Study of chiral crystallization of achiral molecules

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Abstract

Chirality is an important concept which has many consequences and applications in many fields of science and especially in chemistry. The project *"Study of chiral crystallization of achiral molecules"* focuses on chirality of crystals and more specifically on chiral symmetry breaking for these crystals. Some molecules, although achiral, are able to form chiral crystals. This report presents some general information about crystal space groups and chiral crystals. The enantiomeric ratio of chiral crystals obtained under different experimental conditions for three different achiral molecules is discussed. These molecules are sodium chlorate, benzophenone and dimethylchalcone. All the performed methods of cristallization and analysis of these molecules are described in this report. Results of analyses for each molecules are presented, explained and discussed.

Keywords: chiral crystals, space groups, chiral symmetry breaking

1 Introduction

The concept of chirality was introduced in the nineteenth century. However, chirality is still one of the subjects at the top of scientific research. The concept has many consequences and applications in many fields of science and especially in chemistry. Chirality is a general concept based on the geometric caracteristics of an object. A chiral object is an object which has a mirror-image non superimposable to itself. Chirality deals with molecules but also with macroscopic objects such as crystals. Scientists, K.Kondepudi[1] heading the list, are involved in study of chiral crystals. Some molecules, although achiral, are able to generate chiral crystals. Chirality is then due to the crystal structure, having two enantiomorphic forms. Cubic chiral crystals are easily identifiable. Indeed, they deviate polarized light.

There is a scientific relevance to study the distribution and the ratio of the two enantiomorphic crystal forms of an achiral molecule not only in a sample but also in numerous samples prepared under specific conditions. The relevance of this type of study is, for instance, a better comprehension of homochirality. The experimental conditions act upon the breaking of chiral symmetry. Enantiomeric excess is not obviously easy to induce. Nevertheless, a constant stirring of the solution during the crystallization will generate a significant rupture of chiral symmetry in the sample and will offer an interesting and accessible case study.

This project, *"Study of chiral cristallization of achiral molecules"*, is based on scientific publications and more particulary on two of them. These two publications focus on the cristallization of sodium chlorate[1] and dimethylchalcone[2], two molecules which are achiral and generate chiral crystals. The enantiomeric distribution of the crystals was studied for the two molecules on the one hand in the case of non stirred crystallization and on the other hand in the case of stirred crystallization. The conclusions of the publication established that strirring induces a chiral symmetry breaking. Indeed, in the non stirred samples, enantiomeric ratio is about 50% of L-crystals and 50% of D-crystals whereas, in the stirred samples, enantiomeric ratio is about 90% of L-

crystals and 10% of D-crystals or 10% of L-crystals and 90% of D-crystals[1]. The two publications also bring to the fore that the chiral symmetry breaking is totally random and that if you take into account the results of numerous samples globally the enantiomeric ratio for the whole results is about 50% of L-crystals and 50% of D-crystals.

Homochirality and symmetry breaking is obtained by the stirring of solutions. This very precise stimuli induces an autocatalytic and competitive process. All except one of the nuclei are destructed and redisolved until one become large enough to produce new seeds of the same handedness which produce a large number of new nuclei of the same enantiomorphic structure and so on[1].

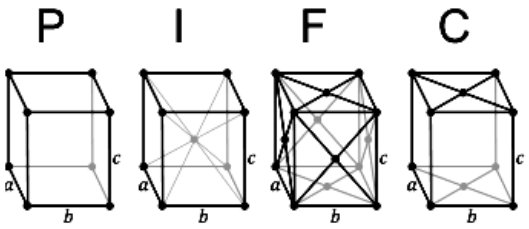
1.1 Review on Space Groups

Space groups will be extensively discussed in this project, therefore a brief introduction on the topic will be made. The space group of a crystal is a mathematical description of its symmetry. The space groups consist of a combinaison of the 32 point groups operations (such as rotations) and the 14 Bravais Lattice. Bravais Lattice are given from the seven crystal systems and centering informations.

Crystal Systems are well known by chemists and are refered by names : **cubic**, **tetragonal**, **monoclinic**, **triclinic**, **rhombohedral**, **orthorombic**, **hexagonal**. Each one can then be extended with different centering refered as,

- **P**, *Primitive Centered*. The lattice points are at the corners only.
- **I**, *Body Centered*. Same as P with a new lattice point in the center of the cell.
- **F**, *Face Centered*. Same as P with lattices in the center of each faces.
- **A**/**B**/**C**, *Centered on one Face*. Same as P with lattices centered on a given (A, B or C) face.

`Figure1` shows the orthorombic system example.



`Figure1.` Bravais Lattice of orthorombic crystals.

To obtain space groups from Bravais Lattice information about rotation and translations are added. The screw axis is noted by a number x with a rotation of $\frac{2\pi}{x}$ and the translation is then added as a subscript y refering to y times the $\frac{1}{x}$ porition of the parallel lattice vector. For example 3_2 represent a 120 rotation followed by $\frac{2}{3}$ translation along the lattice vector. Because of the periodicity of crystals, a translation of the fraction $\frac{y}{x}$ of the unit cell along the axis correspond to a translation of the fraction $\frac{x-y}{x}$ along the axis in the opposite direction, so a 3_1 screw axis is the mirror image of a 3_2 screw axis.[3]

On the 230 theoretical space groups in which molecules cristallize, 65 are chiral. `Table1` lists the 65 chirals groups.

1.2 Type of Crystallization

1.2.1 Chiral Crystals

Optically active crystals may be produced from different kind of materials. It is obviously possible to create chiral crystals *from chiral molecules* but creating chiral crystals *from achiral material* is also possible. However, a third case emerge from the definition of chirality. Conventional chirality involves most of the time the presence of stereogenic center but chirality can also be obtained *from achiral materials that have chiral conformations.*

1.2.1.1 From chiral molecules Chiral molecules may crystallize as chiral crystals in two separate cases : either the solution is

Table1. The 65 chiral space groups[3, 4],

System	Class	Chiral Space Groups
Cubic	23	$P23$, $F23$, $I23$, $P2_13$, $I2_13$
	432	$P432$, $P4_232$, $F432$, $F4_132$, $I432$, $F4_332$, $P4_132$, $I4_132$
Tetragonal	4	$P4$, $P4_1$, $P4_2$, $P4_3$, $I4$, $I4_1$
	422	$P422$, $P42_12$, $P4_122$, $P4_12_12$, $P4_222$, $P4_22_12$, $P4_322$, $P4_32_12$, $I422$, $I4_122$
Monoclinic	2	$P2$, $P2_1$, $C2$
Triclinic	1	$P1$
Rhombohedral	3	$P3$, $P3_1$, $P3_2$, $R3$
	32	$P312$, $P321$, $P3_112$, $P3_121$, $P3_212$, $P3_221$, $R32$
Orthorombic	222	$P222$, $P222_1$, $P2_12_12$, $P2_12_12_1$, $C222_1$, $C222$, $F222$, $I222$, $I2_12_12_1$
Hexagonal	6	$P6$, $P6_1$, $P6_5$, $P6_2$, $P6_4$, $P6_3$
	622	$P622$, $P6_122$, $P6_522$, $P6_222$, $P6_422$, $P6_322$

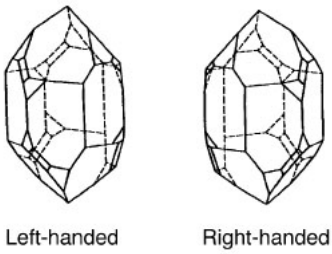


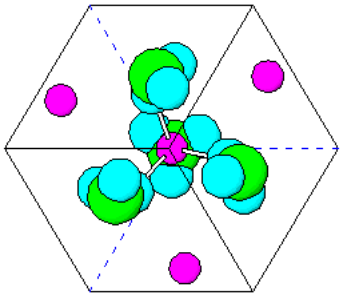
Figure2. enantiomorphic quartz crystals.[4]

enantiomericaly pure (*ie* only one of the enantiomers is present in solution) or the solution resolves spontaneously. The later case was discovered by Pasteur when he managed to separate the two enantiomers of a $(\pm)$*sodium ammonium tartrate* solution by crystallization. Chiral molecules that resolves spontaneously represent less than 10% of the known cases, all the other crystallizing as optically inactive *racemates*. The preparation of chiral crystals from chiral reagents is not the concerned by this study.

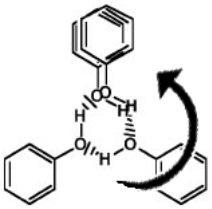
1.2.1.2 From achiral molecules Because 65 space groups are chiral, some achiral material may produce chiral crystals. It is well known to chemists that amorphous silicon dioxide when crystallized to quartz produce chiral crystals (space group $P3_121$ which belongs to the trigonal group) because of an helix arrangement of the molecules[4]. Figure2 represent enantiomorphic quartz crystals.

This case can be well illustrated by several inorganic compounds such as sodium chlorate[1], sodium bromate[5], lithium sulfate[5]... and were extensively studied. But if the crystals are redisolved in a solvent or melted, they revert back to an achiral system which can undergo a new chiral crystallization. Repeated experiments done by Kondepudi et al.[1] shows that crystals prepared from an unstirred solution of sodium chlorate approach a 1:1 ratio of each enantiomorphs but, more interrestingly, under certain conditions they managed to introduce an asymetric crystallization, in other words they suceeded in producing almost pure L or D crystals without any chiral reagents nor solvents. Koshima et al.[4] carried out extensive research on organic achiral molecules that crystallize as chiral crystals by searching the Cambridge Structural Database. They concluded that about 8% of achiral organic molecules are though to crystallize in chirals space groups.

A common example of an achiral organic molecule that undergoes chiral crystallization is phenol. When crystallized, the molecule are arranged in an helix which can take two types of orientation[4]. Figure3 shows the stacking arrangment of phenol.



`Figure4.` Sodium chlorate crystals structure generated with the software ATOM via ICSD data[6].

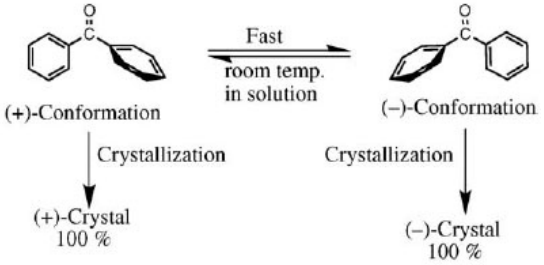


`Figure3.` Helix-like stacking of phenol molecules in their crystal[4].

In the course of this project, we studied the crystallization of sodium chlorate. The crystals belong to the cubic space group $P2_13$ [6]. Again, the simple arrangment of the molecules is sufficent to produce chiral crystals. `Figure4` shows the unit cell of sodium chlorate crystals which reveals to be chiral.

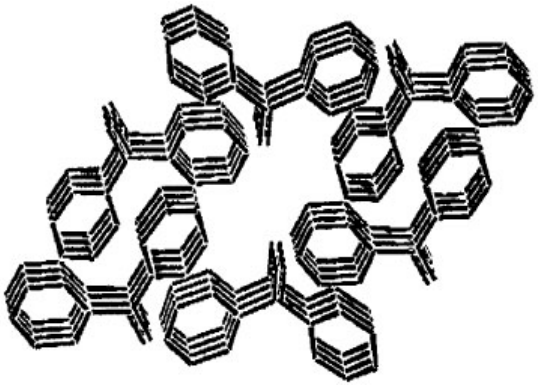
All the examples provided here illustrate the fact that the orientation of achiral molecules can produce chiral crystals.

1.2.1.3 From achiral molecules that have chiral conformations Some organic achiral molecules possess a chiral conformation. Benzophenone readily explains that concept : in solution benzophenone conformation keeps changing due to the low energy rotation barrier and is achiral on average. Upon crystallization the conformation is frozen and the molecule presents two different torsion angles, making it chiral[4].



`Figure.` Chiral Conformations of Benzophenone[4].

Then a chiral stacking may also take place resulting in a resolution of the pseudo-chiral molecule[4].



`Figure.` Stacking of benzophenone in a crystal [4].

1.3 Detecting Chiral Crystals

Several methods can be used to detect if a crystal is chiral or not and to discriminate both enantiomorphs. These includes the *visual inspection of the morphology of the crystals*, the *use of polarizating sheets* (or *polarizated light binocular*), *circular dichroism spectrography*, *X-Ray analysis* and *High Accuracy Universal Polarimeter (HAUP)*. We will focus on the three used in this project.

1.3.1 Morphology of Crystals

The resolution of $(\pm)$*sodium ammonium tartrate* was successfuly carried out by Pasteur who succeeded in separating the different enantiomorphs crystals by a simple visual inspection

of their morphology. However, this seems to be difficult to reproduce[4].

1.3.2 Polarized light

Polarizating sheets and polarizating binocular are the most convenient and fast way to identify chiral crystals. However, this technique only work for the space groups belonging to the cubic system and seems to be ineffective with small crystals[4]. Still, it works fine for crystals of several millimeters wide. This method was selected to study the chirality of sodium chlorate crystals[1].

To operate one must select two polarizating sheet with a light source and cross them in order to have an extinsion of the polarizated light. Then the sample is introduced between the sheets and the top sheet is slowly turned until the color change of the crystals appear. In the case of sodium chlorate's crystals, it has been observed that one group of crystals will turn red and the enantiomorph crystals will turn blue.

1.3.3 Circular Dichroism

Circular dichroism spectroscopy works by the differential absorption of circulary polarized light, $\triangle A$, by L/D enantiomers. This can be directly linked to Beer Lambert's law $\triangle A = (\varepsilon_{left} - \varepsilon_{right})\, Cl$ with ε the molar extinction coefficient for cicularly polarized light (either left or right-handed). The circular dichroism is then given by $\triangle\varepsilon = \varepsilon_{left} - \varepsilon_{right}$.

2 Methods

2.1 Sodium Chlorate[1]

100g of ACS *sodium chlorate* were dissolved in 100 ml of distilled water and placed on a magnetic stirrer at 50°C for 30minutes to ensure that all the sodium chlorate was dissolved. The solution is then filtered to eliminate any potential impurities, which could have impact on the crystallization process and alter the results. The obtained solution is equally divided into six samples of 20 ml, the rest of the solution was discarded. Three samples were poured in Petri dishes in order to realize a non-stirred crystallization and the three other samples were poured in beakers and stirred at 100rpm in order to realize a stirred crystallization. Crystallization was obtained through evaporation at room temperature and was carried on during a period of two days for the non stirred samples and during a period of three days for the stirred ones. The experiment was run three times.

2.2 Benzophenone

Although it is possible to crystallize benzophenone by melting/cooling, a procedure similar to the one used for sodium chlorate was designed. 60g of ACS *benzophenone* were dissolved in 40ml of *o-xylene* and stirred at 40°C for 30 minutes. The solution was then filtered to prevent potential impurities to contaminate the crystallisation process. The obtained solution is equally divided into four samples of 20ml, the remaining part was discarded. Two samples were poured in Petri dishes in order to realize a non-stirred crystallization and the two other samples were poured in beakers and stirred at 100rpm in order to realize a stirred crystallization. Crystallization was obtained through evaporation at room temperature and was carried on during a period of one day for the non stirred samples and stirred ones.

2.3 Dimethylchalcone[2]

1.0g of *sodium metal* was slowly added in five portions to 30ml *anhydrous methanol* on an ice-water bath. 21.6 gr (0.18 mol) of *p-toluadehyde* was poured at once in the solution and 24.0g (0.18mol) of *p-methylacetophenone* were added dropwise at a rate of a few drops per seconds. No vigorous reactions were recorded. *Dimethylchalcone* will precipitate after a few minutes but the product was left in solution for 24 hours in order to achieve completion. The resulting solid was then filtered and washed with distilled water and cold ethanol. The resulting solid was purified by recrystallization from hot ethyl acetate as follows : *dimethylchal-*

cone crystals were poured in hot (50°C) *ethyl acetate*. The solution was then filtrered and evaporated. The recrystallization was performed three times to achieve significant purity. Three samples of 4g *dimethylchalcone* in 10ml *ethyl acetate* were poured in rounded flasks with a magnetic stirbar and immersed in a water bath over a magnetic stirrer. The solutions were heated to 80°C until all the *dimethylchalcone* dissolved. Heating was then stopped and strirring was interrupted in one flask only. The solution were cooled to room temperature in a few hours. Crystals were then filtered and dried under reduced pressure. The final step consist of a bromination of *dimethylchalcone*. The solid were crushed to a fine powder and placed into three flasks equipped with stirbars and placed in a water bath at 30°C to prevent the condensation of bromine. Flasks were connected to a flask containing liquid bromine and placed above a oil bath at 130°C at a moderate distance to vaporise slowly the bromine[1]. Gaseous bromine diffused to the *dimethylchalcone* powder flasks during a period of 48 hours. Stirring of the crystals powder improved absorption by the powder and reaction between gaseous bromine and *dimethylchalcone*. The resulting *dibromodimethylchalcone* was then freed of bromine by reduced pressure.

3 Results and discussion

3.1 Sodium chlorate

Cristallization of sodium chlorate was performed three times. Nine non-stirred samples and nine stirred samples were analyzed.

3.1.1 Non-stirred samples

From the obtained samples, every crystal was analyzed individually under a polarizated light binocular to determinate the enantiomeric ratio of the entire set. The pair of polarizers was adjusted and the color of each crystal, blue or red, was recorded.

[1]The bromine should not be set to ebullition to prevent glassware from explosion.

`Table2.` Results of the non-stirred experiment of Sodium Chlorate.

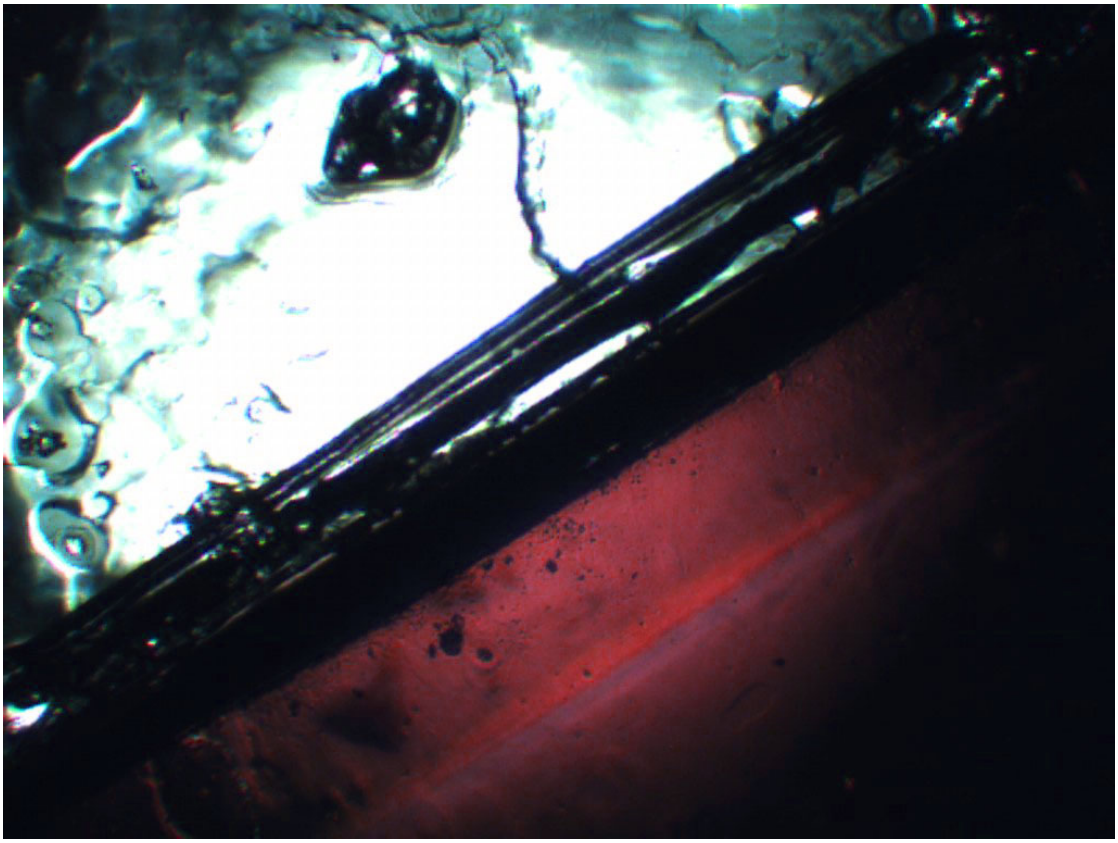
sample number	blue crystals	red crystals
1	28	27
2	23	26
3	24	23
4	21	22
5	25	27
6	24	21
7	24	26
8	27	25
9	22	21

`Table2` summarized the results for the samples. Proportions of the two enantiomorphs are close to 50% and the observed small fluctuations are statistical. These equal proportions correspond to a state of chiral symmetry. The obtained results reflect what was predicted and are in accordance with the scientific publications. `Figure6` shows two adjacent crystals observered under a polarizated light binocular.

3.1.2 Stirred samples

Crystals obtained by stirred crystallization were very small, shattered and highly conglomerated. Shattered crystals, which have the disadvantage to spread light in all directions, are hardly observable under a binocular microscope. Therefore, they were covered with a solution, having a refractive index similar to the sodium chlorate refraction index, which allowed the crystals observation under the binocular. However, no colors changes nor extinction were observed.

Two solutions were proposed to solve the problem, but due to lack of time they were not tried. Stirring could be stopped in the experiment after a given time to let the crystals grow. Or, a statistically representative sample of crystals could be taken and placed in a hot saturated sodium chlorate solution which would be cooled at a rate of 0.2°C per day. The later is known to yield very nice and large crystals which sould be easily observable[7].



`Figure6.` Sodium Chlorate ($P2_13$) observed on a polarizated light binocular. 3.2x magnification / enhanced saturation.

3.1.3 Recommendations

We recommend to he who will perform the experiment to pay attention to the crystallization process. To prevent crystals from penetrating into each others, they must be removed from the solution as soon as they are large enough to be observered under polarized light.

3.2 Benzophenone

Two non-stirred samples and two stirred samples were analyzed.

3.2.1 Non-stirred samples

Only cubic crystals can be observed with the polarized sheets technique. Therefore, benzophenone, which crystallizes in the orthorombic space group, can not be study under polarized light. Only the birefringence of the crystals inducing four interruption of polarized light when turning the sample were observed, however this is in no case a sign of chirality.

A visual inspection of the crystals morphology to identify enantiomorphs did not succeed. Solvent and impurties have significant side effects on crystals growth[4]. On all the samples of benzophenone prepared, hardly no crystals could be matched with another. This is due to the high interraction between the solvent and the crystal faces. `Figure5` shows benzophenone crystals grown by melting/cooling (a) and grown from a toluene solution (b).[4]

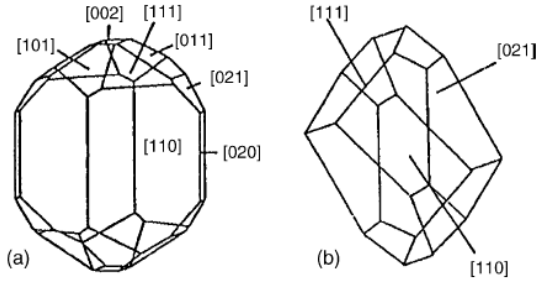


Figure5. Benzophenone crystals grown from melting (a) and from a toluene solution (b).

3.2.2 Stirred samples

Crystals formed a compact slurry and were impossible to examine. It is advisable to decrease the initial concentration of benzophenone to allow crystals to be more scattered in the beaker.

3.2.3 Recommendations

Despite the high boiling point of xylenes, these are very volatile and we recommend to cover the petry dish with filter paper to reduce evaporation and slow down the crystallization.

3.3 Dimethylchalcone

One non-stirred sample and two stirred samples were analyzed. *Dibromodimethylchalcone*'s crystals were dissolved and diluted in chloroform in order to examine the samples in a circular dichroism spectroscopy machine. Results, by working with a circular dichroism spectroscopy machine, are viable only if a maximal limit of 500 Volt for the high-tension is not overpassed. High-tension can be reduced by using a smaller sample-cell in the machine or by a dilution of the analyzed sample.

3.3.1 Non-stirred sample

The spectrum presented no singularities, which could have been the evidence of a chiral symmetry breaking, and so probably characterized a sample with equal enantiomeric ratios.

3.3.2 Stirred samples

The spectrums, obtained by a high-tension higher than 500 Volt, were totally erratic and no chiral symmetry breaking could be put in evidence.

The lack of results for the *dibromodimethylchalcone* is due to the use of a circular dichroism spectroscopy machine instead of a polarimeter which was recommended in the publication. Indeed, enantiomeric excesses in the samples are weak and the circular dichroism machine's sensibility is lower than the polarimeter's one. Kondepudi et al. report that a sample of *dimethylchalcone*'s crystals enantiomericly pure will result, after bromation, in a sample with an enantiomeric excess of only 6%[2]. Moreover, *dimethylchalcone*'s bromation produces not only *dibromodimethylchalcone* but also *tribromodimethylchalcone*. This tribromation of the *dimethylchalcone* was put in evidence by a *NMR* (Nuclear Magnetic Resonance) spectroscopy[2]. Figure6 shows the spectrum of the samples before bromation. The peaks between 0.2 and 0.3 ppm correspond to the six protons of the two methyl radicals and the peaks between 0.7 and 0.8 ppm correspond to the ten other protons of the *dimethylchalcone* plus the protons of the solvent (CDCl3). This spectrum corresponds to the one for *dimethylchalcone*'s one. Figure7, Figure8 and Figure9 show the spectrum of the three samples after bromation and dilution in deuterated chloroform. In these spectrums, peaks between 5.0 and 6.0 ppm correspond to protons of *dibromodimethylchalcone* and *tribromodimethylchalcone* showing thus that *dibromodimethylchalcone* was not the only product of bromation. This information was confirmed by a thin layer chromatography (solvent: *hexane/benzene 1:1*). By integration of the peaks, proportion of *dibromodimethylchalcone* and *tribromodimethylchalcone* can be determined. Spectrums show that, due to undetermined factors acting during the bromation, this proportion varies among the samples.

[2]NMR spectrometer: Brüker 300MHz spectrometer; CDCl3 solvent; temperature of 25°C.

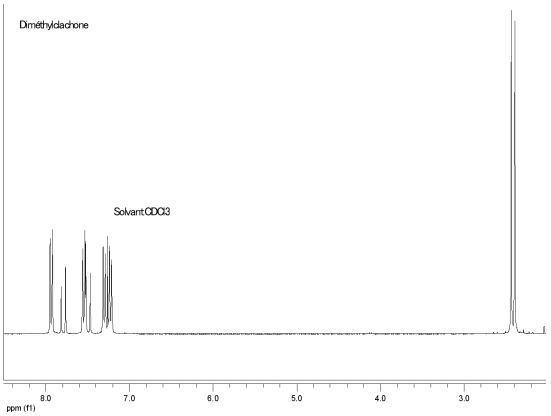


Figure6. Dimethylchalcone before bromination.

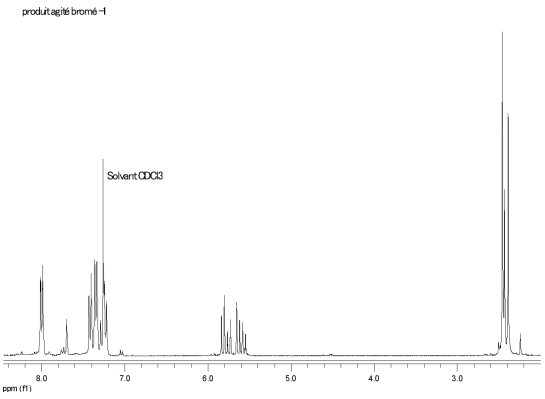


Figure8. Dimethylchalcone after bromination for the stirred sample #1.

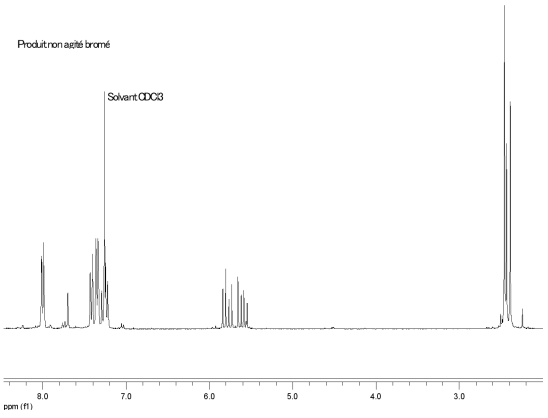


Figure7. Dimethylchalcone after bromination for the non-stirred sample.

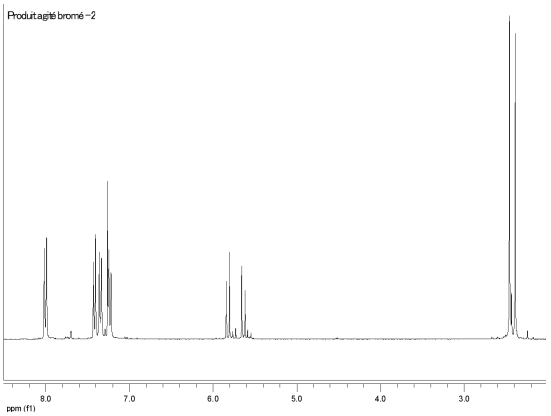


Figure9. Dimethylchalcone after bromination for the stirred sample #2.

A solution would consist in using a chiral chemical shift reagent which will interact with the two enantiomers of *dibromodimethylchalcone* and *tribromodimethylchalcone*'s molecules and so induce a split of the considered peaks. Two peaks for each proton would demonstrate the existence of two enantiomeric forms in the solution and allow to determine, by integration of each peak, the enantiomeric ratio of the solution.

4 Perspectives and Conclusions

In this project, we performed the cristallization of three molecules and tried to determinate the enantiomorphic ratios of the crystals. We did not succeed to put in evidence the chiral symmetry breaking. However, we looked for the reasons explaining the examining's difficulties and proposed some solutions.

Because we did not have time to test every data found, we propose here some routes that would be interresting to investigate even more. The ICSD minerals database / Retrieve© Software v0.2 was searched for any evidence of minerals that cristalize in the cubic space group[3]. On the 526 matches of the database, 5 caught our attention, see Table3.

[3]Crystals belonging to the cubic group should suits the usage of polarized light as described in **section 1.3.2**.

Table3. Molecules crystallizing in the cubic chirals space groups.

Compound name	**Solubility / 20°C**[4]
Sodium Bromate	36,4g / 100ml H2O
Zinc Sulfate	30g / 100ml H2O
Sodium Phosphate	28,5g / 100ml H2O
Barium Nitrate	8,7g / 100ml H2O
Tin (IV) Tetraiodide	in organic solvents

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Vandercammen Juliana
Ingénierie moléculaire et biomoléculaire
Faculté des Sciences appliquées

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