±)	significance
FI	seed mass	−0.25	0.38	no	−0.14	0.39	no
F2	dipped and withdrawn seeds	−0.21	0.38	no	−0.12	0.39	no
F3	unwashed seeds	0.13	0.38	no	0.24	0.39	no
F4	ground and stored seeds	0.28	0.38	no	0.21	0.39	no
F1*F3	interaction effect	0.34	0.37	no	0.51	0.38	yes

Table 6. Tabulation of Significance of Factors 1 and 2 and Their Interaction and Quadratic Curvature of the Factors, from DoE Matrix 7

factor		effect (°C)	conf int	significance
F1	duration of overheat	−2.61	±2.51	yes
F2	overheat temperature	2.99	±2.98	yes
F1*F1	quadratic	2.7	±4.59	no
F2*F2	quadratic	−9.01	±5.58	yes
F1*F2	interaction	−4.62	±4.28	yes

Table 7. Tabulation of Significance of Three-Vessel Hot-Plate Experiments

	condition		effect (°C)	conf int (±)	significance
(a)	U	previously used flasks	17.57	9.1	yes
	Na	new flasks on first cycle	−9.34	6.15	yes
	Nb	new flasks on subsequent cycles	−8.00	5.58	yes
	S	silanized flasks	−0.23	5.58	no
(b)	U + Nb	used flasks	−4.16	3.8	yes
	S	silanized flasks	4.16	3.8	yes
(c)	U + Nb	used flasks	−4.12	6.15	no
	D	DECON-treated flasks	4.12	6.15	no

so this was investigated by experiments in conical flasks on a hot plate, although a DoE system was employed. This used five factors and two dummies. The factors were overheat, fresh versus reused vessels, fresh versus reused solutions, fresh versus reused seeds, and seeded versus unseeded crystallizations. There is a clash between the latter two factors which was overcome in the analysis by combining them into one factor with three levels. The fourth set was an eight-run DoE full factorial using overheat, long versus short cycle time and fresh versus reused seeds.

The PXRD charts were obtained using flat, lightly ground samples on a Rigaku Miniflex diffractometer.

Data analysis was carried out using MODDE 8.0 software (Sartorius Stedium Biotech, Umea, Sweden), with multiple linear regression to derive the effects and analysis of variance for the statistical evaluations.

■ **RESULTS**

The main item of interest was the metastable zone width (MZW) associated with the various seeding options. The results are presented in Tables 1−9, but further details of the

Table 8. Tabulation of Significance of Factors from the Plackett−Burman DoE Matrix

factor		effect (°C)	conf int (±)	significance
F1	overheat temperature	3.56	2.3	yes
F2	vessel (used)	3.36	2.3	yes
F3	solution (used)	−0.23	2.27	no
F4 f	fresh seeds	−4.24	3.28	yes
F4 u	used seeds	−8.15	3.28	yes
F4 n	no seeds	12.39	3.08	yes

results, the raw data, and the associated statistics are shown in the Supporting Information. The four preliminary matrices comprised only 117 crystallization cycles, so these results must be prefaced with the warning that they have limited reliability. The first two matrices were analyzed in conjunction and the results shown in Table 1.

The seeding temperature was highly significant, the cooling rate and the method of stirring were significant, the concentration was barely significant, but neither the overheat temperature nor the seed size were significant. In the third DoE matrix, the cooling rate was significant, but neither the seed size nor the seeding temperature was significant, Table 2.

The results of the fourth matrix are shown in Table 3.

The cooling rate was significant, faster cooling leading to wider MZWs. Loose seeds were more effective than dipped and withdrawn seeding, in turn more marginally effective than tethered seeds, surprisingly, although the error bars indicate the low significance of the difference between the latter two results. In the non-DoE seeding experiments, two 50 mg of seeds were ineffective, but one 15 mg of seed, 5 mg of coarse seeds or 50 mg of fine seeds were significantly more effective in reducing the MZW over the unseeded situation. This erratic behavior mirrors that observed by others.[14,15]

Two measures of crystal detection are recorded in Table 4, first crystal detection and elbow of proliferation. The first crystal detection is the first movement of the turbidity curve away from the baseline. The elbow of proliferation is the point of change of slope of the turbidity curve; see ref 13 for a discussion of the relative merits of these crystallization detection measures. In the results from the 14 factor, 144 cycle 4(5)-block DoE experiment, the main item of interest again was the MZW associated with the various seeding options. Only the method of stirring and the block effects were significant, and these are discussed later. The crystallization results are summarized in Table 4.

The results of the 18-run, 4-factor DoE are summarized in Table 5. No main effects were significant.

The results of the non-DoE hot-plate experiments with the increased overheat temperature and duration are summarized

in Table 6. Both the temperature and duration of overheat were significant.

The results of the comparative three-vessel hot-plate experiments are shown in Table 7. Reuse of flask was highly significant and pretreatment of flask surfaces showed some effect in reducing the MZW, which presumably reflects an increased nucleation rate.

The results of the eight-run five-factor Plackett−Burman design[10] run on a hot plate are shown in Table 8. Seeding was significant as was overheat temperature and reuse of vessel but not the reuse of solution.

The results of the eight-run three-factor full factorial experiment are shown in Table 9. The overheat temperature was significant, but only in three of the four measures of crystallization.

■ DISCUSSION

The first objectives of the work reported here were to explore the applicability of the DoE approach to the investigation of the seeding process but at the same time to establish the sensitivity of seeding to a range of crystallizing conditions and to gain insight into the seed size issue. Larger seeds have been repeatedly been reported as being more effective that smaller seeds in promoting magnesium sulfate crystallisation,[15,23] which appears contrary to intuition, since larger seeds have a smaller surface area than smaller seeds of the same mass. This is discussed in detail in the next section

The results from the four preliminary DoE sets consistently suggested that there was no effect of seed size, that magnetic stirring was much more effective than overhead stirring in promoting crystallization, and that an overheat of 6 °C was ineffective in altering the MZW. All of these observations were confirmed by later experiments. The other factors investigated includig overheat temperature[17] were not confirmed, suggesting that the erratic crystallization of magnesium sulfate solutions needed more replication for statistical certainty.

The major DoE experiment (Table 4) was originally designed as a 38-run, 3 cycles per run experiment divided into 4 blocks to test 14 factors. There were eight independent runs in each block plus six repeats distributed overall to check reproducibility. The crystallizations were intended as student projects, so the idea of blocking was to reduce each block to manageable proportions that could be completed within the limited practical time available. Each block investigated 7 of the 14 factors, and the blocks were interlinked so that they could be analyzed both separately and together. Blocks 1 and 2 could be analyzed as a pair, as could blocks 3 and 4. Then, all of the blocks could be combined and analyzed. A mistake was made with block 4 in that the first half of block 4 was run with the second half of block 3. However, this erroneous block,

Table 9. Tabulation of the Significance of Factors from the Final DoE Matrix, a Full Factorial Eight-Run Experiment

factor		effect (°C)	conf int (±)	significance	effect (°C)	conf int (±)	significance
		(a) seeded runs, first crystal detection			(b) seeded runs, elbow of proliferation		
F1	overheat temperature	0.49	0.43	yes	0.31	0.39	no
F2	cycle time	0.23	0.43	no	0.02	0.39	no
F3	seeds (used)	0.01	0.43	no	0.04	0.39	no
		(c) unseeded runs, first crystal detection			(d) unseeded runs, elbow of proliferation		
F1	overheat temperature	0.85	0.50	yes	0.70	0.48	yes
F2	cycle time	−0.16	0.50	no	−0.20	0.48	no
F4	overheat duration	−0.06	0.50	no	−0.10	0.48	no

because of the interlinked design, could also be added to the combined analysis once the correct Block 4 had been run. So in total 14 factors were examined eventually in 48 runs (144 cycles). The design and the factors are set out in detail in the Supporting Information. Of these factors, F13, the overhead versus magnetic stirring was consistently significant and had a significantly larger effect than any of the others, as can most easily be seen in Table 4. This result confirmed that of the earlier runs. The only other large effects were the block factors, that is, the overall differences between the average values for the MZWs in the different blocks. This will be discussed later.

Seed presentation was another factor investigated.[18] Previously, momentary dipping was accomplished with a single crystal, but we wished also to investigate collections of seeds. So the use of teabags was investigated. Teabags are robust, water and temperature resistant, extremely porous and good at retaining particles. Two types of construction are available, those made of a non-woven cloth and thosemade of a 200μm plastic mesh. Both were used: no difference in performance was apparent, althouth the plastic mesh typecould not be used withvery fine seeds,of course. It is intuitive that loose seeds should be at least as effective as confined seeds. It would be expected that seeds retained in the teabag for the duration of the experiment would be at least as effective as dipping the seeds in the teabag into the solution and immediately withdrawing. that it appears not to be the case suggests that it may be a relection of the limited number of cycles involved (42 in total). Plastic teabags are reported to shed nano- and microparticles[21] when immersed at 95°C in aqueous solutions.Ot is not clear whether this applies at lower temperatures or to non-woven teabags. In any case we did not find any difference in crystallization behavior between solution in which teabags had been used or not. Seed washing should remove dendrites and other fine particles.[22] It is discussed in detail later. The further checks in the subsequent 18-run DoE experiment (Table 5) of several of the other factors again confirmed their lack of significance. These observations support the belief that the number of experiments in the first four DoE experiments were generally insufficient to establish reliable conclusions about the significance of factors.

In summary, there are many factors that might have been thought to be significant, namely, seed size, seed mass, seed preparation and presentation, seed addition temperature, stirring rate, seed storage, seed dehydration, solution overheating, and seed washing, but these further experiments have not found any evidence of significant effects. On the other hand, two factors, namely, the method of stirring and the block factors, which might not have been thought important, were very significant.

Stirring and Seed Size. The dominant most significant and consistent result is the method of stirring. Magnetic stirring is much, much better than overhead stirring at inducing crystallization, even though the stirrer blade area of all the overhead impellers was much larger than that of the magnetic stirrer bars and even though the material of the overhead impellers, glass, was much harder than that of the stirrer bars, which were of polyfluoroethylene. That the hardness of the stirrer blades is important in promoting crystallizations has been long known.[24] When the area, shape, and material of the stirring systems was equalized by mounting a stirrer bar on a glass rod to make an overhead stirrer, see Figure 1, positioned 1 mm above the base of the vessel, the difference between the rates of inducement of crystal proliferation was even more
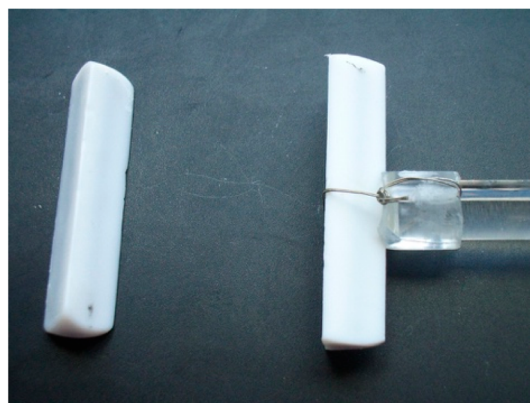

Figure 1. Triangular magnetic stirrer bar, left, and similar bar designed to act as an overhead stirrer.

dramatic than when an overhead stirrer of conventional design was used.

We judge all this to be due to the grinding of seeds on the bottom of the vessel by the magnetic stirrer bar. There will be a continuous generation of increasing area of fresh seed surfaces which has always been considered to be effective in promoting seeding.[25] Further confirmation of the effectiveness of seed grinding was obtained when using a conventional cylindrical bar with a central raised rim to promote easy magnetic stirring in place of the triangular stirrer bar. This was used in the first two DoEs. The effect of this proved to be intermediate between the triangular stirrer bar and overhead stirring in reducing the MZW, which would be consistent with the much-reduced area of contact between stirrer bar and the base of the crystallization vessel. At first sight, the explanation of the generation of nascent surfaces would appear incompatible with the effect under seed preparation, F4 of Table 4. Grinding the seeds and then either using them immediately or storing them and then using made no difference to their effectiveness. The difference must be that grinding in air and then immersing in the solution is not the same as grinding in solution where the surface exposure is immediate. The lack of significance even of the storage factor, F8, or the seed preparation factor, F4, suggests that surface healing must be a rapid process. The factor F10, intact versus dehydrated seeds, although not reaching significance levels, is larger than the other seed factors. The surface dehydrated crystals were slightly more effective than intact seeds. One could imagine that this was due to the generation of immediate fresh surfaces of the heptahydrate in the solution. The other possible explanation, the vast increase of surface area on dehydration, is negated by the lack of significance of the seed size or seed mass.

This observation of the effectiveness of magnetic stirring is, we believe, the explanation of the contrast between these results and the earlier literature with respect to the importance of seed size, which has been discussed at length previously.[19] The persistent reports in the literature over several decades assert that larger seeds are more effective than smaller sizes, despite the smaller area associated with large crystals. By contrast, we have consistently seen no effect of seed size. Because we were interested in this issue, many non-DoE seedings were conducted, not otherwise reported here. Seeds were sieved or ground and sieved into size ranges of >1 mm, 1 mm to 500 μm, 500−200 μm, 200−106 μm, 106−93 μm, 93−75 μm, and <75 μm. Equal masses were used in several cycles

of seeding, under several different solution conditions, but no effect on the temperature of crystal appearance or proliferation was ever apparent.

All the previous papers in the literature which discussed seed size used overhead stirring in larger vessels. Typical rotation speeds for overhead stirrers in previous studies (e.g., 600−950 rpm[15]) and the much larger stirrer blade diameters leading to high tip speeds will ensure that seeding is much more effective in such conditions than those used in the overhead stirring in this paper. Larger crystals, because of their momentum, are shattered by the impeller blade, but small crystals are carried around the blade in the liquid current. The speed limitation here was primarily due to vortex formation. Any speed above 250 rpm resulted in vortexing with air entrapment which interfered with the turbidity probe. In any case, it was also impossible to run the magnetic stirrer much above those rotational rates. Vortexing can be reduced and mixing improved by the use of baffles.[26] In later experiments, a vessel with built-in glass baffles was used. However, even with that improvement, the tip speeds were but a fraction of that used in the published investigations. So the explanation for the previous results is that large crystals are shattered by impact with the propeller, while small crystals are not. The importance of breaking or abrading crystals of magnesium sulfate in order to bring about seed proliferation has been studied.[27] A second implication of this assertion, discussed further below, is that magnesium sulfate crystals are relatively inert[19] in the seeding process unless activated in solution by grinding. the stirring rate, provided it is sufficient to disperse whatever species causes crystallization in the bulk of the solution to take place, is unlikely to make any difference under the conditions used here, although there is contrary evidence in the literature.[28] What it might have been affected is the balance between crystallization in solution and heterogeneous crystallization on surfaces. The stirring rate sometimes affected the variability of the measurements. If the stirring was insufficient and the crystals were large, then there might be insufficient lift to bring the crystals into the orbit of the turbidimeter, so no turbidity would be registered. If large crystals were formed but only occasionally entered into the optical path of the turbidimeter, the averaging over 20 s might reduce the turbidity to within the inherent noise levels. This occurred in some of the early measurements when examination of the effect of using unstirred solutions was attempted. In the present case, new jacketed crystallizing vessels and lids had different stirring and mixing characteristics due to the presence of baffles and off-center stirring. Although the stirring rate *per se* as a factor, F11, did not make a difference to the crystallization, the different vessel geometry might be expected to induce changes in crystallization behavior. For example, baffles might help retain crystallites in the re-entrant angles of the baffle-to-vessel attachment.

Block Effects. The only effects to show up strongly in Table 4, other than the method of stirring, are the block effects. These are the differences between the average results for one block (for crystallization point, proliferation point, or dissolution point) and the average of all of the other blocks. Since the different blocks were run at different times by different operatives, in different vessels, and with different apparatuses, it is perhaps unsurprising that such differences should occur. Nominally, all experiments should have been conducted in the same way, and conditions should have been equivalent. While we were not aware of any differences in

procedure, it is known that, for example, a crystallization vessel can determine the polymorphic outcome of an experiment, which must be dependent on the increased rate of crystallization of that polymorph, or the suppression of the competing polymorphs. Bohlin[29] has told how a metal vessel or stirrer encourages the crystallization of an undesired polymorph of an AstraZeneca intermediate, a circumstance entirely cured by the substitution of a glass vessel or stirrer. The shape of the vessel can make a difference to polymorphic outcome.[30] Even stirring,[31] bubbles,[32] or vibration[33] can produce differences in crystallization behavior.

If a vessel is reused, then it might have surface scratches, thus altering the crystallization behavior. On the other hand, a new vessel might have a different set of active sites. In any case, the block effect can encompass unidentified effects. One effect that was noticed after the completion of these experiments for example was that the positioning of the tea bag greatly affected the amount of turbulence to which it was subjected, although this did not seem to affect the crystallization behavior. The initial impression was that the block differences were artifacts. The vast changes in the MZW of alum solutions on reuse or in different vessels (Figure 1) ought to have warned of the need to be aware of and to check such factors between the blocks in the magnesium sulfate experiments, but they were totally overlooked. When the dramatic dependence of the MZW on new or reused vessels became apparent, it was realized that this and other factors had been uncontrolled within the blocks. Since substantial differences can be induced by these hidden undetermined factors, it substantiates the observation that the small differences in F1−F12 and F14 are insignificant.

Other Seed and Solution Effects from the Major (Fifth and Sixth) DoE Matrix Results. Information in the literature, other than seed mass or surface area,[34−36] is sparse as to what aspects of seed characteristics might contribute to crystallization, although Nyvlt has examined many seed parameters.[21] We therefore tried to think of as wide a range of seed and seeding characteristics as possible in this study. The lack of significant differences in every seed parameter was surprising and in contrast to assertions in the literature[37,38] even for magnesium sulfate crystallization.[23,27,36] As can be seen in Table 4 under F2, F4, F5, F8, F9, and F14, the mass of seeds added, the condition of the seeds, whether dehydrated, freshly ground, stored, or the mode of addition, whether washed or unwashed, dipped and withdrawn, left in solution, added as loose seed, or confined by gluing to a rod, or confined in a tea bag, made no difference to the temperature of crystal appearance or proliferation.

The expected effect of some of the factors is difficult to forecast. Washing of seeds, for example, might remove a debris of fine crystals from the surfaces and therefore reduce the surface area available for seeding.[23] On the other hand, the consequent addition as a slurry and the generation of new crystal surfaces might have been expected to increase the effectiveness of seeding. Recrystallization of the solution might take place on the surface before reimmersion in the crystallizing solution. There is no way of establishing whether the surface has become activated or annealed by the immersion other than observation of its capacity for catalyzing the seeding process. It is possible that these contrasting effects canceled out in these experiments.

However, for many of the other factors, the effect is readily forecastable. Larger mass of seeds of greater area must encourage crystallization. There is a long history in the

literature and in crystallization practice that a greater seed mass is more effective. For example, to ensure that the desired polymorph of Ritonavir was obtained after the stable polymorph suddenly appeared, a 50% seeding was initially used.[38] Seeding by slurry rather than as dry powder has been recommended.[39] By contrast, in this work, seed mass, seed size, or by implication seed surface area and wet versus dry seeding made no difference.

This implies that something immediate occurs which then is independent of the crystal surface so that the crystal surfaces no longer have any relevance to the seeding process. Some material particles or clusters or structure must be generated in the solution by the seeding. These entities, whatever they are, must then persist in solution perhaps changing into a nucleus before growing into a detectable crystal. We can also deduce that they must be exceedingly numerous because when crystals are first detected, there are very many of them. Since it does not matter whether the seeds are loose, glued, or confined in a tea bag, it would appear that the effective entities must be small enough to pass through the tea bag, which is not a very critical requirement. The most puzzling behavior of all the factors tested is the independence of seed mass and crystallization. It must mean either that the single event mechanism is in evidence or that the critical mass for seeding is much less than has been explored here. In the supplementary DoE matrix, seed masses of 2 mg versus 100 mg were used instead of 10 mg versus 50 mg. Again, no difference to the time and temperature of crystal formation or proliferation could be seen. The turbidity and the rate of its increase at the point of crystal detection appeared independent of the seed mass or other factor.

Seeding in industrial practice is commonly at 0.1−0.2%, perhaps up to 5% of the crystallizable component of the solution.[40] The lowest mass investigated here was only about 0.002% of the mass of the magnesium sulfate in solution. In the dipped and withdrawn case, only a small fraction of this can transfer to the solution, because there appears to be no change in the amount of crystals withdrawn. If this miniscule portion is effective, all the other material must be redundant.

Seed addition temperature might have perhaps been expected to be significant because the seeds would have spent longer in the cooling solution when added at a higher temperature. However, as has been pointed out, seed growth unaccompanied by proliferation is observed at temperatures above the secondary nucleation threshold;[41] given the lack of effect of seed size, this result is completely compatible. As can be seen in the effects and error bars of Table 4, the concentration of solution, F3, and the overheating, F12, made no discernible difference to the MZW values obtained.

There are limitations both on the ranges possible for all these parameters and on the total effectiveness of the distinctions between low and high. It would perhaps have been desirable to have used a larger mass of seeds for the high condition in the DoE and a smaller mass for the low. However, one would not wish to increase the concentration of the solution significantly by the use of a larger mass, while the smaller mass is limited by the ability to weigh accurately both the mass of a single crystal and the mass of the collection for the small mass, larger crystal runs. In the subsidiary DoE, Table 5, the levels of the seed mass were widened, but again there are operational limits.

Although the overheat experiments were consistently insignificant, this was because of a poor choice of high and

low levels as is shown by the results in Tables 6, 8, and 9; see Discussion.

Non-DoE Hot-Plate Experiments, the Results of Which Are Shown in Tables 6 and 7. These were exploratory experiments preliminary to formal DoE matrices. It is often desirable to precede DoE experiments with an assessment of suitable levels and to forestall difficulties in procedures. However, the results from these, although not probative in themselves, added weight to the further results and are therefore discussed here. Those experiments, the results of which are shown in Table 6, were intended to assess the effect of overheat temperature and duration on the MZW. When it had become clear as a result of a chance observation that 6 °C overheat was insufficient, the levels were adjusted. Overheating of 44 °C has been shown to have a dramatic effect on MZWs in the case of vanillin crystallization,[42] hugely more than a 30 °C overheat. For this reason, overheating between 10 and 48 °C was explored. Only the overheat temperature was significant. That the duration was insignificant probably means that the overheat temperature is rapid in breaking up the solution structure.

A problem that appears not to have been mentioned in the literature is the continuous shrinkage of MZWs with repeated crystallization. The first unseeded crystallization cycle of magnesium sulfate in a new vessel, for example, typically produces an MZW of 12−23 °C or greater. However, on repeating the cycle, this rapidly drops. If one can examine the vessel during the cooling cycle, but before crystallization, a ring of crystals will be found especially around the metal shafts of probes but also just above the meniscus on the sides of the crystallizing vessel. The extent of the incrustation seems to depend in our experience on the diameter of the shaft. It also seems probable, although we have not checked this, that the material of the shaft and its state of polish could make a difference. If these crystals fall into the solution, clearly such adventitious seeding could interfere with the recorded seeding. Even if they do not detach, the inevitable splashing during stirring could result in a similar interference. However, this is not the only source of problem. We have evidence from melt crystallization (unpublished) of templating of surfaces by crystals. This has previously been noted.[43] Since the surfaces are inorganic, it seems likely that no amount of heating of the solution will eliminate these potential heterogeneous seeding points. Figure 2 which shows the change of MZW of alum solutions[44] with repeated seeding and change of vessel illustrates the potential for interference of the intended seedings by adventitious seeding.

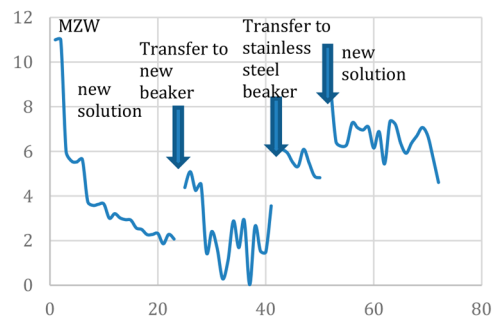Clearly, very large numbers of cycles both in new and in well-used vessels are needed in order to ascertain the

Figure 2.

magnitude of these effects or to swamp these effects and obtain satisfactory repetitions.

The source of the magnesium sulfate was also important. When there was a supply problem with the usual laboratory suppliers, some "Care" samples were purchased from pharmacies. These however contained minute amounts of fine insoluble particles, too small in total quantity to identify, but sufficient to act as heterogeneous seeds. A similar problem was also noted after several crystallization cycles, irrespective of the source of the magnesium sulfate, in that fine scum appeared on the surface. It was thought that this might be barium sulfate or calcium sulfate leached from the glass vessel. It is a known problem in the pharmaceutical industry with vials of solutions for injection.[45] Again, the amounts were too small to identify but enough to interfere with the measurements.

The issue of vessel reuse was addressed in DoE sets 8 and 9, Tables 7 and 8. The effect of different vessel treatments on the MZW was examined in DoE 8. Flasks previously used for magnesium sulfate crystallization were compared with new flasks and with silanized new flasks. The results show that new flasks produced significantly larger MZWs than used flasks, but this difference disappeared completely with one use. On the other hand, the silanized flasks continued to show significantly larger MZW with reuse. Table 7a would suggest that silanized vessels were without effect. This is because the average MZW of silanized vessels coincidentally produced the same value as that of the average of new and used vessels. When the silanized flasks are compared with equivalent unsilanized flasks in Table 7b, the true effect is apparent. A further set of experiments assessed the difference between used flasks, new flasks, and flasks treated with the powerful detergent Decon 90. Insufficient experiments were conducted to give a statistically significant result. The overall conclusion from this set of experiments is that the nature of the vessel and its history is important, a conclusion confirmed by the results of the DoE experiment below.

Plackett−Burman DoE: Overheat and Reuse of Vessel and Solution and Seeds, Table 8. The opportunity was used to check the effect of seeding in this experiment. Although it had always been assumed that seeding as opposed to the lack of seeding was effective in reducing the MZW, we had never formally checked this in a DoE set. As Table 5 shows, seeding is highly effective.

The overheat levels were 2 and 12 °C; 12 °C proved to increase the MZW significantly. This result was confirmed by that of the eight-run full factorial DoE: the overheat factor is discussed further there.

The other significant factor was the reuse of vessel, confirming the observations just discussed. Unstirred freshly prepared solutions of magnesium sulfate in a vessel not previously used for the crystallization of magnesium sulfate solution typically have an MZW of 23 °C or more. Even when stirred and seeded with fresh seeds, the MZW is typically 12 °C. Unseeded runs in reused vessels and solutions have MZW values ranging from 7 to 3 °C, while seeded ones range from 4 to 1 °C. These values are from hundreds of runs not reported here but from surplus runs (preliminary cycles, demonstration cycles, overnight cycles). These values mirror those reported by Mason and Strickland-Constable[46] but also confirm the rather different value ("1 °C of undercooling") provided by Mullin.[47] Implications of these observations is reserved for the final section of this discussion, where the support that these results give to Qian and Botsaris' embryo coagulation theory[48]

are discussed. We were convinced from our interpretation of the seeding mechanism that there would be carry over of active entities in the reused seeds and solution, which is why these factors were explored in several of these experiments. Neither of them proved significant. The reason may either be that they were not in fact significant or that the time lag between the original crystallization and their reuse had destroyed their effectiveness. The only way to test this critically would be to have two coupled crystallization vessels with a slight time lag between them so that new seeds and a portion of solution for induction of crystallization could be reused immediately, but the equipment for this was not available.

Results of the Eight-Run Full-Factorial DoE, Table 9. The factors intended to be explored in this experiment were overheat, cycle time, and seed reuse. The overheat factor of 12 °C was significant, but the other factors were not. It was then realized that the unattended overnight runs could be analyzed for overheat, duration of overheat, and cycle time because a selection of ramps had been used. Again, only overheat was significant. Table 9 shows overheat and duration of overheat and the comparison of seeded and unseeded runs from the combined experiments. As in previous experiments, the duration of overheat was insignificant. The reason for the inclusion of cycle time was because it was realized that transformation of the crystalline phase was occurring; see Figure 3 below. This shows the effect of transformation from
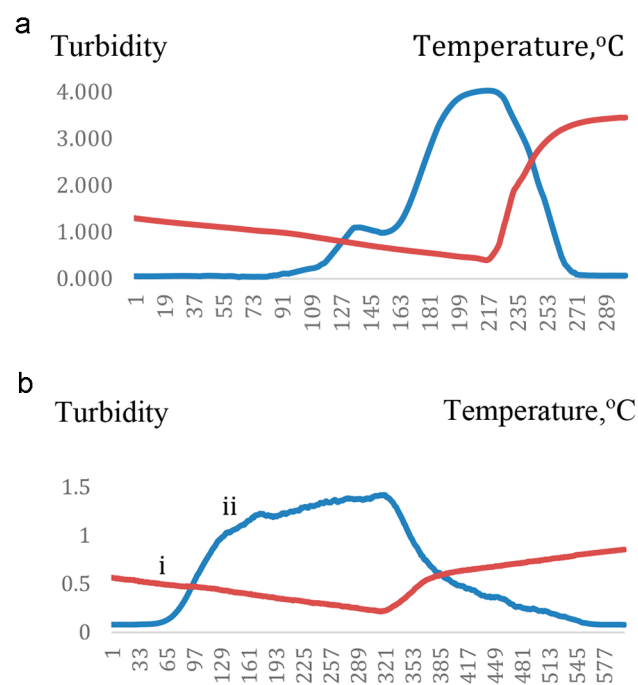

Figure 3. (a, b) Turbidity curves are shown in blue, and temperature ramps are shown in red. The scales at the foot of the curves are cycles at 20 s intervals.

the initially formed metastable magnesium sulfate hexahydrate to magnesium sulfate heptahydrate, stable at the temperatures of crystallization. Curves showing clear evidence of transformation such as that of Figure 3a were observed in a handful of cases out of more than a thousand cycles. However, the competition between the transformation and the turbidity increase due to the cooling ramp nearly always resulted in asymmetry of the turbidity curve shown in Figure 3b. The

rapid rise (i) then gives way to a second stage (ii) in which the dissolution competes with the proliferation resulting in a slower increase of turbidity.

Although it had previously been thought that magnesium sulfate solutions did not show signs of transformation,[13] it is clear from Figure 3, for example, that this is not always the case. If one accepts Ostwald's rule of stages,[49] then there will be many examples of transformation during crystallization. Indeed, a combination of this with McCrone's dicta about polymorph universality[50] leads to the conclusion that most crystallization involves transformation from a less stable to a more stable form during the process. The limitation on this is that only forms reaching their limit of solubility at the particular temperature and concentration present at the point of nucleation can be involved. The larger the metastable zone width, the greater the chance of more competing forms being present. That this does not appear to have been noted during solution crystallization studies, although of course intrinsic to the production of polymorphs by crystallization from solution, implies that transformation is usually rapid and therefore unobserved.[51] In the case of magnesium sulfate crystallization, it is undoubtedly the hexahydrate which appears first, then converts to the heptahydrate, stable in any solution below 46 °C but above 3 °C.[52] We have checked this by PXRD of rapidly harvested crystals from freshly crystallized solutions; see Figure 4a. IR spectra confirmed these observations.

The transformation in the cycle at low temperature, Figure 3a, appears to be more rapid than that at the higher temperature, Figure 3b. There could be numerous reasons for this: the stability of the hexahydrate is less at the lower temperature so that a greater thermodynamic driving force applies, the particle size might be different, and there is always the erratic nature of crystallization and transformation to contend with. The curve shape is highly dependent on the ramp segments in their rate and duration, and therefore an irregular curve of itself may not relate to a transformation process but rather to the change of solubility with temperature. The results of this eight-run DoE experiment are less than satisfactory. One problem with this experiment was that a 12 °C overheat was applied, but evaporation resulted in many cycles with a greater dissolution temperature and therefore a lesser overheat, so the effect appears much weakened. Because of the high water content of magnesium sulfate heptahydrate and its steep change with temperature, a small loss of water by evaporation results in a large change in solubility. Consequently, the intended 12 °C overheat for the "high" setting had to be adjusted to 10 °C overheat in order to maintain the matrix for the analysis. One benefit of these results is that they suggest that the critical temperature of the overheat may lie near 10 °C.

Since inevitably there are many unseeded runs overnight, we were able to get a second result out of the cycles, namely, a full factorial with temperature and duration of overheat and cycle time for those unseeded runs. This option was not available for previous DoE sets because seeding factors were dominant there. That the cycle time would prove insignificant is obvious in retrospect. The scales of Figures 3a,b show that the transformation takes place typically in 15−30 min. Only if no transformation had occurred would a lower dissolution temperature be apparent. The results also show that seeding reduces the average MZW for the unseeded runs from 3.13 °C to first crystallization and 3.29 °C to proliferation and for seeded runs, 1.56 and 1.88 °C respectively. These figures also
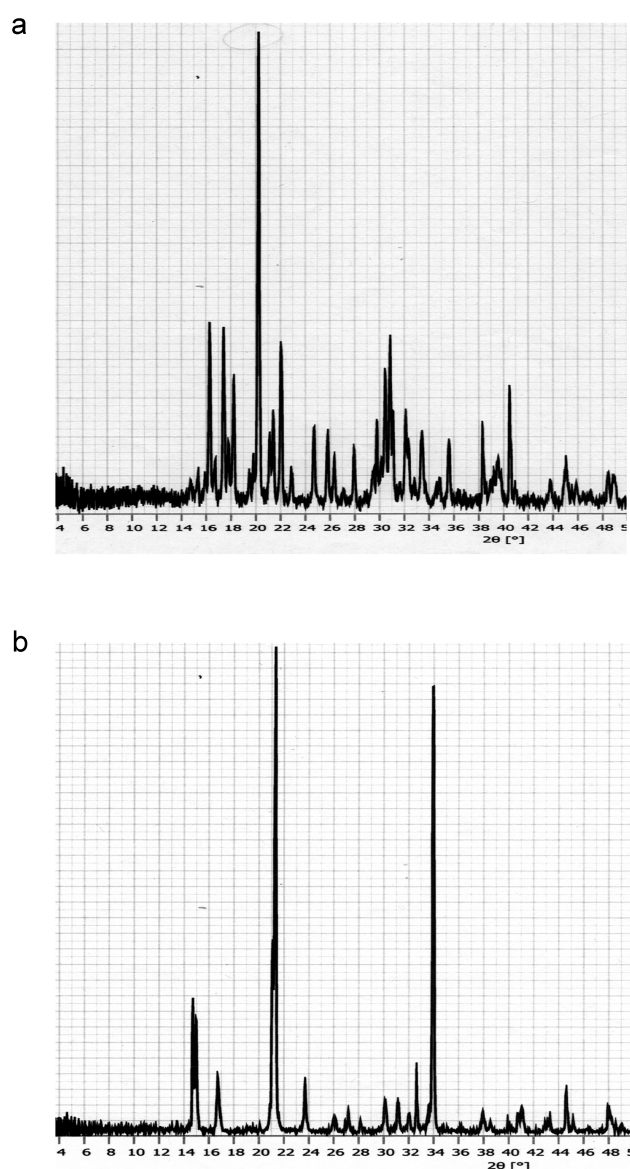
Figure 4. (a) X-ray powder diffraction chart of crystals harvested immediately after proliferation from a magnesium sulfate crystallization. This is the powder pattern of magnesium sulfate hexahydrate. (b) X-ray powder pattern of crystals harvested 1 h after proliferation. This is the powder pattern of magnesium sulfate heptahydrate.

show that proliferation after initial crystallization is longer in the seeded runs than in the unseeded ones. This is presumably due to the greater driving force at the greater supersaturation in the unseeded runs.

Implied Mechanism. All of these observations support the single event view[53−55] of seeding for magnesium sulfate crystallization. Despite assiduous efforts, no effect on the MZW could be observed for any seeding factor other than the stirring method. In particular, the mass of seeds added had no effect. After seeding, the first crystal detection might be delayed for 20 min and the proliferation for 20 min more. Kadam, Kramer, and ter Horst[53] have shown that only one crystal needs to be formed in order for secondary nucleation to take place in their experiments. By contrast, the key event in the crystallization of magnesium sulfate is undoubtedly the generation of an active particle by grinding. What is not

explained in the accounts of the single nucleus mechanism is how all the other events which could lead to the formation of primary nuclei are suppressed. One possible mechanism for such a suppression is described by Qian and Botsaris' embryo coagulation theory.[55] This asserts that the function of the seed is to catalyze the coagulation of colloidal prenucleation clusters ("embryos") in solution. In the absence of these, seeding will not occur. We believe that our results are consistent with this. The results presented here go further: magnesium sulfate seeds are inert unless freshly ground in solution to generate an active site. Once the nucleation process is put into motion through this active site, all of the colloidal agglomerates coalesce, so the solution is depleted of them. Consequently, no further active sites can have any effect, and the single event model of nucleation/crystallization is apparent.

The question arises as to why the prenucleation clusters can arise in the first instance during a crystallization process which depletes the solution of those embryos. The answer must be that embryos are generated and depleted continuously during the crystallization process, but as the solution reaches finality in the crystallization process and nears equilibrium, there is a reduced driving force for the mopping up of embryos.

Another puzzle is the role of the vessel surfaces, including that of the probes. The possibility of retained crystals at and above the meniscus has been canvassed earlier. The possibility of the templating of glass or metal surfaces was also mentioned. To these must be added the more generally accepted phenomenon of minute crystals trapped in crevices. Whatever the reality, the existence of foci for potential crystal growth is apparent, even against the background knowledge of the inertness of magnesium sulfate crystals in the seeding process. Frenkel et al. has suggested that the radius of curvature is a necessary feature of seed activity.[56] We have some support for this in the effectiveness of chemically or electrically etched tungsten wires[57,58] in encouraging crystallization. It is possible to etch to a point of only a few atoms,[57] which represents a very tiny radius indeed. Perhaps the trapped crystals or the surface templated features are tiny and have small radii that encourage nucleation.

Strickland-Constable et al.[9] and Miers[8] as mentioned previously observed and reported on the delay between the introduction of a seed and the subsequent first observation of crystallization particularly with magnesium sulfate solutions. We have previously commented upon the delay in crystallization caused by conformational confusion in acylanilides[59] and in sugars. It is possible that the time to nucleation in the case of magnesium sulfate is due to the competition between the numerous hydrated forms, ranging from undecahydrate[51] to monohydrate.[60]

The overwhelming advantage of the DoE approach is that it enables several factors to be examined in the same number of cycles that it would have taken to examine only one factor in a traditional experiment. Any interactions between factors will also show up. One disadvantage is that the whole experiment must be completed before any result can be extracted so that, for example, if instrument failure occurs, it may invalidate all the cycles run up to that point. Another disadvantage is that any error will affect not just the one cycle but be spread throughout much of the experiment, although thereby the individual measurements should be each less affected. It is easy for unnoticed errors to occur, for example, spontaneous preseeding, especially with narrow MZWs, especially when one

is trying to examine the effects of seeding at different temperatures.

Particularly for magnesium sulfate crystallization, the rational behavior tends to be swamped by the random, stochastic effects of the nucleation process. For that reason, the DoE approach was particularly valuable.

■ CONCLUSIONS

DoE design is shown to be applicable to investigation of seeding processes. DoE enabled the huge number of experiments which would have been needed by traditional methods to be reduced to manageable proportions, thus allowing many seeding parameters to be established. The lack of influence of seed size in the seeding of magnesium sulfate solutions, which is contrary to previous literature reports, is shown to be dependent on seed breakage. Magnetic stirring is therefore very much more effective than overhead stirring in inducing crystallization of magnesium sulfate solutions. The extensive experimental results presented here support both the single event mechanism and Qian and Botsaris' embryo coagulation mechanism. But they also hint at deeper complexity than that underlying the seeding process. The importance in the seeding process of vessel reuse is established. It is also shown that at least a 10 °C overheat is needed to remove seed precursors from the preceding cycle.

The original objective of the study, to establish the reasons for the delay of crystallization of supersaturated magnesium sulfate solutions, was not achieved. This work however established that this approach was incapable of doing so. Work is ongoing by terahertz spectroscopy (Koelbel et al.[61]) and by X-ray scattering of solutions (Light, Irving, and Threlfall, in progress) to monitor changes of crystallizing solutions immediately preceding and during nucleation and crystallization.

■ ASSOCIATED CONTENT

ⓈI Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acs.cgd.1c01257.

> Details of the statistical analyzes, histograms of results, justification for the DoE matrices explored, and pictures of the crystallization vessels (PDF)

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■ REFERENCES

(1) Davies, O. L. *Design and Analysis of Industrial Experiments*, 2nd ed.; Oliver & Boyd: Edinburgh, 1956.

(2) Nunn, C.; DiPietro, A.; Hodnett, N.; Sun, P.; Wells, K. M. High throughput Automated Design of Experiment and Kinetic Modelling to Aid in Process Development of an API. *Org. Process Res. Dev.* **2018**, *22*, 54−61.

(3) Sjovall, S.; Hansen, L.; Granquist, B. Using DoE to achieve reliable drug administration: A Case Study. *Org. Process Res. Dev.* **2004**, *8*, 802−807. (a) Saranteas, K. E.; Botsaris, G. D. *Separation and Purification by Crystallization*; ACS, 1997; Chapter 14, pp 150−171. (b) Gijsen, H. J. M.; van Bakel, H. C. C. K.; Zwann, W.; Hulshof, L. A. Optimisation of the Preparation and Isolation of 5-Amino-2,4,6-triiodophthalic acid dichloride. *Org. Process Res. Dev.* **1999**, *3*, 38−43. (c) Walter, T. S.; Diprose, J.; Brown, J.; Pickford, M.; Owens, R. J.; Stuart, D. I.; Harlos, K. A Procedure for Setting up High-throughput Nanolitre Crystallization experiments 1. Protocol, Design and Validation. *J. Appl. Crystallogr.* **2003**, *36*, 308−314. (d) MacFhionn-ghaile, P.; Svoboda, V.; McGinty, J.; Nordon, A.; Sefcik, J. Crystallization Diagram for Antisolvent Crystallization of Lactose: Using Design of Experiments to Investigate Continuous Mixing-Induced Supersaturation. *Cryst. Growth Des.* **2017**, *17*, 2611−2621.

(4) McCabe, J. F. Application of Design of Experiment (DoE) to Polymorph Screening and Subsequent Data Analysis. *CrystEngComm* **2010**, *12*, 1110−1119.

(5) Cui, Y.; Jaramillo, J. J.; Stelzer, T.; Myerson, A. S. Statistical Design of Experiment in Contact Nucleation as a Means of Creating Seed Crystals for continuous Tubular Crystallizers. *Org. Process Res. Dev.* **2015**, *19*, 1101−1108.

(6) Nordstrom, F. L.; Svard, M.; Rasmuson, A. C. Primary Nucleation of Salicylamide: The Influence of Process Conditions and Solvent on the Metastable Zone Width. *CrystEngComm* **2013**, *15*, 7285−7297.

(7) Liu, J.; Svard, M.; Rasmuson, A. C. Influence of Agitation and Fluid Shear on Nucleation of m-Hydroxybenzoic acid Polymorphs. *Cryst. Growth Des.* **2014**, *14*, 5521−5531.

(8) (a) Miers, H. A.; Isaac, F. XLVII. The Refractive Index of Crystallising Solutions, with Especial Reference to the Passage from the Metastable to the Labile Condition. *J. Chem. Soc.* **1906**, *89*, 413−454. (b) Miers, H. A. Growth of a Crystal: Being the Eighteenth Robert Boyle Lecture Delivered before the Oxford Junior Science Club. *J. Oxford Junior Sci. Club*, May 20, 1911.

(9) Strickland-Constable, R. F.; Mason, R. E. A. Breeding of Nuclei. *Nature* **1963**, *197*, 897−898.

(10) Stowe, R. A.; Mayer, R. P. Efficient Screening of Process Variables. *Ind. Eng. Chem.* **1966**, *58*, 36−40.

(11) Ting, H. H.; McCabe, W. L. Solubility of Magnesium Sulfate Heptahydrate. *Ind. Eng. Chem.* **1934**, *26*, 1207−1208.

(12) Mydlarz, J.; Jones, A. G. Solubility and Density Isotherms for Magnesium Sulfate Heptahydrate-Water-Ethanol. *J. Chem. Eng. Data* **1991**, *36*, 119−121.

(13) Coles, S. J.; Threlfall, T. L. A Practical Guide to the Measurement of Turbidity Curves of Cooling Crystallisations from Solution. *CrystEngComm* **2020**, *22*, 1865−1874.

(14) Ting, H. H.; McCabe, W. L. Supersaturation and Crystal Formation in Seeded Solutions. *Ind. Eng. Chem.* **1934**, *26*, 1201−1207.

(15) Kubota, N.; Fujiwara, M. Minimum Crystal Size for Secondary Nucleation of Potassium Alum in a Stirred-vessel Crystallizer. *J. Chem. Eng. Jpn.* **1990**, *23*, 691−696.

(16) Yang, H. Relation between Metastable Zone Width and Induction time of Butyl Paraben in Ethanol. *CrystEngComm* **2015**, *17*, 577−586.

(17) Zhang, X. Y.; Wang, X.; Hao, L.; Yang, X.; Dang, L.; Wei, H. Solubility and Metastable Zone Width of DL-Tartaric Acid in Aqueous Solution. *Cryst. Res. Techn.* **2012**, *47*, 1153−1163.

(18) Yang, H.; Florence, A. J. Relating Induction Time and Metastable Zone Width. *CrystEngComm* **2017**, *19*, 3966−3978.

(19) Coles, S. J.; Threlfall, T. L. A Perspective on a Century of Inert Seeds in Crystallisation. *CrystEngComm* **2014**, *16*, 4355−4364.

(20) Nordstrom, F. L.; Svard, M.; Malmberg, B.; Rasmuson, A. C. Influence of Solution Thermal and Structural History on the nucleation of m-Hydroxybenzoic acid Polymorphs. *Cryst. Growth Des.* **2012**, *12*, 4340−4348.

(21) Nyvlt, J. The Metastable Zone Width of Ammonium Aluminium Sulfate and Mechanism of Secondary Nucleation. *Collect. Czech. Chem. Commun.* **1982**, *47*, 1184−1188. Ness, J. N.; White, E. T. Collision Nucleation in an Agitated Crystallizer. *AIChE Symp. Ser.* **1976**, *72* (153), 64−73.

(22) Hernandez, L. M.; Xu, E. G.; Larsson, H. C. E.; Tahara, R.; Maisuria, V. B.; Tufenkji, N. Plastic Teabags release billions of Microparticles and Nanoparticles into Tea. *Environ. Sci. Technol.* **2019**, *53*, 12300−12310.

(23) Lal, D. P.; Mason, R. E. A.; Strickland-Constable, R. F. Collision Breeding of Crystal Nuclei. *J. Cryst. Growth* **1969**, *5*, 1−8.

(24) Shah, B. C.; McCabe, W. L.; Rousseau, R. W. Polyethylene vs. Stainless Steel Impellers for crystallization Processes. *AIChE J.* **1973**, *19*, 194−194.

(25) Larson, M. A.; Bendig, L. L. Nuclei Generation from Repetitive Contacting. *AIChE Symp. Ser.* **1976**, *72* (153), 21−27. Botsaris, G. D.; Sutwala, G. Nuclei Breeding in the presence of impurities. *AIChE Symp. Ser.* **1976**, *72* (153), 7−10. Strickland-Constable, R. F. Mechanism of Production and Growth Inhibition of Collision Crystallites. *J. Chem. Soc., Faraday Trans. 1* **1979**, *75*, 921−924.

(26) Atherton, J. H. Unpublished video showing dramatic increase of mixing both of particles and of solutions when baffles are inserted in a vessel.

(27) Stickland-Constable, R. F. *AIChE Symp. Ser.* **1976**, *72* (153), 33−40.

(28) Vesga, M. J.; McKechnie, D.; Mulheran, P. A.; Johnston, K.; Sefcik, J. Conundrum of gamma-Glycine Nucleation Revisited: to Stir or not to Stir? *CrystEngComm* **2019**, *21*, 2234−2243.

(29) Bohlin, M. Lecture, Aspects of Polymorphism and Crystallization Symposium; Hinckley, Leics, June 23−25, 1999.

(30) Capes, J. S.; Cameron, R. E. Effect of Polymer Addition on the Contact Line Crystallisation of Paracetamol. *CrystEngComm* **2007**, *9*, 84−90.

(31) Liu, J.; Svard, M.; Rasmuson, A. Influence of Agitation on Primary Nucleation in Stirred Tank Crystallizers. *Cryst. Growth Des.* **2015**, *15*, 4177−4184. Nordstrom, B.; Malmberg, M.; Svard, M.; Rasmuson, A. Influence of Solution Thermal and Structural History on the Nucleation of m-Hydroxybenzoic Acid Polymorphs. *Cryst. Growth Des.* **2012**, *12*, 4340−4348. Liu, J.; Rasmuson, A. Influence of Agitation and Fluid Shear on Primary Nucleation in Solution. *Cryst. Growth Des.* **2013**, *13*, 4385−4394.

(32) Matsumoto, M.; Ohno, M.; Wada, Y.; Sato, T.; Okada, M.; Hiaki, T. Enhanced Production of alpha-Form Indomethacin Using the Antisolvent Crystallization Method Assisted by N_2 Fine Bubbles. *J. Cryst. Growth* **2017**, *469*, 91−96.

(33) Liu, J.; Svard, M.; Rasmuson, A. Influence of Agitation and Fluid Shear on nucleation of m-Hydroxybenzoic Acid Polymorphs. *Cryst. Growth Des.* **2014**, *14*, 5521−5531.

(34) Frawley, P. J.; Mitchell, N. A.; O'Ciardha, C. T.; Hutton, K. W. The Effects of Supersaturation, Temperature, Agitation and Seed Surface Area on the Secondary Nucleation of Paracetamol in Ethanol Solutions. *Chem. Eng. Sci.* **2012**, *75*, 183−197.

(35) Tung, H.-H.; Paul, E. L.; Midler, M.; McCauley, J. A. *Crystallization of Organic Compounds*; Wiley, NJ, 2009.

(36) Sung, C. Y.; Estrin, J.; Youngquist, G. R. Secondary Nucleation of Magnesium Sulfate by Fluid Shear. *AIChE J.* **1973**, *19* (5), 957−962.

(37) Rousseau, R. W.; Li, K. K.; McCabe, W. L. The influence of Seed Crystal Size on Nucleation Rates. *AIChE Symp. Ser.* **1976**, *72* (153), 48−52.

(38) Bauer, J.; Spanton, S.; Henry, R.; Quick, J.; Dziki, W.; Porter, W.; Morris, J. Ritonavir: An Extraordinary Example of Conformational Polymorphism. *Pharm. Res.* **2001**, *18*, 859−866.

(39) Beckmann, W. Seeding the Desired Polymorph: Background, Possibilities, Limitations and Case Studies. *Org. Process Res. Dev.* **2000**, *4*, 372−383.

(40) Beckmann, W. *Crystallization*; Wiley-VCH: Weinheim, 2016.

(41) Threlfall, T. L.; Coles, S. J. A Perspective on the Growth-only Zone, the Secondary Nucleation Threshold and Crystal Size Distribution in Solution Crystallisation. *CrystEngComm* **2016**, *18*, 369−378.

(42) Hussain, K.; Thorsen, G.; Malthe-Sørenssen, D. Nucleation and Metastability in Crystallization of Vanillin and Ethylvanillin. *Chem. Eng. Sci.* **2001**, *56*, 2295−2304.

(43) Weygand, C.; Baumgaertel, H. Ueber ein natuerliches System der Polymorphen Formen des p'-Methylchalkons. *Liebigs Ann. Chem.* **1929**, *469*, 225−256 at p.231. Schaum, K.; Riffert, E. Changes in the state of aggregation and Polymorphism. Introduction and I. The crystallization of undercooled melts. *Z. Anorg. Allg. Chem.* **1922**, *120*, 241−260.

(44) Barrett, P.; Glennon, B. Characterizing the Metastable Zone Width and Solubility Using FBRM and PVM. *Trans IChemE* **2002**, *80*, 799−805.

(45) Boddapati, S.; Butler, L. D.; Im, S.; DeLuca, P. P. Identification of Sub-visible Barium Sulfate Particles in Parental Solutions. *J. Pharm. Sci.* **1980**, *69*, 608−610.

(46) Mason, R. E. A.; Strickland-Constable, R. F. Breeding of Crystal Nuclei. *Trans. Faraday Soc.* **1966**, *62*, 455−461.

(47) Mullin, J. W. *Crystallisation*, 2nd ed.; Butterworths: London, 1972.

(48) Qian, R. Y.; Botsaris, G. D. The effect of Seed Preparation on the Chirality of Secondary Nuclei. *Chem. Eng. Sci.* **2004**, *59*, 2841−2852.

(49) Ostwald, W. Studies of the Formation and Transformation of Solid Substances. *Z. Phys. Chem.* **1897**, *22U*, 289−330. Threlfall, T. L. Structural and Thermodynamic Explanations of Ostwald's Rule. *Org. Process Res. Dev.* **2003**, *7*, 1017−1027.

(50) McCrone, W. *Fusion Methods in Chemical Microscopy*; Interscience, NY, 1957.

(51) Liu, Y.; Gabriele, B.; Davey, R. J.; Cruz-Cabeza, A. J. Conceiving Elusive Crystal Forms. The Case of Paracetamol. *J. Am. Chem. Soc.* **2020**, *142*, 6682−6689.

(52) Duncan, T. M.; Reimer, J. A. *Chemical Engineering Design and Analysis*, 2nd ed.; Cambridge University Press, 2019.

(53) Kadam, S. S.; Kramer, H. J. M.; Ter Horst, J. H. Combination of a Single Primary Nucleation Event and Secondary Nucleation in Crystallization Processes. *Cryst. Growth Des.* **2011**, *11*, 1271−1277.

(54) Kulkarni, S. A.; Kadam, S. S.; Meekes, H.; Stankiewicz, A. J.; Ter Horst, J. H. Crystal Nucleation Kinetics from Induction Times and Metastable Zone Widths. *Cryst. Growth Des.* **2013**, *13*, 2435−2440.

(55) Kadam, S. S.; Kulkarni, S. A.; Ribera, R. C.; Stankiewicz, A. J.; Ter Horst, J. H.; Kramer, H. J. M. A New View on the Metastable Zone Width During Cooling Crystallization. *Chem. Eng. Sci.* **2012**, *72*, 10−19.

(56) Cacciuto, A.; Auer, S.; Frenkel, D. Solid-liquid Interfacial Free Energy of Small Colloidal Hard-sphere Crystals. *J. Chem. Phys.* **2003**, *119*, 7467−7471.

(57) Bourque, H.; LeBlanc, R. M. Electrochemical Fabrication of Scanning Tunneling Microscopy Tips without an Electronic Shut-off Control. *Rev. Sci. Instrum.* **1995**, *66*, 2695−2697.

(58) (a) Milhim, A. B.; Mrad, R. B. Electrochemical Etching Technique: Conical-long-sharp Tungsten Tips for Nanoapplications. *J. Vac. Sci. Technol. B* **2014**, *32*, 031806. (b) Ju, B.-F.; Chen, Y.-L.; Ge, Y. The Art of Electrochemical Etching for Preparing Tungsten Probes with Controllable Tip Profile and Characteristic Parameters. *Rev. Sci.* *Instrum.* **2011**, *82*, 0132707. (c) Kerfriden, S.; Nahle, A. H.; Campbell, S. A.; Walsh, F. C.; Smith, J. R. The Electrochemical Etching of Tungsten STM Tips. *Electrochim. Acta* **1998**, *43*, 1939−1944.

(59) Hursthouse, M. B.; Huth, L. S.; Threlfall, T. L. Why do Organic Compounds Crystallise Well or Badly or ever so Slowly? Why is Crystallisation Nevertheless Such a Good Purification Technique? *Org. Process Res. Dev.* **2009**, *13*, 1231−1240.

(60) Genceli, F. E.; Lutz, M.; Spek, A. L.; Witkamp, G.-J. Crystallization and Characterization of a New Magnesium Sulfate Hydrate $MgSO_4 \cdot 11H_2O$. *Cryst. Growth Des.* **2007**, *7*, 2460−2166.

(61) (a) Kolbel, J.; Li, Q.; Threlfall, T. L.; Zeitler, J. A. Measuring Solute Concentration with Terahertz time-Domain Spectroscopy in Single and Multicomponent Systems. *Anal. Chem.* **2022**, *94*, 1713. (b) Li, Q.; Kolbel, J.; Threlfall, T.; Zeitler, J. A. Flow Cell to Study Crystallization Processes in-situ by Time-domain Terahertz Spectroscopy. *IEEE Trans. Terahertz Sci. Technol.* **2022**, *12*, 193−198. (c) Li, Q.; Koelbel, J.; Davis, M. P.; Korter, T.; Bond, A.; Threlfall, T.; Zeitler, J. In-situ Observation of Crystallising Magnesium Sulfate Heptahydrate Solution with Terahertz Transnmission Spectroscopy. ChemRxiv, accepted; *Cryst. Growth Des.*, submitted.

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