Deracemization via temperature cycling or Viedma ripening

Further development of an undergraduate experiment

Introduction: Viedma ripening

All proteins in earth's organisms are built exclusively from amino acids that have an L configuration. Although scientists had long speculated that the origin of life's enantiospecificity is likely to have been caused by amplification of a statistical fluctuation in enantiomeric excess in the primordial soup towards the L configuration, no viable mechanism for such an amplification was known, until 2005. In that year, Viedma [1] performed a groundbreaking experiment with sodium chlorate ($NaClO_3$), which has the interesting property that, although being achiral in solution, it crystallizes as a racemic mixture of enantiomeric crystals: a mixture of pure R and pure S crystals. Viedma studied a saturated solution in contact with a sodium chlorate crystals that were continuously being ground by small glass beads that were being stirred by a small bar magnet. Surprisingly, after a day, all crystals were either 100% R or 100% S. By repeating the experiments, Viedma showed that the outcome was completely random, meaning that the process seemed to illustrate exactly the complete amplification of a statistical fluctuation in enantiomeric excess.

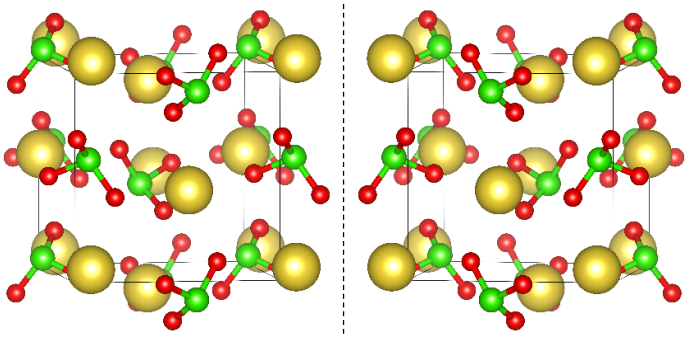

Figure 1 – *Two non-superimposable mirror images of the unit cell of R and S sodium chlorate crystals*.

In 2008, Noorduin *et al*. proved that this experiment could be extended to chiral organic molecules being racemized in solution [2]. Figure 2 shows that such a system is analogous to the sodium chlorate system, because the solution phase as a whole, containing equal amounts of the R and S enantiomers in equilibrium with each other as a result of the racemization reaction, is intrinsically achiral, just like the sodium chlorate solution.

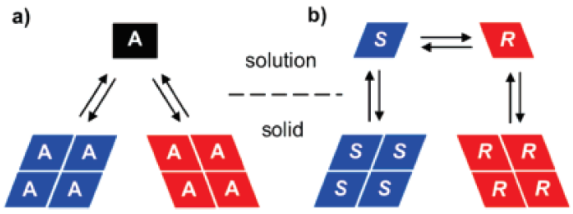

Figure 2 – *Comparison of **a)** the sodium chlorate system and **b)** the system of chiral molecules being racemized in solution. (Figure copied from [2])*

In an ultimate extension, Steendam *et al*. showed in 2014 that a reaction starting from only achiral reactants and producing a racemic mixture of R and S product, can yield a fully enantiopure product (*i.e.* either 100% R or 100% S), as long as reactants and products are in equilibrium with each other in solution and the products are in equilibrium with enantiopure crystals that are continuously being ground [3].

Further developments

Since the initial discovery of Viedma ripening and its extension to chiral molecules, a better understanding of the underlying mechanism has been obtained. Also, research has shown that similar results can in general be obtained even quicker by temperature cycling [4].

The potential for procedures such as Viedma ripening and temperature cycling for the production of enantiopure compounds or the deracemization of racemic mixtures is high. Earlier methods for obtaining enantiopure compounds include:

1. enantioselective reactions, which rely on the use of (enantiopure) chiral reactants or chiral catalysts (an important class of which is formed by enzymes);
2. deracemization or chiral resolution via diastereomeric resolution, which depends on the formation of diastereomeric salt crystals together with a chiral resolving agent (such as in [5]);
3. chromatographic separation using chiral column materials;
4. spontaneous resolution, when the racemic mixture crystallizes as as a racemic conglomerate (which is the proper term to indicate a mechanical mixture of crystals of the two pure enantiomers).

Method 1, has a maximal yield of 100% enantiopure compound, but needs expensive chiral starting compounds or catalysts. Methods 2 and 3 similarly rely on other chiral compounds as well. Method 4 is the only one not relying on chiral compounds, but is far from being generally applicable. Finally, methods 2,3 and 4 have a maximal yield of 50% enantiopure compound. The financial implication is that enantiopure compounds are expensive. Procedures that provide enantiopure compounds without relying on other enantiopure compounds and provide them in a yield of 100% could drastically reduce production costs.

Viedma ripening and temperature cycling for the production of enantiopure compounds or the deracemization of racemic mixtures do exactly that and, as such form an important addition to the available methods, but to be fair, they are no silver bullets either, since they are also limited by constraints:

- the compound should be racemizable in solution without forming a significant amount of side products.
- the compound should crystallize from a racemic mixture as as a racemic conglomerate (just like in spontaneous resolution)

Especially the second constraint drastically limits the number of applications. About 90% of chiral organic compounds do *not* crystallize as a racemic conglomerate, but rather as a single racemic compound: a crystal in which both enantiomers are present in equal amounts in the unit cell.

A way around this limitation is the use of salts: each combination of an ionizable chiral compound with a counterion again has about 10% chance of forming a conglomerate [6]. (And by using artificial intelligence to determine likely candidates, the odds might even be significantly enhanced [7]).

An educational experiment

Although the findings described above are *groundbreaking*, the experiments are relatively simple. That last fact notwithstanding, they seem not yet to have made it to the educational literature. We would like to change this!

In 2014, we already set up a masterclass for high-school students based on the results in [3]. In it, we performed the experiment described in the paper, but in two different solvents. In one solvent, the reaction mixture is a solution, in the other, it is a solution in equilibrium with a conglomerate. By spiking both mixtures with a tiny amount of enantiopure product – taking care to spike the full solution with slightly more product – and measuring the optical rotation of product solutions, we could show the students that after half a day of grinding, the enantiomeric excess (*ee*) in the suspension had overtaken the *ee* of the solution. When enough students participated, we would separate them in two groups and spike the solutions with the R product in one group and with the S product in the other group.

Although the experiment did what it was supposed to do, and almost all results were consistent, they were not *very* convincing, because the conversion was far from complete and, importantly, the specific rotation of the product is very small (on the order of 2°). This would mean that in practice we could show the students that the rotations we measured on our polarimeter were about ±0.002° for the solutions and about ±0.004° for the suspensions.

Previous internships

Previously, two students did their internship within our department, with the goal to set up a robust and convincing experiment that can be carried out in a short time (a couple of hours to a single day) by undergraduate students and that illustrates the concept of deracemization via Viedma ripening and/or temperature cycling by using a DIY polarimeter for the measurement of the optical rotation of the product.

This meant we did not immediately aim for the Steendam-type of experiment (producing an enantiopure compound from only achiral starting products). The reason for this is that the quality of such an experiment strongly depends on the specific rotation of the synthesized product, which is hard (or impossible) to predict and must therefore be determined experimentally. This means a lot of trial and error with no guarantee on a good outcome whatsoever.

Deracemization of amino acids

Instead, we focused on experiments and compounds described in [6] and employed the use of conglomerate formation to deracemize amino acids. In particular, we started by reproducing the deracemization of phenylalanine by combining it with 2,5-xylenesulfonic acid to form a conglomerate. Because of its specific rotation of about ±35°, we expected that changes in *ee* could probably be easily observed via measuring optical rotation. Instead of Viedma-ripening, we employed temperature cycling, since that produces results faster. Although we managed to reproduce the deracemization of phenylalanine, it turns out that its solubility in water is too low to measure large rotations. For that reason, we switched to proline, which also has an appreciable specific rotation and additionally has a good solubility in water. We managed to measure significant and reproducible rotations of solutions of enantiomerically pure proline using a DIY polarimeter. However, we currently have not found a suitable conglomerate-former that would enable us to deracemize *rac*-proline.

Deracemization of organometallic complexes

Another interesting option that arose is the use of Werner-type octahedral metal-organic complexes, such as tris(ethylenediamine)cobalt(III) salts and tris[tetrammine-μ-dihydroxocobalt(III)]cobalt(III) ('hexol') that can be resolved into their strongly optically active Λ and Δ isomers.

Although we found favourable conditions in which to racemize tris(ethylenediamine)cobalt(III) salts, we did not yet manage to *de*racemize the racemate (maybe rather counterintuitively, racemization is a prerequisite for the *de*racemization of the racemate).

The current internship

The current project would start where the previous ones stopped:

- By trying to find suitable conglomerate-formers for proline and attempting to use these to deracemize *rac*-proline.
- By optimizing conditions for the deracemization of phenylalanine and developing a cuvette with a longer effective path length (allowing the measurement of appreciable changes in optical rotation in a shorter time frame).
- By trying to find conditions under which tris(ethylenediamine)cobalt(III) salts or other organometallic complexes can be deracemized.

References

[1] Viedma, C., Chiral Symmetry Breaking During Crystallization: Complete Chiral Purity Induced by Nonlinear Autocatalysis and Recycling, *Phys. Rev. Lett.* 2005, **94**:065504

[2] Noorduin, W.L., Izumi, T., Millemaggi, A., Leeman, M., Meekes, H., Van Enckevort, W.J.P., Kellogg, R.M., Kaptein, B., Vlieg, E., and Blackmond, D.G., Emergence of a Single Solid Chiral State from a Nearly Racemic Amino Acid Derivative, *J. Am. Chem. Soc.*, 2008, **130**, 1158–1159

[3] Steendam, R.E., Verkade, J.M.M., Van Benthem, T.J.B., Meekes, H., Van Enckevort, W.J.P., Raap, J., Rutjes, F.P.J.T. and Vlieg, E., Emergence of single-molecular chirality from achiral reactants, *Nat. Comm.*, 2014, **5**:5543

[4] Intaraboonrod, K., Lerdwiriyanupap, T., Hoquante, M., Coquerel, G., and Flood, A. E., Temperature cycle induced deracemization, *Mendeleev Commun.*, 2020, **30**, 395–405

[5] Shiraiwa, T., Sakata, S., Fujishima, K. and Kurokawa, H., Asymmetric Transformation of (*RS*)-2-Phenylglycine via Formation of Salt with (1*S*)-10-Camphorsulfonic Acid, *Bull. Chem. Soc. Jpn.*, 1991, **64**, 191–195

[6] Spix, L., Alfring, A., Meekes, H., Van Enckevort, W.J.P. and Vlieg, E., Formation of a Salt Enables Complete Deracemization of a Racemic Compound through Viedma Ripening, *Cryst. Growth Des.*, 2014, **14**, 1744–1748

[7] Devogelaer, J-J., Meekes, H., Tinnemans, P., Vlieg, E. and De Gelder, R., Co-crystal Prediction by Artificial Neural Networks, *Angew. Chem. Int. Ed.* 2020, **59**, 21711–21718

[8] Werner, A., Zur Kenntnis des asymmetrischen Kobaltatoms. I., *Berichte der Deutschen Chemischen Gesellschaft* (in German), 1911, **44** (2) 1887–1898.