

# The Art of *in situ* Cryocrystallization

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The molecular and crystal structure of low-melting organic compounds and molecular liquids can be determined by *in situ* cryocrystallization technique. This also enables a detailed understanding of the various types of non-covalent interactions present between the molecules in the crystal.

## Introduction

*In situ* cryocrystallization is a technique to determine the molecular structure of a compound, which is a liquid at room temperature, using the technique of Single Crystal X-Ray diffraction (SCXRD). This allows for the determination of the position of all the atoms in the molecule of interest. Furthermore, once the crystal packing is determined, it allows for the study of intermolecular interactions between the molecules. It is very important to understand the arrangement of molecules in the crystal lattice and this depends on the shape and size of the molecule. This method can also be made applicable to compounds which are low melting solids, ionic liquids, and gaseous hydrates at room temperature.

The crystallization of a liquid in a glass capillary and the determination of the structure enable an unequivocal characterization of drugs, pharmaceuticals, agrochemicals and technologically important materials. In this article, we explain the *in situ* cryocrystallization technique and discuss relevant studies in which it was performed on different ionic liquids, solvents, gas hydrates and low melting solids. The single crystal X-ray diffraction technique is very useful to determine the 3D structure of compounds which are 'solids' under normal conditions of temperature and pressure. It is easy to crystallize any solid compound from a solution or melt of the given compound. But, for compounds which are liquid at normal temperature and pressure, different procedures have to be adopted to obtain the 3D structure of their molecules. It is of interest to know that the first crystal

## Keywords

*In situ* cryocrystallization, states of matter, OHCD.

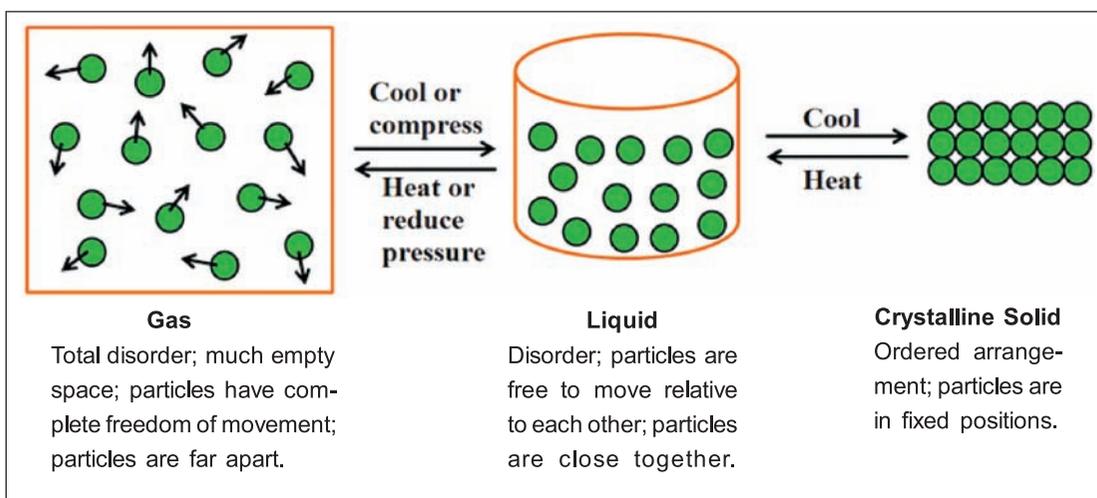


structure determination of a liquid was done in India in the Department of Physics at Indian Institute of Science, Bangalore on a liquid named 'dimethyl sulfoxide' [1]. This technique lies at the realm of cutting-edge research and this aspect has been nicely highlighted in a very recent research article by J A K Howard [2].

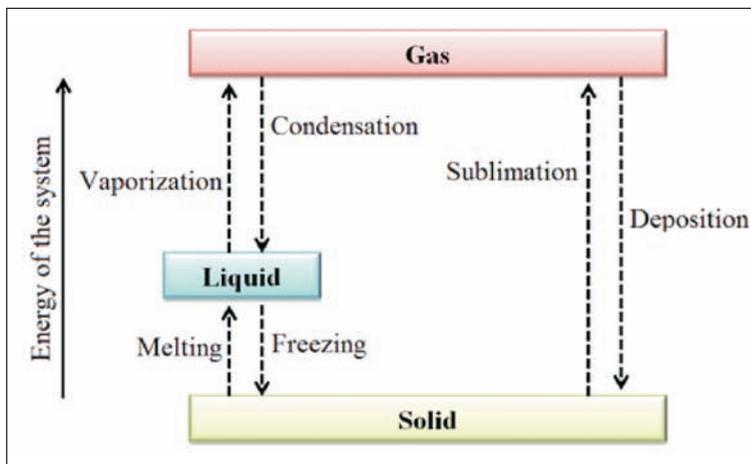
## States of Matter

The fundamental difference between the states of matter is the distance between particles. In solid and liquid states, particles are closer together in comparison to the gas phase (*Figure 1*). At ordinary pressure, the molecules of a gas are so far apart that intermolecular forces have an insignificant effect on the random thermal motion of the individual particles. As the temperature decreases and the pressure increases, intermolecular attractions become more important, and there is an increasing tendency for molecules to form temporary clusters. The arrangement of the building blocks in solids tends to be highly ordered, with each repeating motif occupying a fixed position with respect to the others. In liquids, the molecules are able to slip around each other, introducing an element of disorder and creating some void spaces that decrease the density. The molecules in gases exhibit maximum randomness and disorder with practically no restrictions on the position of molecules.

**Figure 1.** Schematic representation of the different states of matter in terms of density and disorder.



**Figure 2.** Energy of the system with respect to the phase of the substance.



The state of a given substance at a particular temperature and pressure depends on two factors: the kinetic energy of the particles and the strength of the attractions between the particles. Non-covalent interactions between molecules are not as strong as covalent interactions which hold the atoms together in a molecule. But, they are strong enough to control the physical properties such as boiling and melting points, vapour pressures, and viscosities [4]. These properties are strongly dependent on the strength and directionality of these interactions which play an important role in the generation of different supramolecular assemblies. In the solid phase, the molecules are not free to move and are more compact. That is the reason for the decrease in the energy of the system when a liquid is converted to a solid (*Figure 2*).

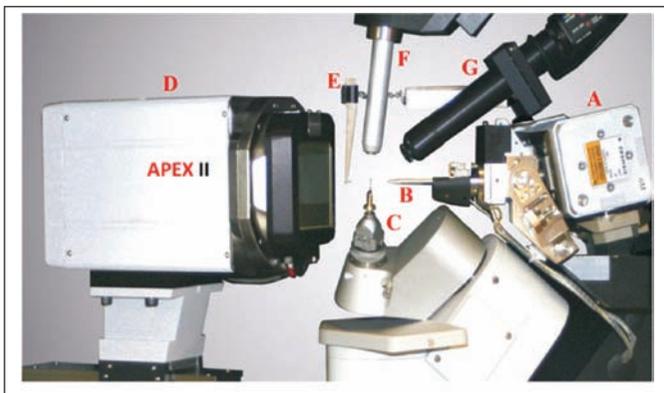
### Methodology

In the methodology discussed here, we start from a liquid and the temperature of the system is lowered to enable conversion into a solid. The nature of the crystallized solid may vary from being amorphous to polycrystalline. We delineate below the detailed strategy for the entire process.

### Single Crystal X-Ray Diffraction (SCXRD)

For single crystal X-ray diffraction (SCXRD), the instrument



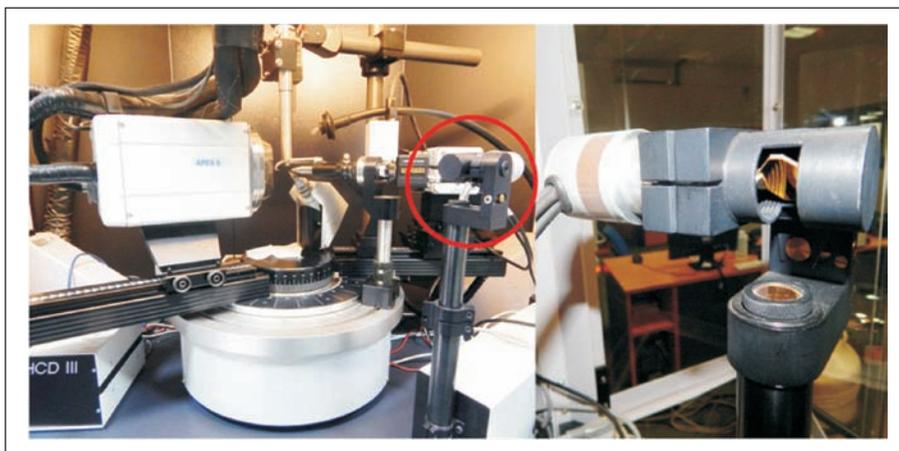


**Figure 3.** SCXRD instrumentation setup (A: Sealed X-ray tube, B: Incident beam collimator, C: Goniometer, D: APEX II detector, E: Beam stop, F: Oxford Cryosystem,

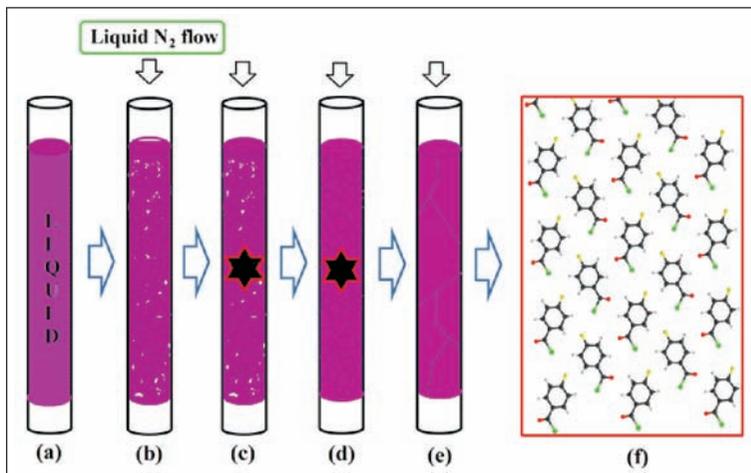
used is the Bruker APEX II CCD diffractometer equipped with OXFORD cryosystem as a low temperature attachment (*Figure 3*). In the beginning, a DSC (Differential Scanning Calorimetry) [4] plot of the target liquid is recorded. The DSC curve provides useful information (freezing point and melting point) about the nature of the crystallizing sample.

OHCD (Optical Heating and Crystallization Device) (*Figure 4*) is an apparatus by which the process of crystallization can be controlled inside the Lindemann glass capillary under liquid nitrogen flow [4]. CO<sub>2</sub> gas laser is used as a source of heat to generate the molten zone after the formation of the polycrystalline solid inside the capillary. This heating-cooling process has to be repeated several times until a homogeneous single crystal is

**Figure 4.** OHCD device.



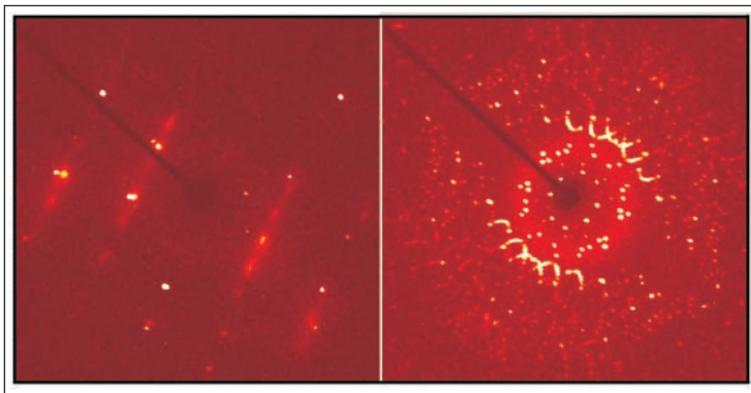
**Figure 5.** Crystallization steps inside the Lindemann glass capillary under liquid nitrogen flow: (a) liquid, (b) nucleation of molecules inside the capillary, (c) scan by  $\text{CO}_2$  laser, (d) formation of small single crystals, (e) formation of good quality crystal, (f) final molecular and crystal structure of the crystallized liquid.



formed. *Figure 5* highlights the relevant steps for growing a good quality crystal from a liquid:

1. The capillary is filled up with the target liquid; both sides of the capillary are sealed; a small portion above the top of the liquid meniscus is kept empty; the capillary is mounted on the goniometer; the liquid nitrogen flow is maintained during the entire experiment of crystal growth and data collection.
2. On formation of a polycrystalline solid, the zone-melting technique is used (application of OHCD) for melting and recrystallization; control conditions (laser power, scan time for the laser, length of the crystallization region) needed for the growing crystal are set in the software; the lower portion of the capillary is heated with  $\text{CO}_2$  laser to generate a molten zone; this molten zone is moved up along the capillary to a certain region ( $\sim 2.5$  cm).
3. The laser power is increased from zero to a value which is sufficient to melt the polycrystalline solid inside the capillary; when the  $\text{CO}_2$  laser position is moved away from the molten region, the region again gets converted to a solid; this process is repeated several times until better quality crystals, suitable for performing the diffraction experiments, are obtained.
4. During this process, formation of air bubbles is prevented inside the capillary; the power of the  $\text{CO}_2$  laser is controlled to





**Figure 6.** Still photograph (left) and rotational photograph (right) of the cryocrystallized sample before the data collection.

ensure that the capillary does not crack; after a few laser scans, (the shape of the diffraction spots is important: they must be nearly spherical rather than having a diffused nature) (*Figure 6*).

5. The data is collected and the crystal structure is determined.

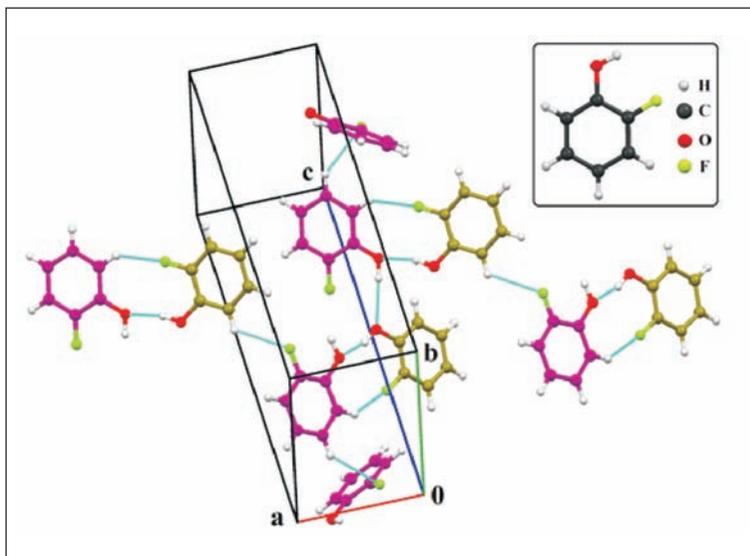
In the absence of OHCD, it is possible to grow a single crystal inside the capillary under liquid nitrogen conditions. For this, the liquid nitrogen flow is focussed on the capillary (filled up with the target liquid) at an angle  $\sim 45^\circ$  with respect to the Lindemann capillary; along the capillary length, the target position is moved from bottom to top; the flow of liquid nitrogen leads to the solidification of the liquid; on moving the position of the nitrogen flow on the capillary, the region of the polycrystalline solid again melts into the original liquid; this process of annealing is repeated manually (the time can vary from 1–5 hours depending on the nature of the crystallizing sample) until a good quality single crystal is obtained.

### Representative Case Studies: Low Melting solids

2-Fluorophenol and 3-fluorophenol [6] are examples of low-melting solids. Melting points are  $16^\circ\text{C}$  and  $8\text{--}12^\circ\text{C}$  respectively. At room temperature ( $25^\circ\text{C}$ ), they are liquid, but in a refrigerator maintained at  $5^\circ\text{C}$ , they exist as solids. For such substances, it is very easy to grow single crystals with the help of *in situ* cryocrystallization technique. 2-Fluorophenol crystallizes in an orthorhombic crystal system with the chiral space group  $P2_12_12_1$



**Figure 7.** Packing network of 2-fluorophenol having two molecules (different colour code) in the asymmetric unit showing strong O-H $\cdots$ O and weak C-H $\cdots$ F intermolecular interactions down the *ac* plane.

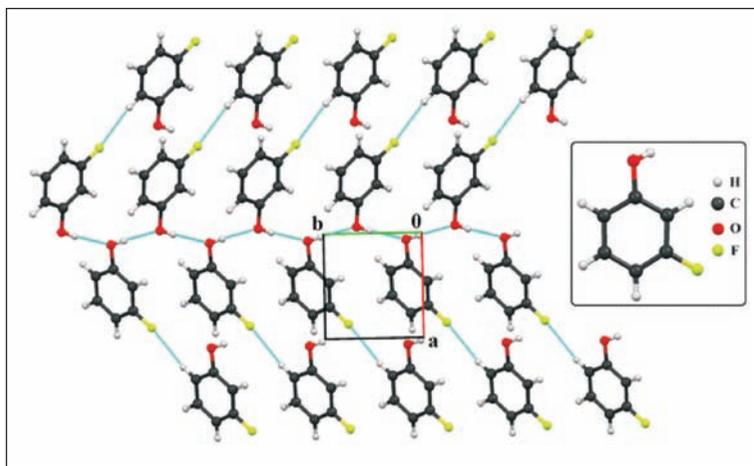


having two molecules in the asymmetric unit. The cell parameters are  $a = 5.7168(7)\text{\AA}$ ,  $b = 9.9997(19)\text{\AA}$  and  $c = 17.868(2)\text{\AA}$  and all the interaxial angles ( $\alpha$ ,  $\beta$  and  $\gamma$ ) are  $90^\circ$ . The molecules pack *via* strong O-H $\cdots$ O hydrogen bond and weak C-H $\cdots$ F intermolecular interaction down the *ac* plane. These synthons are interconnected with each other via the strong O-H $\cdots$ O chain and weak C-H $\cdots$ F chain along the *c*-axis and *a*-axis respectively (Figure 7). 3-fluorophenol crystallizes in the monoclinic crystal system with the space group  $P2_1$  with one molecule in the asymmetric unit having cell parameters  $a = 5.6747(9)\text{\AA}$ ,  $b = 5.0760(4)\text{\AA}$  and  $c = 9.4753(13)\text{\AA}$  and the angle  $\beta$  is  $107.832(11)^\circ$ . Here, the molecules form a chain along the *b*-axis via the strong O-H $\cdots$ O hydrogen bond and finally it forms a sheet with weak intermolecular C-H $\cdots$ F interaction down the *ab*-plane (Figure 8).

### Liquids

Alkanes are hydrocarbons composed of only carbon and hydrogen atoms connected by single bonds. In the solid state, *n*-alkane molecules are packed together by van der Waals interactions. Ethane, propane and butane molecules form layered structures in the solid state. The molecule, *n*-hexane [7], which is a liquid at room temperature, crystallizes in the centrosymmetric triclinic



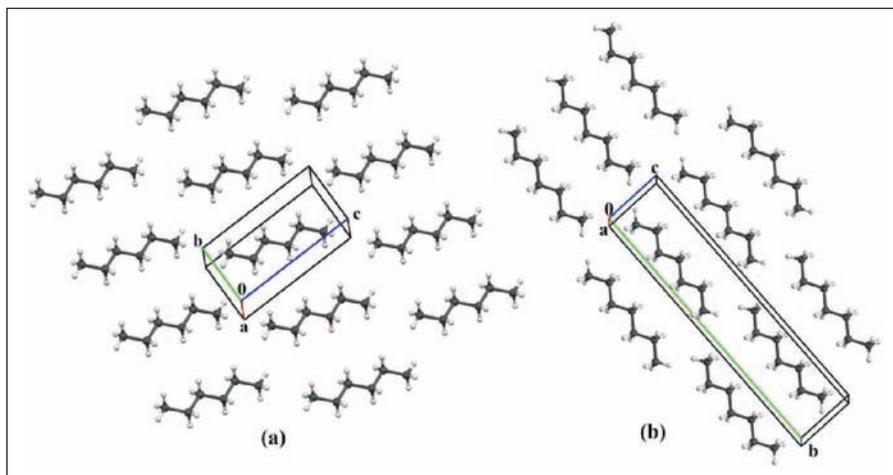


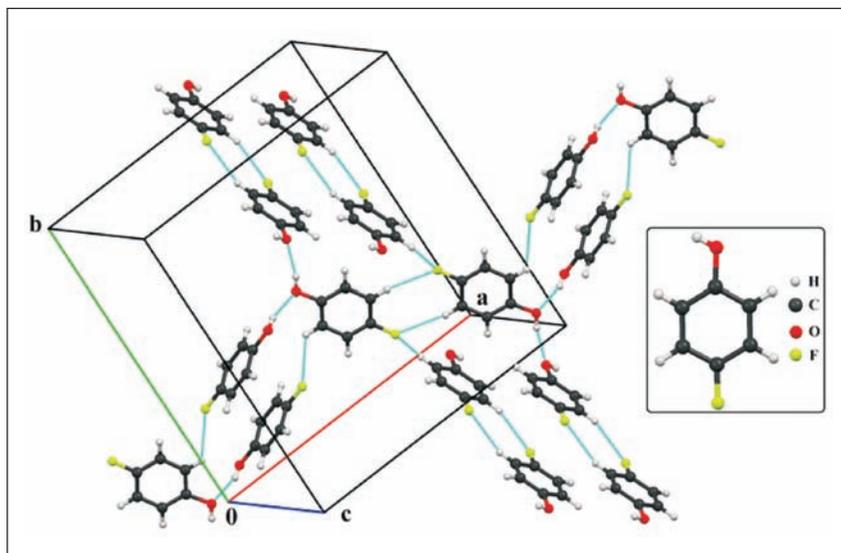
**Figure 8.** Packing network of 3-fluorophenol along the  $c$ -axis showing the zigzag arrangement of molecules associated with strong  $O-H\cdots O$  chains and connected laterally  $C-H\cdots F$  interaction.

$P$ -1 crystal system, the axial lengths for the unit cell being  $a = 4.17\text{\AA}$ ,  $b = 4.70\text{\AA}$  and  $c = 8.57\text{\AA}$ .  $n$ -heptane [7] also crystallizes in the same crystal system with the lengths  $a = 4.18\text{\AA}$ ,  $b = 4.75\text{\AA}$  and  $c = 20.16\text{\AA}$ . The packing of the molecules  $n$ -hexane and  $n$ -heptane are shown in *Figure 9*. It is to be noted that the parallel chains of molecules are slightly displaced, relative to each other. This is to ensure the packing of the methylene groups into the void space of the neighbouring chains.

This technique is also useful for unsaturated hydrocarbons. The crystal structures of 1,7-octadiene ( $C_8H_{14}$ ), 1,9-decadiene ( $C_{10}H_{18}$ ), 1,7-octadiene ( $C_8H_{10}$ ) and 1,9-decadiene ( $C_{10}H_{14}$ ) following *in situ* crystallization from liquid have been analysed by Bond and

**Figure 9.** Packing of (a)  $n$ -hexane and of (b)  $n$ -heptane viewed down the  $bc$  plane.





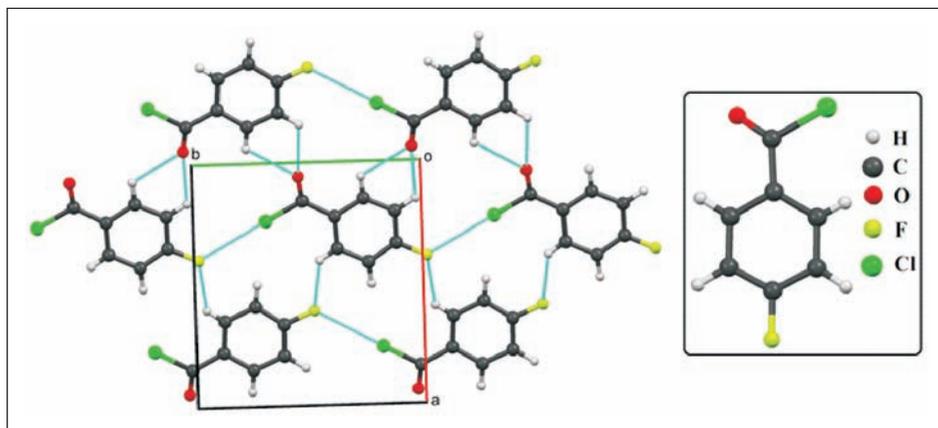
**Figure 10.** Packing network of 4-fluorophenol down the *ab* plane connected via C-H...F intermolecular interactions and O-H...O H-bond

co-workers [8]. Furthermore, liquids containing strong donor (O–H) and acceptor (O) along with weak donor (C–H) and acceptor (F) have also been explored. For example, 4-Fluorophenol [9] is a liquid at room temperature (25°C). It crystallizes in a rhombohedral crystal system with the space group  $R\bar{3}$ , having the cell parameters,  $a = 22.620(2)\text{Å}$ ,  $b = 22.620(2)\text{Å}$ ,  $c = 5.5690(11)\text{Å}$  and  $\alpha = 120^\circ$ . In this case, the overall crystal packing is stabilized via strong O-H...O hydrogen bond, C-H...F dimer and the  $\pi\cdots\pi$  stacking down the *ab*-plane (Figure 10). 4-Fluorobenzoyl chloride [10] has a melting point of 10–12°C. It crystallizes in the monoclinic crystal system with centrosymmetric space group  $P2_1/c$  having the cell parameters,  $a = 9.7208(14)\text{Å}$ ,  $b = 9.1128(13)\text{Å}$ ,  $c = 7.5178(11)\text{Å}$  and  $\beta = 96.727(2)^\circ$ . In this case, the molecules are found to pack via C-H...F, bifurcated C-H...O intermolecular interaction and hetero halogen...halogen (F...Cl) interaction, the distance being  $3.153\text{Å}$  (Figure 11).

### Crystal Structure of Ammonia

To obtain information about the electron density distribution in ammonia molecule, a single-crystal of ammonia,  $\text{NH}_3$ , is grown in a thin-walled capillary at 178 K, and high-resolution X-ray





diffraction data are obtained for this compound at 160 K [11]. In order to get a single crystal of ammonia, a thin-walled capillary (diameter 0.3 mm) is filled with condensed dried ammonia, sealed, and transferred to a diffractometer with a low temperature device. A single crystal is grown directly on the diffractometer at 178 K using a zone-melting technique producing a molten zone with a focused  $\text{CO}_2$  gas laser. Ammonia crystallizes in the cubic crystal system, with space group  $P2_13$ , at 160 K with  $a = 5.1305(8)$  Å,  $Z = 4$ ,  $V = 135 \text{Å}^3$ , and density ( $\rho$ ) =  $0.838 \text{ g cm}^{-3}$ . From X-ray data, it is observed that the H–N–H bond angle is  $109.0(2)$  and the N–H bond length is  $0.838(3)$  Å. Bond angle H–N–H is close to the tetrahedral one and corresponds to  $sp^3$  hybridization of the nitrogen atom (Figure 12). Multiple strong N–H $\cdots$ N hydrogen bonds are the key structural feature responsible for the crystal formation.

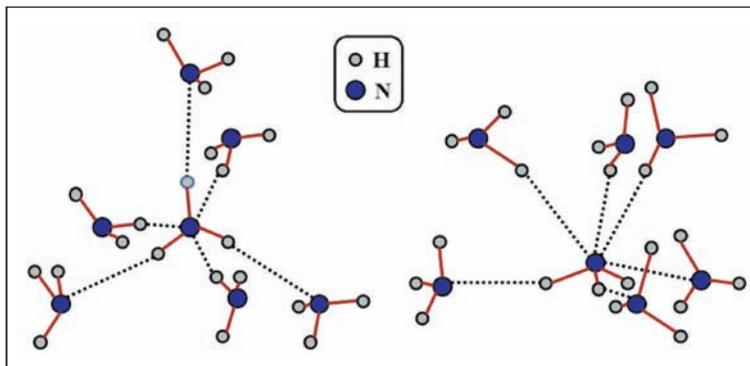
### Advantages

With the help of *in situ* cryocrystallization, we can obtain 3D molecular connectivity as well as the existence of intra- and intermolecular interactions which are responsible for the formation of crystals. This technique has been successfully applied to compounds which are low-melting solids and molecular liquids at room temperature. In future, this opens up the possibility of exploring the structure of molecules which exhibit drug and pharmaceutical activity. This has immense potential in the field

**Figure 11.** Packing network down the *ab*-plane of 4-fluorobenzoyl chloride indicates weak C–H $\cdots$ O and C–H $\cdots$ F intermolecular interaction along with halogen $\cdots$ halogen (F $\cdots$ Cl) contact.



**Figure 12.** Hydrogen bonding scheme of  $\text{NH}_3$  molecule in the crystal in two different projections.



of computational protein–ligand interactions to enable a better understanding of the biological function at the active site.

### Suggested Reading

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