Fundamentals and Applications of Supramolecular Chemistry
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Lecture 27

W6L27_Characterization of Polymorphs

So, in the previous lecture, we looked at different types of polymorphism. So, we looked at conformational polymorphism, examples of conformational polymorphism, packing polymorphism, and then we looked at pseudopolymorphism.

Now that we have some idea about how we are able to obtain polymorphs by changing the crystallization conditions, let us look at the statistical probability or the possibility of the occurrence of polymorphs. That is, how frequently a polymorph is obtained when we try to crystallize different kinds of compounds.

So, the occurrence of polymorphs. So, there are some compounds that have been crystallized millions of times, but no polymorphs have been obtained.

For example, benzoic acid, D-glucose, and naphthalene. These three compounds are just three examples I'm giving you. These have been crystallized millions of times, but no polymorphs have been obtained. Obviously, when these have been crystallized, they have been done at ambient pressure.

So, pressure is another factor that plays an important role in polymorphism, and there is a field called high-pressure polymorphism, where you can actually apply pressure, decrease the volume available to the crystallizing molecules, and then when crystallization takes place and crystals are formed, they are actually formed under the application of high pressure.

So, in the absence of high pressure, under ambient pressure conditions—that is, at normal atmospheric pressure—when you have tried to crystallize benzoic acid, glucose, D-glucose, and naphthalene, they do not form any polymorphs.

On the other hand, there are some compounds; for example, carbamazepine has a large number of polymorphs, and then we have thiourea, 2-thiobarbituric acid, and pyrazine-2-carboxamide. So, these four examples tell us that these compounds have given us a large number of polymorphs whenever we have attempted crystallization.

Even if today, you try to crystallize these compounds by changing the conditions, you might get different kinds of polymorphs, and you might also get solvates, which is also very important as we realize in the pharmaceutical industry.

And then there are some compounds depending on the amount of effort you put into crystallizing those molecules; you will be able to discover polymorphs. So, there was one very famous statement made by a scientist named Walter McCrone who said that the number of polymorphs you get is directly proportional to the amount of time and money you invest in that compound.

So, if you know that a compound is biologically very relevant, has good pharmaceutical activity, and is important in the treatment of, say, a particular disease, then the industry has invested a lot of time and money to be able to screen the compound for all possible polymorphs.

And this is something that Walter McCrone did say: that you can get more polymorphs if you invest more time and money in that particular compound, trying to crystallize it under different conditions. The crystallization space is extensively sampled, and then you might be able to get polymorphs.

So, there are compounds in which substantial efforts have been put, and polymorphs have also been obtained. Because now, this particular phenomenon has gained relevance in the pharmaceutical industry, and a large amount of effort is put in either manually by a team or using different kinds of solvents and different kinds of apparatus.

This has been partially replaced by so-called robotic crystallization, where you have a robot that has chambers made, and you can at one time put in around 500 to 700 crystallizations using a small amount of the compound.

Say the particular compound is a very important compound in the drug industry; say you have got, you know, 20 to 30 mg of it. Now you actually put, say, 1 or 2 to 3 mg in small regions of this robotic space that is available, and then the robot is actually programmed to put in the necessary solvents in the right amounts.

Once the crystals are formed, it will take the crystal and put it on the diffractometer to check the unit cell for you. In this way, we are able to extensively screen a large number of crystallization conditions and produce a large number of crystals, which is done using robotic processes.

But then again, this is a lot of effort and a lot of experimental inputs to be able to get polymorphs, and this is where the computational efforts have been extended to predict polymorphism.

And when you are doing the computational investigation, we need to keep in mind that all the calculations are being done primarily, to start with, at 0 Kelvin; that is, we are ignoring the role of temperature in the crystallization process and also the fact that temperature does play an important role in giving you different kinds of polymorphs, and the overall stability of polymorphs can also vary depending on the temperature of crystallization.

But to start with, the computational investigation of polymorphism has been actively pursued, where there is a very important research area that has been developed, called crystal structure prediction.

Where we hypothetically generate a large number of crystal structures in a small energy window, say between 1 and 2 kcal per mole. And out of these hypothetically generated crystal structures, say we take the top 100 low-energy crystal structures, and then we are able to, and we know that the line is a narrow energy window.

Now we can go ahead and crystallize that compound and see whether we can experimentally realize the low-energy forms of the particular compound. And most of the time, it is not the most stable one that is sampled; it is the kinetic form that is slightly higher in energy compared to the thermodynamic form that is actually experimentally realized.

So, this field of crystal structure prediction is very, very interesting because it allows us to a priori predict the different kinds of polymorphs and the energetics associated with these polymorphs, which can be formed.

You can actually look at the hypothetical crystal structure of these particular polymorphs. Then, if you experimentally realize these polymorphs, it is a win-win situation because you predicted something, and now you have also experimentally realized it, which vastly decreases the amount of experimental resources you actually put into the field of polymorphism.

That amount of money is actually reduced substantially if computational resources are also developed to investigate polymorphism. So, now let us go a bit more into the details of how we characterize polymorphs.

The last number of techniques that have been developed in the past two decades has benefited tremendously from the development of different kinds of techniques that contributed to the understanding of the phenomenon of polymorphism.

So, to start with, we will first look at the basic technique that we have already explored, which is called the optical examination of crystals under a microscope. The number 2

technique is thermal methods of characterization, where we look at a technique called differential scanning calorimetry and combine it with another microscopic technique called hot stage microscopy (HSM), and DSC is differential scanning calorimetry.

And in order to characterize the amount of solvent present in a crystal, we can do a thermogravimetric analysis, which we call TGA, that actually looks at the weight of the crystal as a function of temperature.

So, we will look at some of these aspects. Number 3, the most important technique for unequivocal characterization of polymorphs, is single crystal X-ray diffraction or powder X-ray diffraction to begin with.

So now these techniques are also very important. Then there are spectroscopic techniques. For example, IR, Raman and solid-state NMR, to look at the local environment and the changes associated with the bonding in the local environment that get reflected in the spectroscopic properties now of these solids, because polymorphs are characterized by different arrangements of the same set of nuclei.

So, depending on the kind of interactions the different nuclei experience, it will also have an effect on the spectroscopic properties, and this can be investigated at a very subtle level by exploring the subtle changes in the chemical environment with concomitant changes in the chemical bonding associated with the functional groups.

These spectroscopic properties can be extremely useful to monitor, and then we can look at computational approaches, for example, the lattice energy calculations, and then we can examine the physicochemical properties, which are different for different polymorphs.

For example, we can do IDR, which is called the intrinsic dissolution rate, the solubility, and the equilibrium solubility, which is a measure of the bioavailability of the polymorphs of interest. I already told you the melting point, the lattice energy, and the density are going to be different. So, we have already looked at the optical examination of crystals. We have discussed it. Now let us look at the thermal methods.

Now, thermal methods are extremely sensitive to the characterization of polymorphs, and the first thing we need to keep in mind is that when you are doing an experiment, or a DSC experiment, the thermal history of the sample is very important. The thermal history of the crystals or the crystalline solid is very important; that is how you have prepared your crystals.

That is how the crystals were obtained, whether the crystals were obtained under kinetic conditions or thermodynamic conditions, and at what temperature the crystallization was

done. So, the thermal history of the crystals is extremely important, and what we do here is essentially a DSC experiment where we heat the compound and, as a function of temperature, look at the heat flow. And we start with, say, room temperature, and then say, at 130 degrees centigrade, the compound melts.

So this corresponds to the onset temperature, which is the melting point. So, the onset value is the melting point of my solid substance, and the area under this curve is associated with the enthalpy change of going from solid to liquid.

And the ratio of this delta H to T, when you take this quantity, gives you the entropy change associated with the process. And because these experiments are being done in hermetically sealed aluminum pans, when the process of melting takes place, the temperature does not increase; whatever heat flow happens to the system goes into melting the compound only.

So, this delta H can be equated to Q by T, where Q is the amount of heat absorbed by the substance to melt; therefore, the temperature does not increase, and that is why these processes are endothermic: because heat is absorbed by the substance to melt.

On the other hand, now say you have melted the compound; now you stop the heating process. You obviously assume here that the compound has gone from the solid to the liquid state; it is a melt, and now you perform the reverse experiment, where you start cooling the sample.

When you start cooling the sample, you will see that at the temperature at which it should have crystallized, crystallization does not take place; instead, it happens at a slightly lower temperature.

So, there is a hysteresis in the process; there is a slight delay in the process; this is called hysteresis, and then the crystallization takes place at a particular temperature, and then it comes back to the initial state. So, this is an idle representation of a DSC; you have melting, and then you have recrystallization.

In most of the experiments, the recrystallization process does not occur at the time of the experiment. This is because the melt does not recrystallize; there is a hysteresis, there is a thermal arrest, and it is more like a supercooled liquid.

And it is only after enough time has been given, which means almost when you have allowed the conditions of thermodynamic equilibrium to be established, that the melt now slowly converts into the solid state.

Many times, it has been observed that you keep the melt in the sealed pan for around 24 to 48 hours, and then it obviously comes down to room temperature. You leave it for

another 24 to 48 hours, and when you take it out, you will see the solid again.

So, definitely when you brought it back to the initial room temperature, it did not become solid; you left the pan for 1 to 2 days, and it became solid. So, it has now converted to the solid phase.

Now, the next question is whether it converted back to the initial phase or has converted into a new polymorph, and that can be determined by again doing the DSC experiment.

You will see that it is now possibly melting at a lower temperature; say it melts at 120 degrees centigrade. So, now the melt has converted into another polymorph.

So, the initial polymorph, say I can refer to as P1, and now you have another polymer P2, and the melting points are different. One was 130 degrees centigrade, and the other is 120 degrees centigrade.

So, the melting point is different for the two polymorphs, and this can be very clearly established from a DSC experiment, which is very, very sensitive to the solid-state formulation of the particular substance, the solid-state structure.

Similarly, you can now actually follow up with these events, of say melting. So, say you have the solid melting to liquid and then the liquid back to solid.

This process is taking place now; it goes back to the solid from the liquid again. Now, this can also be followed visually using hot stage microscopy, where you have an optical microscope, a plate on which you normally examine your crystals, but now instead of that particular plate, you put a hot plate and then close the hot plate.

Then you heat the plate, and you can see that as the crystal or solid melts, you will physically be able to visualize the changes that happen on the hot stage plate.

So, the hot stage plate combined with the optical examination allows you to see the changes that are happening in DSC at a more physical level, with more visualization.

You can visualize all the effects that are happening, and you can follow this process over multiple heating and cooling cycles.

So, the DSC experiment itself, to start with, need not be stopped after one complete cycle of heating and cooling. You can actually continue this for at least two cycles.

So, you can go from heating. First, you can start the first heating cycle where you go from heating and melt it. Then you bring it back, cool it, and again heat it, and again cool it.

Now, this is one complete cycle where you have heated and cooled it, and then you do not stop the experiment; you again do heating and cooling, which is two complete cycles.

And this allows you to understand more deeply whether any possible events have happened, where one polymorph has converted into another, because you had a melt, and that will allow you to see whether you actually accessed a new polymorph or not, what it is, how it looks, and so on and so forth.

So, DSC combined with HSM gives a 1-to-1 mapping of all the events that are taking place. Another very interesting thing that can be seen in an HSM is if you have a solvent included in the crystal structure. So, say you have a solvatomorph that includes acetone, tetrahydrofuran, ethyl acetate, methanol, and benzene.

Then you will see that when the crystal is heated at a particular temperature, desolvation takes place and bubble formation occurs. So, you have the crystal, and you will see nice bubbles forming, which actually correspond to the solvent molecules and are being released into the environment.

So, you see the solvent molecules being released into the environment. So, there is a loss of solvent, and then the anhydrous form melts at a particular temperature. So, you have the desolvation, followed by the melting of the anhydrous form of the particular compound.

So, you can see this thing very clearly in an HSM experiment. DSC and HSM actually go hand in hand. And if you would like to quantify the amount of solvent loss as a function of temperature, then you do the TGA, which is the thermogravimetric analysis, where you actually measure the percentage weight change as a function of temperature, and then you will see that you get this kind of TGA response, a thermogravimetric response where you will have some percentage of weight loss, say at a particular temperature here, and then you will have this much weight loss.

When you reach this particular temperature, say X degrees centigrade, you will experience further weight loss. This remains fixed, and you will have more weight loss taking place.

So, this is the next set of weight loss you have. So, this is W2 weight loss; this is W1. How much weight is being lost as a function of temperature? And you can actually map this experimental weight loss with the theoretical weight loss because you know how much substance you started with and which solvent is coming out.

So theoretically, you know how much would come out and what you started with. Calculate the amount of loss that should have occurred and match it with the percentage weight loss from the experiment, which allows you to unequivocally establish the amount of solvent loss taking place as a function of temperature in a thermo-gravimetric experiment.

Another very important parameter, when you do these particular experiments, such as DSC or TGA, is the scan rate. You can actually heat at very fast rates, or you can heat at a slow rate. Normally, the recommended rates are 10 degrees centigrade per minute and 5 degrees centigrade per minute, but in some cases, you can also do 100 degrees centigrade per minute, which is extremely fast heating, and you can also do it at slow rates like 1 degree centigrade per minute.

The slower the rate of heating, the more control there is over the process, so heating and cooling should happen at the same rate for a given experiment. So, you can also combine this; if you want, you can do it at a fast rate and then cool at a slow rate.

But a slow rate of cooling is also encouraged because then the chances of getting the solidified mass are high since you are cooling it very, very slowly. So, you are kind of trying to maintain the thermodynamic equilibrium that can favor the formation of the crystalline phase when you cool it at extremely slow rates.

And the same thing applies when you want to do an HSM; you would like to see that the HSM experiments are also being done at very, very slow rates. And there you can again do it at 1 degree Celsius per minute. So, this is a very important factor when it comes to deciding the DSC, the rate at which you want to conduct the DSC experiments.

And there are many more complicated processes that happen in DSC experiments. For example, there can be a solid-to-solid phase transition before melting occurs. So, you can start with one particular phase that can convert into another phase and then that particular phase melts, okay.

So, you can have a solid-to-solid phase transition, and then that particular form can melt. So, if you have a solid-to-solid phase transition, that is referred to as enantiotopic; the two polymorphs are enantiotopically related.

Whereas, if you have a set of polymers, say A and B, and each polymer melts at its own characteristic melting temperature, that is, each polymer is stable over the entire range of temperatures before it melts, then these two polymers are monotropically related, A and B.

We will look at these aspects in a bit more detail later on, but this is to tell you that the thermal events of characterization are extremely important in the process of crystallization.

And now we go to the next technique, which is the most important technique: single crystal XRD. So, single crystal XRD is now a highly automated technique. You have to put in the crystal, and you collect diffraction data, and the necessary software is there that will integrate your data and also determine the structure.

It will define the structure, and it will perform all the steps systematically and give you the final crystal structure. This is in itself an independent research area that we call X-ray crystallography, which is the science of determining crystal structures.

But today, single crystal X-ray diffraction is highly automated, and the final crystal structure tells you that you have one particular polymorph with an independent set of unit cell parameters and something called a space group.

For example, I am giving just one example, $P2_1/c$, which is called a symbolic representation of a space group. If you are not aware of it, do not worry about it, but we call this quantity a space group.

So, one particular polymorph has a specific set of lattice parameters; another polymorph, say P2, has another set of unit cell parameters in a space group. These are different for different polymorphs, and it is not necessary that you always need to have a different space group.

You can also have the same space group but different lattice parameters. In other words, the molecule can now choose another arrangement with a different box or a different unit cell while still maintaining the same space group.

So, different possibilities exist, but what is more important is that it gives you the atomic positions and the three-dimensional connectivity between the atoms to form the molecules.

And you can look at the three-dimensional connectivity and the overall three-dimensional arrangement, and because the three-dimensional arrangement is going to be very different, that will allow you to characterize it from X-ray diffraction.

The next important technique that is parallel to single crystal XRD is powder X-ray diffraction. This is a very, very important technique. Now, because this is called the fingerprinting response of a particular phase, this is like a fingerprint response.

So, just like infrared spectroscopy is a fingerprint for every organic molecule that you have synthesized in the lab.

Powder XRD is like a fingerprint for a particular phase or a particular polymorph of a compound, and if you record the powder XRD of a particular polymorph, say P1, of any compound that you have synthesized, the intensity as a function of 2θ gives you a set of diffraction peaks.

And now, when you record it for another polymorph, you can see this is a very qualitative way of representing it: some of the peaks are the same, but most of the peaks are different. The red one represents P2, and the green one represents P1.

So, because the solid-state arrangement is different, the diffraction events that are happening corresponding to the different planes in the crystal will also give rise to an interference pattern corresponding to the different planes, which will actually come at different 2 theta values.

So, the two theta values are going to be different for different polymorphs, and that is the reason why this technique is called a fingerprint technique for every polymorph of a given substance.

And what is important is that once you determine the unit cell parameters, say P1 and P2, you can do something called profile fitting, which can actually characterize the phase unequivocally. Now that you know the unit cell parameters and the space group for the P 1 phase, if you run the calculation and do the modeling of this profile, you will actually generate a theoretical or simulated powder pattern where the 2 theta positions calculated will exactly match the 2 theta experimental positions.

So, there is a peak-to-peak correspondence between the theoretical powder pattern simulated from your determined crystal structure. So, once you have solved the crystal structure, you can actually generate a simulated pattern or a theoretical pattern, and then you can compare it with the experimental pattern.

And do a peak-to-peak correspondence; then you can further characterize that phase on which you have recorded the powder pattern unequivocally by fitting the profile to the experimental lattice parameters for a particular phase.

And then once you fit the profile, you can see the difference; the difference profile will come here. The difference profile will essentially be like a baseline that does not have any peaks because the difference is equal to 0.

So, this is essentially featureless, featureless in the sense that the I (obs) - I (calc) is approximately equal to zero. And if this is not equal to 0, then it means that you have

unaccounted peaks either in terms of intensity or in terms of the 2 theta values, and it possibly indicates that either you have not modeled your phase accurately or there is another phase that is present in your powdered sample.

But if you do it on the crystals, chances are you have checked all these crystals slowly by doing the unit cell determination, and now you take these crystals and record the powder pattern, then the chances are almost near 100 percent that you have got a pure phase because you have checked all the crystals of that phase by the unit cell determination.

So, powder XRD allows us to unequivocally establish the phase purity, and in the case of concomitant polymorphs, you will see that both P1 and P2 are present in the bulk phase.

So, you have got the bulk phase; you will see that both polymorphs P1 and P2 are present in the bulk phase as well. So, with this, we can characterize polymorphs using these different techniques. In the next lecture, we will further look at other aspects of polymorphism.

Thank you.