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Crystallization of Sodium Chlorate with d-Glucose Co-Solute Is Not Enantioselective**

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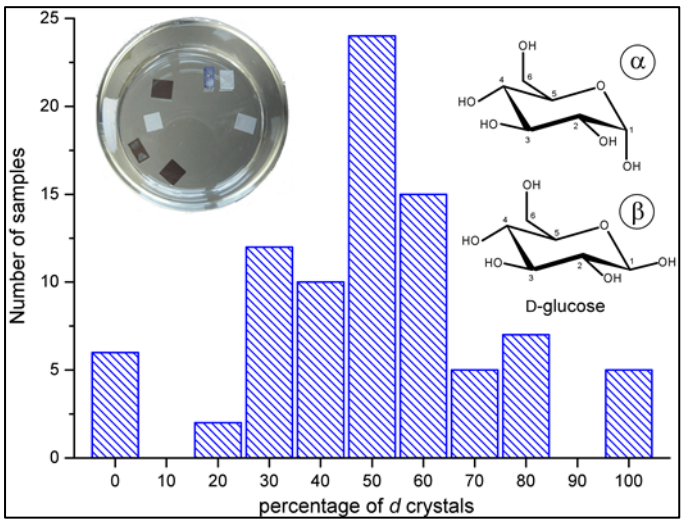
Supporting information:
Tables of numbers of d- and l-NaClO3 crystals obtained in crystallizations with D-glucose and maltodextrin are available. This material is available free of charge via the Internet at http://pubs.acs.org

Graphical abstract:



Keywords:

d-glucose; sodium chlorate; asymmetric crystallization; optical rotation; chirality

Abstract

New evidence is presented that crystallization of sodium chlorate in the presence of the chiral co-solute d-glucose does not bias the growth of levorotatory crystals over dextrorotatory. The weighted mean percentage of *d*-$NaClO_3$ was found to be $W_d = 50.4 \pm 1.4$, compared to previous experiments of Kipping and Pope showing $W_d = 31.7 \pm 0.9$ [J. Chem. Soc. Trans. **73** (1898) 606]. Possible explanations for the discrepancy between the historical results and the present work are discussed. The present work casts doubt on a result that has been accepted for over a century, concerning the ability of a chiral molecule to influence asymmetric crystallization, which has been used to promote theories on the possible origins of biological homochirality.

Main text

Chirality is one of the cornerstone symmetries of chemistry and biology. For a long time there has been intense interest in understanding how chirality can be transferred from one substance to another, also known as enantioselectivity. Enantioselectivity evidently occurred at some time during the evolution of life, leading to the presence of biological homochirality.[1-4] Asymmetric synthesis is a fundamental goal of synthetic chemistry, but yet often very difficult to achieve even with careful choices of chiral starting materials, use of asymmetric catalysis, and sometimes borrowing from nature's arsenal of enantioselective pathways.[5]

Apart from molecular chirality, e.g., in chiral carbon centers, there also exists chirality in the packing of achiral building blocks. A well-studied example is the inorganic compound sodium chlorate, which packs in a chiral space group $P2_13$, and whose crystals exhibit optical rotary dispersion to give *d* (dextrorotatory) and *l* (levorotatory) forms.[6]

In 1898, Kipping and Pope (K+P) published quantitative results showing that crystallization of sodium chlorate from solution yielded on average no preference for *d* or *l* crystals, although it was found that statistical fluctuations meant that any given sample could show a random preference.[7] In a remarkable demonstration of enantioselectivity in that work, K+P also showed that 20 wt % of co-solute chiral d-glucose could bias the crystallization, showing an overall preference in favor of *l*-$NaClO_3$ (~68% *l*).[7] This result suggests a strong interaction between molecular chirality and chiral packing, and has fuelled speculation that biological homochirality could have had its origins in interactions with abundant inorganic minerals, such as quartz (SiO_2) or calcite ($CaCO_3$).[8-10] The crystal space group of quartz is chiral, giving rise to *d* and *l* forms, and in general any crystal surface with structures that break inversion symmetry could be considered chiral. K+P also showed that 6 wt % of d-mannitol gave a preference for growth of *l*-$NaClO_3$ (~59% *l*).[7] Enantioselectivity by a chiral

co-solute during crystallization was apparently confirmed by Niedermaier and Schlenk (N+S) in 1972, who showed that d-mannitol gave a preference for *d*-$NaClO_3$ (100% *d*) and d-dulcitol gives mostly *l*-$NaClO_3$ (~99% *l*).[11] In a tantalising note on unpublished results, Pagni and Compton state that d- and l-arabinose, and d-sucrose, apparently showed no enantioselectivity for crystals of $NaClO_3$.[6] Enantioselectivity has also been demonstrated by other chiral influences, e.g., by seeding, and with β or positron radiation;[12] the results of bias in $NaClO_3$ crystallization are summarized in a review by Pagni and Compton.[6]

In 1990, Kondepudi et al. showed that an achiral external influence—rapid stirring—caused the crystallization to yield nearly limiting enantiomeric excesses (ee) in favor of *d* or *l*-$NaClO_3$ in any given vessel.[13] The high ee is explained by the enhanced secondary nucleation from a single initial seed; it is not, however, possible to predict whether the outcome will favor *d* or *l*, since the creation of the initial seed is random. Although this method causes asymmetric crystallization, it is not enantioselective in terms of cause and effect.

More recently, Petrova and Swift studied the crystallization of $NaClO_3$ in agarose gel.[14] The primary structure of the gel molecule consists of both l- and d-galactose monomers. The authors found that increased gel concentrations showed increasing bias towards one of the crystal enantiomers. They found that growth in 0.75 wt % aqueous agarose gel at 6°C showed a preference for *d*-$NaClO_3$ (~61% *d*), although remarkably this changed to preference for *l*-$NaClO_3$ at 24 °C (~57% *l*). Interestingly, they found that addition of small amounts of methanol to the prepared gel caused significant bias towards *l*-$NaClO_3$ (up to ~77% *l*): a result that they attributed to changes in the gel structure possibly caused by the increased hydrophobic environment of the solvent. At the conclusion of their report, Petrova and Swift note that they did not observe any bias to crystallization of $NaClO_3$ in aqueous solutions containing 1 wt % of d-galactose, perhaps suggestive that the helical tertiary structure of agarose and not molecular chirality of the galactose monomers was indeed responsible for their gel results.[14]

In line with the observed bias of crystallization caused by chiral solute molecules, Soai et al. have recently shown that *d* and *l*-$NaClO_3$ can bias the outcome of an autocatalytic asymmetric synthesis.[15, 16] It was found that (*S*)-5-pyramidyl alkanols are obtained with up to 98% ee in the presence of a crystal of *d*-$NaClO_3$ resulting from the enantioselective addition of diisopropylzinc to

pyrimidine-5-carbaldehydes; the corresponding (*R*)-alkanols are promoted by *l*-$NaClO_3$.[16] The high degree of selectivity is no doubt due to the autocatalytic nature of the reaction mechanism, which is able to effectively amplify any small bias in chirality. Nevertheless, the results show that some chiral interaction occurs between the inorganic crystalline $NaClO_3$ and the organic reagents, even in toluene solution.

If a chiral molecule can bias enantiomorphism in crystal growth, we ask the question: what are the specific, or possibly co-operative, molecular interactions between molecule and seed? With a view to understanding in more detail the interaction between a chiral molecule and a chiral crystal, we thought it prudent to first re-visit the experiments of Kipping and Pope: the results of this visit have been quite surprising: we outline our findings below, along with possible explanations for discrepancies between our results and the works of Kipping and Pope, and of Niedermaier and Schlenk.

A batch of crystallizations was prepared as follows: 400 g of sodium chlorate (Sigma Aldrich, ReagentPlus 99+%) was dissolved by stirring in 444 g of de-ionized water (Fisher, HPLC grade). The sodium chlorate solution concentration was 90% of saturation at 20°C, and the density was measured as 1.407 g cm^{-3} (20°C). After complete dissolution of the sodium chlorate, 120 g of d-glucose (Sigma Aldrich, 99.5%) was added to the solution and stirred to dissolve. The density of the final solution (d-glucose, sodium chlorate in water) was measured to be 1.418 g cm^{-3} (20°C). In later experiments, instead of the d-glucose, we used 90 g of maltodextrin (Sigma–Aldrich DE 4.0–7.0) in a solution of 300 g $NaClO_3$ in 333 g water. The relative proportion of glucose to sodium chlorate was the same as used by K+P, who used 200 g dm^{-3} of solution. In confirmation of tests made by K+P, we found that the glucose did not significantly change the saturation point of sodium chlorate. The final solution was filtered (Whatman 50, nominal pore size 2.7 μm) and transferred to a clean vessel for storage.

A rigorous regime of cleaning was used, to prevent the possible influence of dust, in particular sodium chlorate residue or dust. The crystallizations were carried out in a separate laboratory where sodium chlorate was not routinely handled; the air in the lab consists of conditioned external air. Prior to use, small (~8 cm diameter) pyrex crystallizing dishes were washed and rinsed in filtered de-ionized water, but not dried: excess water was shaken off. The sodium chlorate solution was transferred to the crystallizing dishes by weighing out equal portions (~44 g), and the dishes were covered by a single filter paper (Whatman No. 1) and set aside to crystallize at ambient temperatures ~20°C. The specific rotation of the d-glucose sample was measured using a digital polarimeter (Optical Activity, PolAAr 20) and found to be $[\alpha]_D^{20} = 52.5^\circ$ (c 2.0, H_2O), in agreement with literature values.[17] It should be noted that glucose consists of two anomers (α and β) that can interconvert in solution, known as the mutarotation of glucose. The observed optical rotation results from the equilibrium mixture of anomers, which have different specific rotations: 36.4% α-d-glucopyranose ($[\alpha]_D^{25} = 112^\circ$) and 63.6% α-d-glucopyranose ($[\alpha]_D^{25} = 18.7^\circ$). Representations of the two structures are shown in Fig. 1. The dishes were generally found to have yielded multiple crystals after 5–7 days. The crystals were identified as being dextrorotary (*d*) or levorotary (*l*) using crossed polarizers, separated and counted. For the identification we use a diffuse white light source, a polarizer (polaroid sheet), another

polarizer (the "analyzer"), with the observer looking towards the light. The observer rotates the analyzer polarizer to be at 90 degrees ("crossed" with respect to the first polarizer) so that very little light is passed through. If a crystal of sodium chlorate is placed between the polarizers, the crystal will rotate the plane of polarization and light will pass through the analyzer (the crystal will appear to be light blue). Crystals that are dextrorotary (*d*) will appear darker when the analyzer is rotated a small amount to the right (clockwise, as viewed by the observer). Crystals that are levorotary (*l*) will appear darker when the analyzer is rotated to the left (counter-clockwise). An example of these observations is shown in the image inset to Fig. 1.

The crystallization procedure above was carried out 4 times over the period of nearly 2 months using fresh reagents at each repeat, giving a total of $N_{\text{trial}} = 86$ separate crystallizations. The weighted mean percentage (W_{d}) and corresponding single standard deviation (σ) of *d* crystals was calculated as follows:

$$W_d = \frac{100\sum n_d}{\sum\{n_d + n_l\}},$$

$$\sigma^2 = \frac{1}{N_{\text{trials}} - 1}\left[\frac{\sum\left\{\left(n_d + n_l\right)\left(100n_d/(n_d + n_l)\right)^2\right\}}{\sum\{n_d + n_l\}} - W_d^2\right]$$

where the summations run over each of the N_{trial} crystallizations, which each yielded n_{d} and n_{l} crystals of *d*- and *l*-$NaClO_3$, respectively.

The results of the analysis of the sodium chlorate crystals grown in the presence of d-glucose are summarized in Table 1, and the distribution of *d*-$NaClO_3$ crystals is shown in Fig. 1. The weighted mean percentage of *d*-$NaClO_3$ crystals is found to be $W_{\text{d}} = 50.4 \pm 1.4$. As can be seen, there is no significant overall preference for *d* or *l* sodium chlorate. It was evident that any one individual dish may yield a preference for *d* or *l* crystals, but taken as a whole there is no preference. Such findings are consistent with observations made on crystallizing samples of sodium chlorate alone, i.e., with no chiral influence, as has been noted in several reports, including the work of K+P. Our results are significantly different from those of K+P results for samples crystallized in the presence of d-glucose, who found that *all* samples showed an individual preference for *l* crystals over *d*.

Reference	N_{trials}	N_d	N_l	ee	W_d
K+P no additive[7]	46	1571	1566	+0.002	50.1 ± 1.4
K+P d-glucose[7]	25	781	1679	–0.365	31.7 ± 0.9
d-glucose (present work)	86	624	614	+0.008	50.4 ± 1.4
maltodextrin (present work)	21	306	315	–0.015	49.3 ± 1.8

Table 1. Number of crystallizations, N_{trials} giving overall total numbers of *d* and *l*-$NaClO_3$ crystals counted, N_d and N_l, giving enantiomeric excess, ee = $(N_d - N_l)/(N_d + N_l)$, and weighted mean percentage of *d*-$NaClO_3$ crystals, $W_d = 100\ N_d /(N_d + N_l)$. A breakdown of the individual results is given in the Supporting Information.

To try to understand the discrepancy between the present results and the results of K+P, we look in detail at the experimental procedures followed. We note that K+P do not explicitly give the temperature at which they carried out the crystallization—most likely to have been ambient temperatures (15–25°C)—but they do add that "*... great care was taken to avoid fluctuations in temperature*". We note also that the volume of solution set to crystallize each time was approximately 200 cm^3 in a 6 inch diameter (15.2 cm) basin, whereas each dish in the present work contained approximately 31 cm^3 in an 8 cm diameter basin. Nevertheless, the crystals were obtained in about 1 week in either case. The number of crystals yielded per 100 cm^3 of solution set to crystallize was very similar: 46 (present work) compared to 59 (K+P).

One critical difference could be in the substance called dextrose by K+P, and assumed to be pure d-glucose. It is likely that the samples of glucose available to K+P originated from hydrolysed starch, and it is possible that the material was not fully hydrolysed into glucose monomers. One clue that the dextrose of K+P was not completely hydrolysed comes from their description of the sodium chlorate and glucose solution as being "rather syrupy". We observed that our solutions were very slightly more viscous than water, but not what would reasonably be called "syrupy". Maltodextrin consists of glucose polymer chains of varying lengths; samples consisting of shorter chains have higher DE (dextrose equivalent) values, with pure glucose defined as having DE = 100. To investigate the effect of glucose chains, we studied one batch of 21 samples using maltodextrin (Sigma–Aldrich, DE = 4.0–7.0) instead of d-glucose. The maltodextrin used contains chains of about 20 glucose monomers long, on average. The result $W_d = 49.3 \pm 1.8$ (see Table 1) shows no significant preference for *l*- or *d*-$NaClO_3$, and no difference from the results for d-glucose.

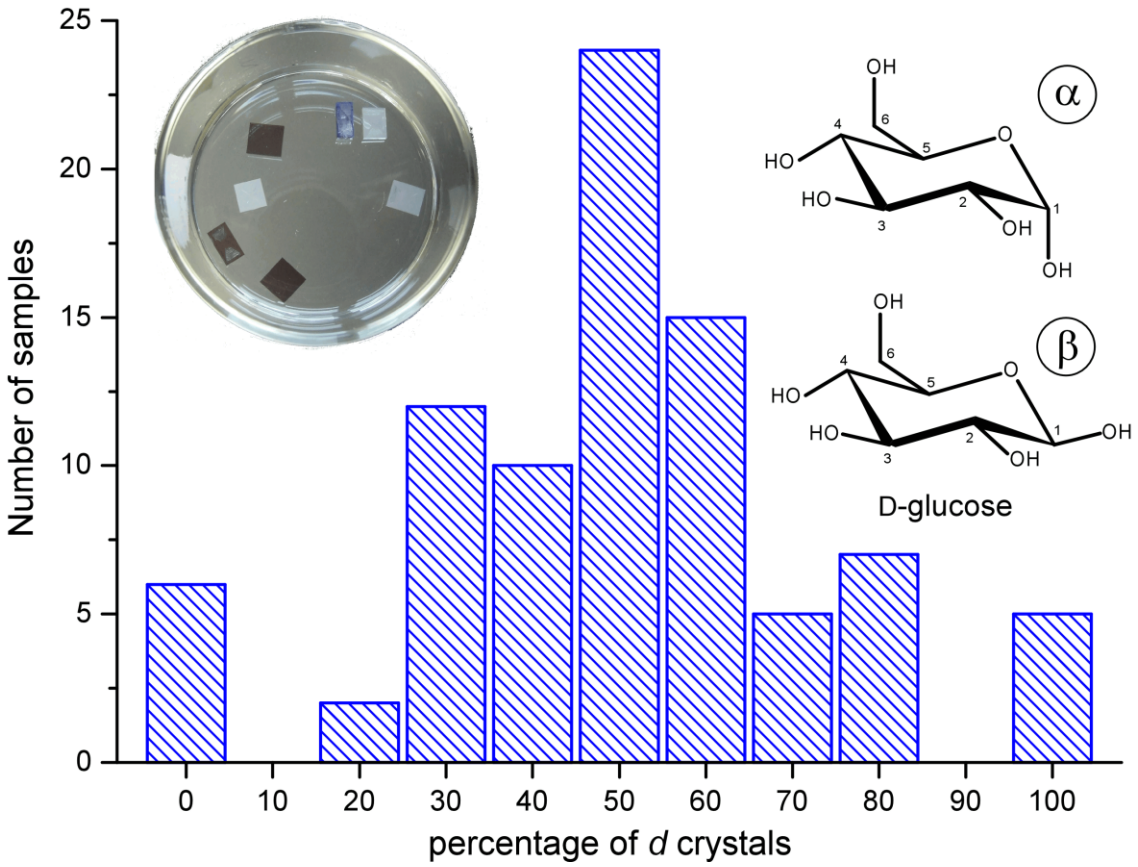


Fig.1. Histogram showing number of samples versus percentage of *d*-$NaClO_3$ crystals obtained per sample $U_d = 100\ n_d / (n_d+n_l)$, where n_d and n_l are, respectively, the number of *d*- and *l*-$NaClO_3$ crystals obtained per sample. The structure inset (top right) shows the structures of the two anomers of glucose,α-d-glucopyranose and β-d-glucopyranose: see text for details. The image inset (top left) is of sodium chlorate crystals viewed through a pair of polarizers as described in the text, showing crystals that appear darker when the analyzer is rotated by a small amount.

Another viable explanation for the results of K+P comes from their description of the experimental procedure where they recycled the materials to produce (in the case of the glucose) the 25 repeated crystallizations:[7]

"*After examination, the crystals were dissolved in the minimum quantity of hot water, the solution mixed with the mother liquor, and, after filtration, the liquid was again put to crystallise.*"

At the point of analysis of the crystals, the mother liquor is bound to be at saturation. If one takes "minimum" to mean the minimum amount of water needed to effect re-dissolution of the grown crystals, then the above description suggests that the liquid set to crystallize was supersaturated, or

saturated at the very least. This would have serious consequences for unintentional seeding: any seeds that were left in or around the vessel could possibly enter the supersaturated solution and grow immediately. In addition, there is no guarantee that any microscopic seeds in the mother liquor were dissolved fully by the above procedures. In a later paper, on the subject of crystallization of sodium ammonium *d*- and *l*-tartrates, K+P acknowledge the influences of laboratory "dust" in potentially biasing experiments towards growing crystals of one form or another.[18] The negative influences of dust, possibly including seeds of specific polymorphs is now well-acknowledged, and possibly better understood than in the early 1900s when K+P carried out their work.

We also look at the work of Niedermaier and Schlenk (N+S). Although N+S did not study d-glucose as a co-solute, they did crystallize sodium chlorate in the presence of d-mannitol and d-dulcitol, and found strong preferences for *d*-$NaClO_3$ (100% *d*) and *l*-$NaClO_3$ (~99% *l*), respectively.[11] It is curious that the result for d-mannitol apparently contradicts the earlier result of K+P using d-mannitol.[7] The main problem with the work of N+S, however, is in the insufficient repetition of the crystallizations, which were limited to only 3 trials for d-mannitol and 1 trial for d-dulcitol! As has been noted above, outwith any possible unintentional seeding, it is possible for any given crystallization vessel to show an extreme enantiomeric excess: to determine a statistically meaningful bias the procedure should be repeated several times. It is possible that N+S were influenced by the previous results of K+P, in conjunction with the exceedingly high enantiomeric excesses that they observed in their small set of trials.

In summary, we have looked at the crystallization of sodium chlorate in the presence of the chiral so-solute d-glucose, and we find no evidence for any bias towards *l*- or *d*-$NaClO_3$ crystals. Our results contradict the results of Kipping and Pope,[7] which have been accepted for over a century, and which have been used to support or promote numerous theories, including autocatalytic asymmetric organic synthesis in the presence of inorganic solids, and theories concerning the origins of biological homochirality.[3, 19]

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