Spontaneous Resolution by Stirred Crystallization**
By J. Michael McBride* and Randall L. Carter

It does not take long for a beginning chemist to develop a naive trust in predictions based on probability. Unlike a physician, who deals with a handful of cases, or an epidemiologist, who deals with hundreds of thousands, a chemist has the luxury of dealing with samples that contain more than $10^{20}$ molecules. For such large samples, predictions based on probability verge on certainty. Chemists are not disturbed that quantum mechanics precludes strict determinism in the behavior of an individual molecule, because it supplies reliable probabilities, and for macroscopic chemical samples molecular probability amounts to collective determinism. A chemist who has difficulty repeating a literature experiment may attribute the problem to unobserved or unreported differences in procedure or materials, or to error in measurement, but not to sampling error. The precise reproducibility from experiment to experiment that is the hallmark of good chemistry depends on having samples with enormous numbers of molecules.

Because of their training, chemists are particularly intrigued by phenomena that seem improbable. Several such cases involve chirality. A familiar and profound example is the occurrence in nature of only one enantiomer of complex biological molecules. How did living systems become resolved? Does biological handedness result from some weak interaction that provided a tiny evolutionary bias in favor of one set of enantiomers, or from one single molecular event that occurred at random but was destined to become a sort of molecular “Adam” by being multiplied into the whole kingdom of living things?

Crystallization provides a more homely example of curious statistics involving chirality. Although growth of a macroscopic crystal involves a large number of molecular events, formation of the original nucleus occurs but once per crystal. It is not uncommon for a solution to deposit only one crystal. Even if 100 or 1000 crystals grow, the number of nucleation events is small by chemical standards, and they differ from most chemical phenomena in that they can often be counted individually as well as measured collectively. Recently, Dilip Kondepudi* et al. of Wake Forest University (USA) have reported results from counting chiral crystals that provide new insights into the mechanism of nucleation.\(^1\)

Their interest in the role of competition and autocatalysis in breaking chiral symmetry in the biosphere,\(^2\) led Kondepudi et al. to repeat 19th Century work by Kipping and Pope on the crystallization of \(\text{NaClO}_3\).\(^3\) This salt is particularly well suited for such experimentation because aqueous solutions of its achiral ions produce beautiful rectangular prisms in the chiral cubic space group \(\text{P}2_1\text{3}\). Since the individual ions are achiral, they can join either enantiomeric lattice. Thus, in contrast to the situation for a racemic solution of intrinsically chiral molecules, there is no necessity that complete crystallization give equal amounts of the two enantiomeric solids. Furthermore, because of their cubic symmetry crystals of \(\text{NaClO}_3\) are not birefringent, so that one may easily identify an individual crystal's handedness by its rotation of linearly polarized light (3.6° mm\(^{-1}\) at 546 nm).

In 1898 Kipping and Pope measured the handedness of 3137 \(\text{NaClO}_3\) crystals from 46 separate crystallizations in order to test surprising reports that crystallization of analogous substances had shown strong bias toward formation of one enantiomorphic form. They were pleased to find that among 3137 crystals 1571 (50.08%) were dextrotrortatory. This fell well within statistical error of the unbiased 50:50 distribution they anticipated.\(^4\)

Kondepudi et al. confirmed these results by counting 525 dextrotrortatory crystals in a sample of 1000. But when they tried stirring the solutions (ca. 100 rpm) to remove the concentration gradients that favor nucleation at the air–liquid interface, they found that each beaker gave a predominance of one enantiomer. There was no systematic bias in favor of one of the enantiomers (dextrotrortatory crystals predominated in 18 batches, levorotatory in 14), but the bias within each batch was remarkably strong. Of the 32 batches, 23 formed only crystals of one enantiomer, for example one batch consisted of 865 levorotatory crystals and no dextrotrortatory crystals. Among 11 829 crystals counted, only 33 had the minority configuration for their respective batch, and 15 of these were formed in one particular crystallization.

In a stirred beaker a single nucleation event apparently gives rise to most or all of the hundreds of crystals formed. The most likely mechanism of this “Adam” effect is that crystals begin nucleating at random, but the first crystal to be struck by the stirrer clones hundreds or thousands of new nuclei. Growth of so many nuclei soon lowers the concentration of the solute below the threshold for spontaneous formation of primary nuclei, so there is no way to begin crystallization of the enantiomer.\(^5\)

We have confirmed this mechanism by video recording a number of stirred crystallizations. Figure 1 presents three stages during crystallization of a supersaturated \(\text{NaClO}_3\) solution that had been prepared by heating near the boiling point, degassing under vacuum, filtering through glass wool, and reheating to near the boiling point. After about an hour of stirring at room temperature,\(^6\) a single crystal had formed on the bottom of the vial and was growing toward the stirrer at 0.2 mm min\(^{-1}\). Figure 1a shows the first collision of the stirrer with the tip of the crystal. After 20 seconds and 35 collisions stirring was stopped. Within another 30 seconds more than 5000 tiny crystals became visible (Fig. 1b). A minute and a half after the first collision these crystals had grown into beautiful small prisms with dimensions of 0.1 to 0.2 mm (Fig. 1c). The speed with which the new crystals developed leaves no doubt that they grew from

\[^{(*)}\text{Prof. J. M. McBride, R. L. Carter}
\] Department of Chemistry, Yale University
New Haven, CT 06511 (USA)

\[^{(**)}\text{This work was supported by the Petroleum Research Fund of the American Chemical Society and by the Office of Naval Research. We thank Professors Lia Addadi and Dilip Kondepudi for valuable conversations.}\]
crystals and thus to provide a powerful new tool for demonstrating has been to study an achiral solute that gives chiral yet been incorporated into the crystal lattice, [I but has been suggested that secondary nuclei arise from an hypothetical layer of adsorbed molecular aggregates that have not yet been incorporated into the crystal lattice, [I but Kondepudi's results show that these nuclei had been incorporated at least enough to acquire the absolute configuration of the bulk crystal.

There have been several previous reports of less dramatic resolutions in unstirred solutions. In 1954 Havinga gave a clear discussion of “spontaneous asymmetric synthesis” in crystallizing the salt of a slowly racemizing allyl ammonium ion. [9] In 1971 Pincock et al. reported spontaneous resolution during crystallization of melted 1,1'-binaphthyl at 150 °C, at which temperature conformational racemization of the molecule in solution is rapid. [10] Like Havinga, they measured resolution by optical rotation after dissolving the crystals at room temperature, where molecular racemization is slow. In 1982 Addadi, van Mil, and Lahav reported spontaneous resolution during crystallization of a substituted divinylbenzene from ethanol, but not from other solvents. [11]

They measured resolution using optical rotation of the cyclobutane formed by solid-state [2 + 2] photocycloaddition. In seven crystallizations the enantiomeric excess ranged from 46 % to “ca. 100 %”. In 1986 Evans et al. reported that solid-state photoysis of chiral crystals of an achiral dibenzo-barrelene gave levorotatory product from all eight crystals formed in one batch. [13] In 1990 Feng and McBride reported that each of the 11 crystals from small-scale crystallization of a diacyl peroxide was of the same chirality. [14]

For achieving practical resolutions Kondepudi’s approach of stirring during crystallization has the advantages of reproducibly giving very high enantiomeric excess and of not requiring the growth and separation of large single crystals. Intentional seeding with dust from a single crystal might be equally effective, but cloning nuclei from the first spontaneously nucleated crystal to strike the stirrer automatically introduces a shower of nuclei at an appropriate level of supersaturation. Intentional seeding has the advantage that the chirality of a given batch is predictable. Either technique should work best when applied to a solution of achiral or rapidly racemizing species that form chiral crystals.

Determining resolution by counting individual crystals has an obvious advantage in precision over the previous approach of measuring optical rotations in solution. It would be very difficult by solution rotation to distinguish 99 from 100 % purity, but Kondepudi had no difficulty in counting ratios of 781:0 or 841:2. Thus his work may form the basis for a new way of studying crystallization; one in which statistical analysis plays an important role. One may now reevaluate previous studies statistically and carry out new studies to look for the contribution of cloning in unstirred systems. It seems clearly to have played a role in Kipping and Pope’s early work. Their data show that 5 of the 46 batches had distributions that departed from 50:50 by more than 2.3 standard deviations (σ), where only 1 in 50 would be expected to do so. The most dramatic had 24 dextrorotatory crystals and 68 levorotatory crystals, thus deviating from expectation by more that 4σ, an event that should occur only one time in 5000. At the other extreme, where 11 of the batches should have departed from 50:50 by less than 0.32σ, in fact only three did so. This suggests that cloning occurred in most, if not all, of their crystallizations.

The role that stirred crystallization may have played in the origin of biological chirality is not clear, but the surprise that many chemists (including the present authors) experienced

nuclei struck from the tip of the single crystal by these few contacts with the stirrer. [17] Subsequent examination of 48 daughter crystals with a polarizing microscope showed that every one was levorotary, as was the initial crystal.

Like many other phenomena with such a reasonable explanation, the results of Kondepudi et al. may not be surprising, in retrospect. As they point out, the idea of cloning crystals during stirred crystallization is familiar to chemical engineers as secondary nucleation. [8] Their novel contribution has been to study an achiral solute that gives chiral crystals and thus to provide a powerful new tool for demonstrating and studying secondary nucleation. For example, it has been suggested that secondary nuclei arise from an hypothetical layer of adsorbed molecular aggregates that have not yet been incorporated into the crystal lattice, [8] but Kondepudi's results show that these nuclei had been incorporated at least enough to acquire the absolute configuration of the bulk crystal.

There have been several previous reports of less dramatic resolutions in unstirred solutions. In 1954 Havinga gave a clear discussion of “spontaneous asymmetric synthesis” in crystallizing the salt of a slowly racemizing allyl ammonium ion. [9] In 1971 Pincock et al. reported spontaneous resolution during crystallization of melted 1,1'-binaphthyl at 150 °C, at which temperature conformational racemization of the molecule in solution is rapid. [10] Like Havinga, they measured resolution by optical rotation after dissolving the crystals at room temperature, where molecular racemization is slow. In 1982 Addadi, van Mil, and Lahav reported spontaneous resolution during crystallization of a substituted divinylbenzene from ethanol, but not from other solvents. [11]

They measured resolution using optical rotation of the cyclobutane formed by solid-state [2 + 2] photocycloaddition. In seven crystallizations the enantiomeric excess ranged from 46 % to “ca. 100 %”. In 1986 Evans et al. reported that solid-state photoysis of chiral crystals of an achiral dibenzo-barrelene gave levorotatory product from all eight crystals formed in one batch. [13] In 1990 Feng and McBride reported that each of the 11 crystals from small-scale crystallization of a diacyl peroxide was of the same chirality. [14]

For achieving practical resolutions Kondepudi’s approach of stirring during crystallization has the advantages of reproducibly giving very high enantiomeric excess and of not requiring the growth and separation of large single crystals. Intentional seeding with dust from a single crystal might be equally effective, but cloning nuclei from the first spontaneously nucleated crystal to strike the stirrer automatically introduces a shower of nuclei at an appropriate level of supersaturation. Intentional seeding has the advantage that the chirality of a given batch is predictable. Either technique should work best when applied to a solution of achiral or rapidly racemizing species that form chiral crystals.

Determining resolution by counting individual crystals has an obvious advantage in precision over the previous approach of measuring optical rotations in solution. It would be very difficult by solution rotation to distinguish 99 from 100 % purity, but Kondepudi had no difficulty in counting ratios of 781:0 or 841:2. Thus his work may form the basis for a new way of studying crystallization; one in which statistical analysis plays an important role. One may now reevaluate previous studies statistically and carry out new studies to look for the contribution of cloning in unstirred systems. It seems clearly to have played a role in Kipping and Pope’s early work. Their data show that 5 of the 46 batches had distributions that departed from 50:50 by more than 2.3 standard deviations (σ), where only 1 in 50 would be expected to do so. The most dramatic had 24 dextrorotatory crystals and 68 levorotatory crystals, thus deviating from expectation by more that 4σ, an event that should occur only one time in 5000. At the other extreme, where 11 of the batches should have departed from 50:50 by less than 0.32σ, in fact only three did so. This suggests that cloning occurred in most, if not all, of their crystallizations.

The role that stirred crystallization may have played in the origin of biological chirality is not clear, but the surprise that many chemists (including the present authors) experienced
on learning of Kondepudi's results shows clearly that mental adjustments are required in order to deal intuitively with "improbable" solid-state phenomena.

German version: Angew. Chem. 103 (1991) 298

[4] Kipping and Pope were lucky. Their 50.08% differs from 50% by only 0.09%, which would be expected to occur once in 14 times of performing their set of experiments.

[5] It is well known that the clusters that precede nuclei are much less stable (and thus more soluble) than larger crystals, because they have such a high fraction of surface molecules.
[6] 10 x 3 mm magnetic stirrer bar at 185 rpm.
[7] In other runs visible multiple crystallization began within two seconds of striking the primary crystal.