Symmetry and Structure in the Solid State
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Lecture – 50
Structure Determination 2

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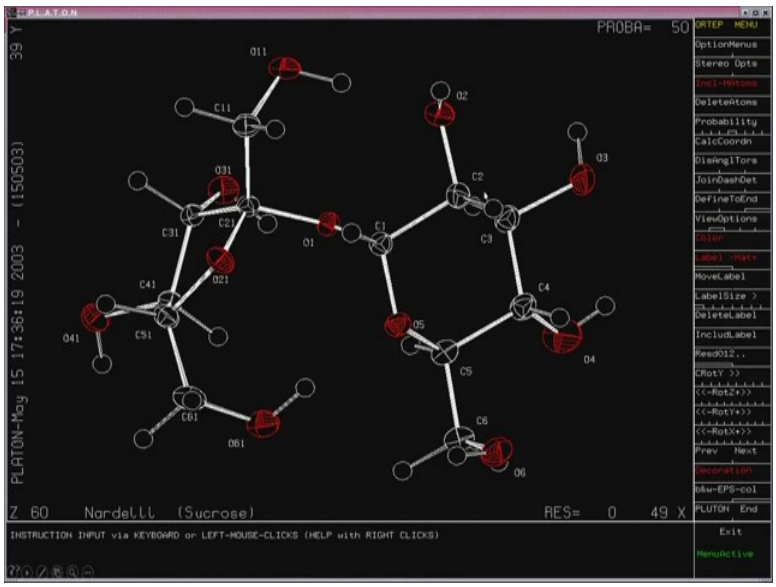

So, we see that this is a representation of the structure drawn by a program, this is a program called Pluton, there are several programs which can do this all type plot. And this is the structure of a sucrose. So, the sucrose structure is determined then, then its positions of the atoms the carbon atoms and oxygen atoms are shown and so on. I was talking about the hydrogen positions, so in a situation like this there are two ways in which hydrogen positions can be determined. One is to put all the atoms in their final positions as we have done the refinement. And, once we have those in the final positions do F calculation, that is you go back to this calculation and then do a F_{obs} minus F_{calc}.

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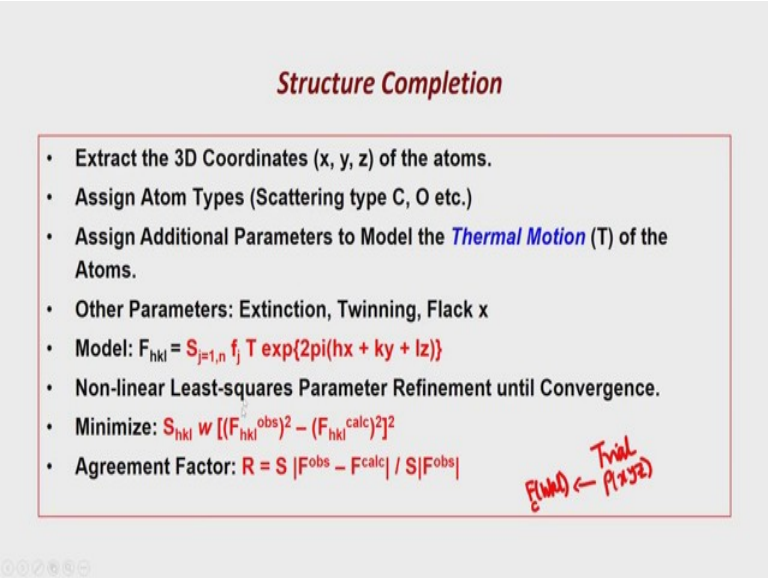

So, all the atoms are in an accurate final accurate positions and so you got a difference of F_{obs} minus F_{calc}. So, that will be now they representation of whatever is left out which is reserved for hydrogen atom positions. Or, for that matter if you have an item which is wrongly put or wrongly introduced in this process, then it will not start showing up when you do a ΔF based Fourier map. So, in the Fourier map now you do not use F_{obs} you used ΔF. So, when you calculate the Fourier map you use ΔF and that means, you will use the whatever is the electronic density that is left out and that is known as a difference Fourier plot because, we are taking the difference of the Fourier values of the observed and the calculated.

So therefore, it is a difference Fourier plot. So, when we do this difference Fourier plot the hydrogen atoms, many of them will show up, as possible small density values were around 1 electron per angstrom cube. And this positions, therefore can be fixed now we can identify their hydrogen positions. So, then we see also the geometry that is associated with those hydrogen positions. In case it is not coming out clearly the hydrogen positions then one can use the stereo chemical information that is available in the molecule. In case of sucrose we know whether it is up or down whichever way the hydrogen positions are going we call them alpha and beta in sucrose nomenclature. So, those hydrogen positions therefore, can be actually fixed at that particular distance.

And angle which is which it is making with respect to the atom for example, this hydrogen atom which is coming out of this carbon atom. The oxygen atom is going down. So, the stereo chemistry around this particular carbon is tetrahedral. So, we have now the 3 directions fixed the 4th direction can be now given to the hydrogen atom. And at the corresponding distance which is normally a normal O-H distance is about one angstrom.

So, we take 1 angstrom from there and fix the hydrogen position. So, then we do not touch the hydrogen position, but we allow the carbon, oxygen and other surrounding atoms to refine. So, whenever the new positions of these carbon comes up, this hydrogen automatically is fixed again at 1 angstrom; that means, this is referred to as a riding hydrogen. So, we call this is as a riding hydrogen because it rides along with these atom. So, whatever happens to this particular atom hydrogen in any case will not leave this is like a rider sitting on a horse.

So, as the horse keeps moving he is not going to be displaced, he is stucked to the back of the horse. So, the hydrogen therefore, is riding on the corresponding atom with which it is attached. So, this is known as the riding atom hydrogen atom refinement in which case the distance is not altered. The hydrogen atom position is therefore, now depends upon how the positions of the other atoms nearby change. The other thing which we can do of course, is one once we have the difference Fourier hydrogen identified and if you still have a good data at our disposal then we can try refining the hydrogen positions as well.

But refine them with respect to the isotropic value. So, that is why you see only circles here rather than ellipsoids, because this is now associated with what we call as an isotropic thermal parameter; only one thermal parameter is associated with respect to x y z and since there is only one thermal parameter you will have a circle instead of a ellipsoid. So, these hydrogen atoms therefore, are represented by circles and this is now a full representation of the particular molecular. So, this is how we get the molecule in the in the asymmetric unit represented in a diagram like this.

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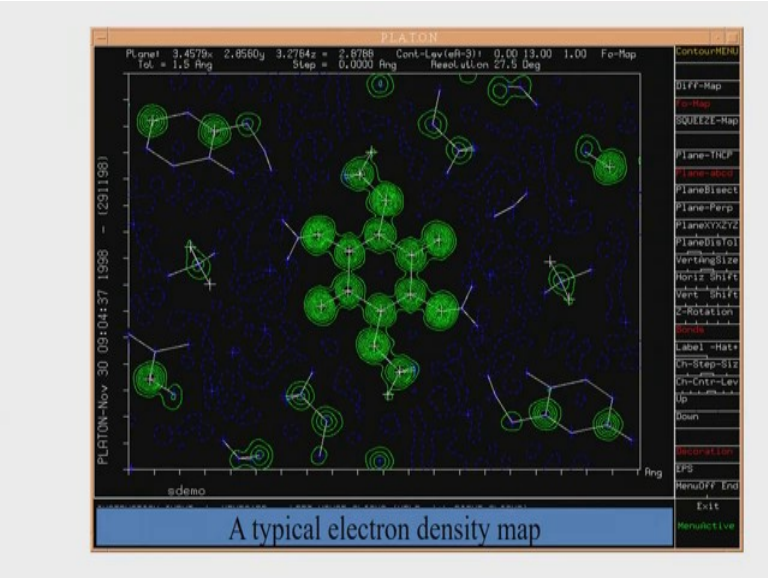

So, just to recapitulate we got into a situation where we had the initial electron density map of some kind. And this particular initial electron density map they could give us some atoms in the structure. We took those atoms to be the correct atoms, calculated the structure factors from the electron density associated with these atoms, that give us the calculated structure factors.

The calculated structure factors and the observed structure factors are subtracted from each other. And the corresponding agreement factor or the reliability index R is calculated and this particular R value should be going down as we improve the F_{obs} minus F_{calc}. If we find that this F_{obs} minus F_{calc} after the so called revaluation goes and makes the R factor higher; that means, either the atoms are in wrong positions or some of the atoms are in wrong position some of them in the right positions. And all these will be revealed in the difference Fourier map.

So, if you do a difference Fourier map it will come with ups and downs, we will study a little bit of how these Fourier maps come up in terms of a numerical example. So, that it makes life easier we will understand, how they associated electron density features come up by taking an example of a Fourier series and checking out how we calculate the Fourier series when we do the difference Fourier plot. So, in this particular case we do a difference Fourier plot and try to improve the condition, right.

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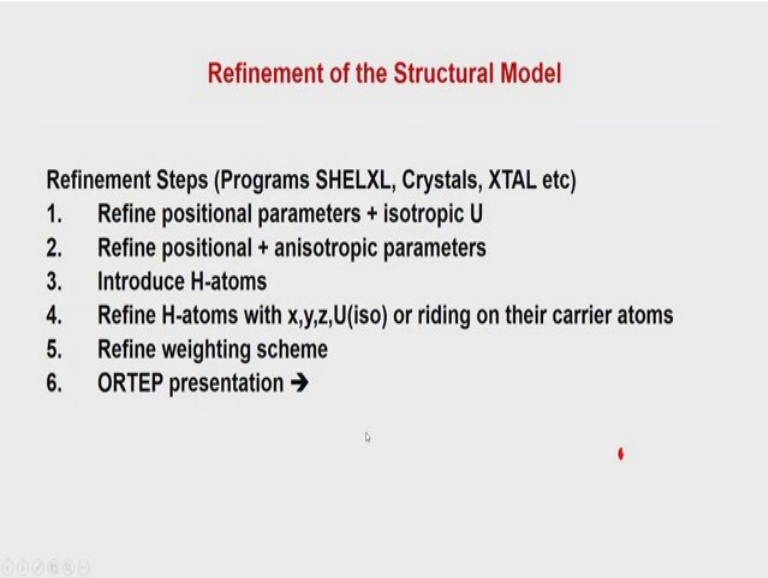

So, as and when these things are all done this is referred to as the refinement of the structural model protocol, we now refined the hydrogen atoms or riding on their carrier atoms as we just discussed. And therefore, we have now completed the refinement of the structural model we go ahead and calculate the so called R factor. These R factors will not go towards 0 they go towards 0, but they will not be 0 because that would mean F_{obs} and F_{calc} are exactly equal to each other which will never happen, due to uncertainties involved. We have so many uncertainties involved like for example, determining the thermal parameters there are certain things determining the position of x y z also there are uncertainties.

The thermal ellipsoid feature will not allow the R factor to go to at 0 because, we cannot pinpoint the exact position of x y z. The only thing that will happen is R factors will go lower if you collect the data at lower temperatures. If you collect the data at higher temperatures then the R factors will not go down, it will have a certain value at which it will sort of remain the same. So, then such situations we call it as convergence. So, we go and find out the optimal value of R and when we reach the optimal value of R, we say that we have reached convergence in the refinement protocol.

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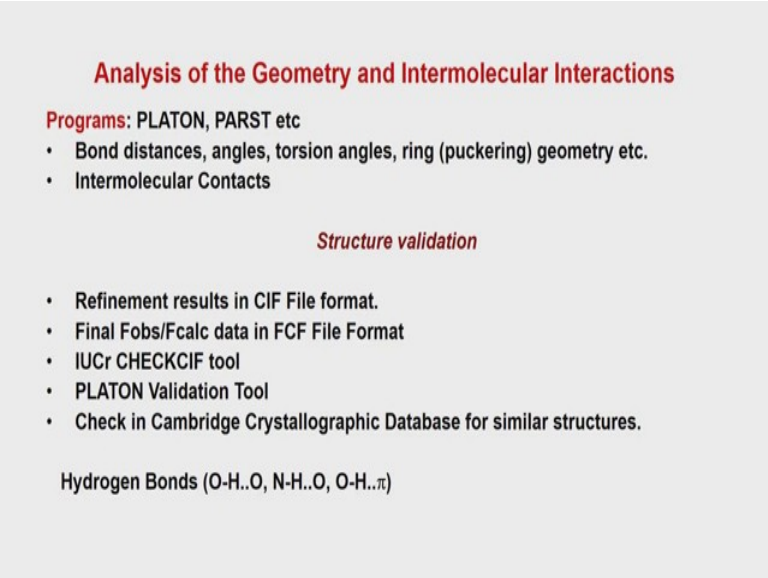

Now, let us see after having done the structure and having got a good R factor and successfully determine the structure, in the space group which we have determined and based on symmetry, we have now the structure information. So, one once we have the structure information how do we analyze the geometry and intermolecular interactions? This is the next that which we will take up and in this particular step we see that we have to determine the geometry of the way in which the atoms are arranged inside the structure.

So that means, we need to find out bond distances, bond angles, torsion angles ring puckering geometry in the case of sucrose you see that we have the puckering of the ring etcetera. So, all the molecular properties can be calculated here, in case there is an inorganic system we can calculate the coordination distances and so on, with respect to the inorganic atom which is present there. In case of organic metallic samples we can also calculate the way in which the coordination sphere develops around a metal atom. We can also calculate because of the fact that we have not just one molecule in the unit cell more than one molecule in the unit cell, we can now construct the rest of the molecules in the unit cell.

So, what we have determined by the structure determination is only the asymmetric unit atoms. So, we can now take the asymmetric unit atoms use the symmetry information which we have in terms of both structure and symmetry in terms of both space groups

and symmetry and then we can then calculate the contacts between molecules. This now refers to the inter atomic contacts. So, we can determine the intermolecular contacts and inter atomic contacts are available here at the bond distances angles and so on.

So, the complete geometry associated with this can be calculated using programs like PLATON, PARST etcetera these are all readymade programs downloadable free of cost. After that we have to see there are no funny contacts. So, suppose now you solve the structure and then the structure develop some contacts which are very much shorter between intermolecular contacts then you know something is wrong. So, anything wrong at this stage of the geometrical calculation one can find out and redetermine the structure.

So, this is a place where the reliability of the structure determination is evaluated. Having got the geometry and the intermolecular interactions evaluated, we have to actually now validate the structure. The validation of the structure is done according to the international union of crystallography rules; say everything is under control of the international union of crystallography as far as structure determination and structure reporting is concerned. So, the refinement results all the results which we have got they are referred to as a crystallographic information file. So, this is referred to as the file CIF. So, one has to have a CIF file available for any structure that is determined.

And that is CIF file will have so many factors, CIF file will consist of the a, b, c α, β, γ the standard deviation that is in the a, b, c α, β, γ the coordinates x y z and their six thermal parameters, if it is anisotropic refinement. The hydrogen positions and their isotropic or anisotropic parameters all these features will be reported under the CIF file. Along with the fact the CIF file will also consist information contains information about the nature of intermolecular interactions in terms of how they are bonded to each other. What is the symmetry operation which takes this molecule 1 to molecule 2 through a hydrogen bonded interaction for example.

All such things are reported in the CIF file. So, one once CIF file is available one can calculate all the geometry that is associated with the structure. Then we also the structure validation is also seen in terms of the F_{obs}, F_{calc} calculated data, that is the final values of the observed and calculated data. And this is known as a FCF file. So, together CIF file and FCF file now represent the geometry and the structure information together, because

one once we have an FCF file it is possible to calculate the electron density map directly. And then verify for yourself that the structure is what is reported in the literature.

So, the CIF value and the FCF values are all available one once the structure is made and the structure validation is checked under the IUCr protocol which is which uses what is known as a check CIF tool. There is also what is known as a PLATON validation tool which checks on other aspects including the aspects associated with intermolecular interactions it will check. The various ways in which one can identify the hydrogen bonds and other kinds of bonds; we will not it is not necessary for us to go into those details because it does not come under the purview of the syllabus of this course.

But that particular course could be a course on advanced crystallography and the information we get from the crystal structures. We can also check in the Cambridge crystal structure database which holds houses all possible structures determined, so far all the CIF files all the FCF files which are now available for all the structures determined from day one. So, the entry is crossing hundred and 50000 entry so far and its adds every day some 15 to 20 structures additionally. So, then we can check the similarity between structures by using the Cambridge structural database; this we will bring us to the issue of how we do crystal engineering analysis I will have some time towards the end of the course to discuss about that.

So, we can also look at hydrogen bonds and various types of hydrogen bonds strength of hydrogen bonds, we can calculate the energies that are associated with it separately. So, essentially the structure one once determined has to be validated before it is put into circulation. Before it is published one has to look at the CIF value and the FCF file format values in order to make sure that they go through the officially approved check CIF protocol. If check CIF says there are no errors then your structure seems to be in order, ok.

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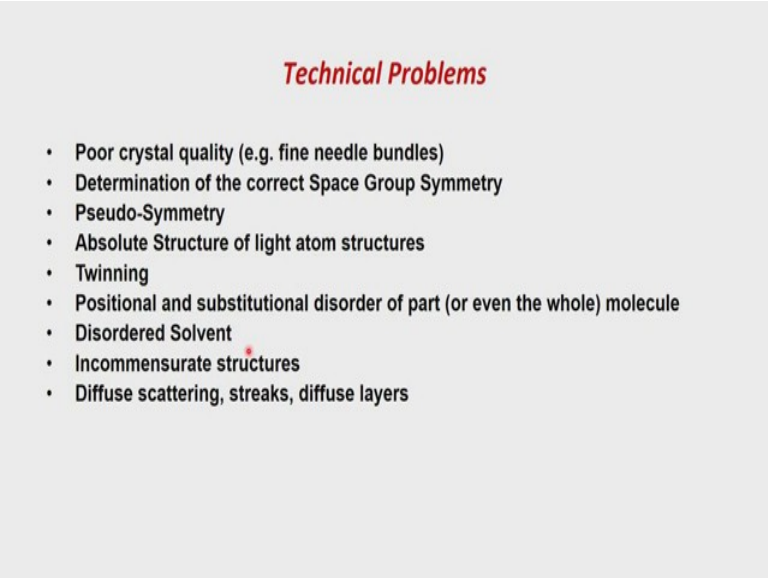

There are certain problems which you will face any experiment we will have problems one is of course, the way in which your diffractometer is set up the laboratory conditions and all that considering all that deficiencies being overcome and you have a perfect system, on which you can do a good data.

Then first major hurdle is the crystal quality. So, this is particularly so in the case of macromolecules where the crystals of proteins and other complexes cannot be so easily grown. It is mainly because of the nature in which they fold themselves and the nature in which the interactions are coming in. So, the poor crystal quality can also occur in very very small crystals as well. So for example if you get fine needle bundles all together, that will not give a good diffraction.

So, that is the poor crystal quality. So, this is one of the technical problems one would come across. And when we collect data on a very poor quality crystal it may be miss leading and it may not gives the structure which is the correct structure; the second one as we always been stressing determination of the correct space group symmetry.

Now if we have a wrong space group symmetry identified, then the structure determination will run into serious technical problems. There is an issue of what is known as a pseudo-symmetry and this issue of pseudo-symmetry can come up with respect to the molecule, it can also come up with respect to the space group that is determined.

So, this anomaly is going to put us into some serious issues I do not think I will have the necessary time to cover the details of this, but this is a warning place where we can also have this pseudo-symmetry as a technical problem. Since, I am just listing out the technical problems. Absolute structure of light atom structures is a question mark we used a flack parameter or whatever, but essentially we make use of the fact that there is anomalous scattering effects.

But the anomalous scattering effects at a given wavelength like for that of copper molybdenum they are so small for lighter atoms, that the determination of the absolute structure is a questionable entity. If you do have a very heavy atom then the value of the anomalous features will be higher and then the absolute structure determination is reasonably accurate. So, this is another technical issue which will come up; the more complicated technical issue which comes up is the so called Twinning. So, some of the crystals will just like we have in humans twinning problems we also have twinning problems in crystals, crystals can grow as twins.

And there are lots of different kinds of twins that can grow some crystals grow as merohedral twins that some crystals grow with a one access unique, but they grow together, all these problems will cause the nature of the diffraction then will be different, because diffraction will be coming from both these crystals. And those two crystals are joined to one another in some fashion unless we know in what fashion there are joined to one another, we will not be able to understand and appreciate the analysis of the data which comes from that.

So, twinning is not a is not a virtue it is a curse for it is a technical problem. Then there is also another issue which will bother in many cases and this is a very serious issue particularly for pharmaceutical compounds positional and substitutional disorder or part or even the whole molecule. Sometimes the whole molecule is disordered; that means, it is not in one position it can be in two possible positions.

The symmetry will allow it, the problem is the symmetry that is associated with the with the determined space group will allow for the positional and substitutional disorder. And this therefore, we will give rise to different kinds of diffraction conditions. It may look as though because of this positional and substitution disorder, the molecule is going into two different crystal systems which is the definition of polymorphism, but it is not the

case it is essentially the possibility of positional and substitutional disorder of the molecule.

The other technical problem we normally face particularly in larger molecules is the disordered solvent. So, the solvent maybe fully disordered or partially disordered because most of the protein crystals crystallize along with solvent water. And this solvent water has no structure of its own, it is disordered and because of that we will get what is known as diffuse scattering. So, apart from your single crystal diffraction which is coming out from the system we will also have diffuse scatter radiation coming out of this disordered solvent. And as a result the actual evaluation of individual reflections coming from the crystal system becomes in an issue and it is not going to be very easy.

There is another thing called the incommensurate structures that is possible because the way in which sometimes the crystals grow. The cell dimensions which we get will be not sufficient to describe the orientation of the molecules in different unit size. That means, the molecules now can orient they have to be exactly the same in the next unit cell that is the definition by translational periodicity, whatever molecule we are getting in a crystal structure in the unit cell should repeat itself in the next unit cell should repeat exactly the same way in the third unit cell. It may so happen that the second third and fourth unit cells the molecules are slightly rotated from each other.

So, it's not an exact replica of the first unit cell, the exact replica of the first unit cell let us say comes at the fourth unit cell, then we call it as the commensurate modulation. So, the unit cell dimension now is not the one unit cell it is that of the fourth unit cell. So, we have to go from first unit cell to the fourth unit cell fourth unit to the eight unit and so on. And that is now the repeat distance, this is a technical issue which can be sorted out. On the other hand if it is not repeating exactly at four unit cells, but somewhere around three and half then it is called incommensurate structure. There are what are called incommensurate way which go through the crystal and one has to determine that.

So, these are all technical issues which will create problems. For example, we will get diffuse scattering, we can get streaks and diffuse layers of reflections and so on. And these reflections can also cause serious problems. So, since we face these technical problems, associated with crystals, one has to be aware of these pitfalls and these pitfalls might also create bad data and one once we have a very bad data set, the structure

determination is going to be difficult. So, whenever we grow a crystal we should look at all these possibilities. And if such possibilities do exist we have to get rid of them or regrow the crystals such that they we get rid of all these. There are certain issues which we cannot get rid of like twinning we cannot get rid of, like incommensurate modulation we cannot get rid of, the presence of disorder and disordered solvents we can get rid of.

So, one has to be aware of these and see how we can account for the for these technical problems, how we overcome or is there any way in which we can solve these problems. Some of the problems which can be solved or for example, we can solve the twinning problem we can solve the incommensurate structure problem. The substitutional disorder positional disorder part we can evaluate, but we cannot solve it. It can still be a part and parcel of the structure which has been determined the disordered solvent of course, will remain disordered. And so also the diffuse scattering streaks diffuse layers and so on.

So, these are all part and parcel of any experimental protocol and experiments do have these kind of difficulties. So, what I therefore, want to say is that I will go back in a few slides and then tell you what we have done with respect to the data collection.

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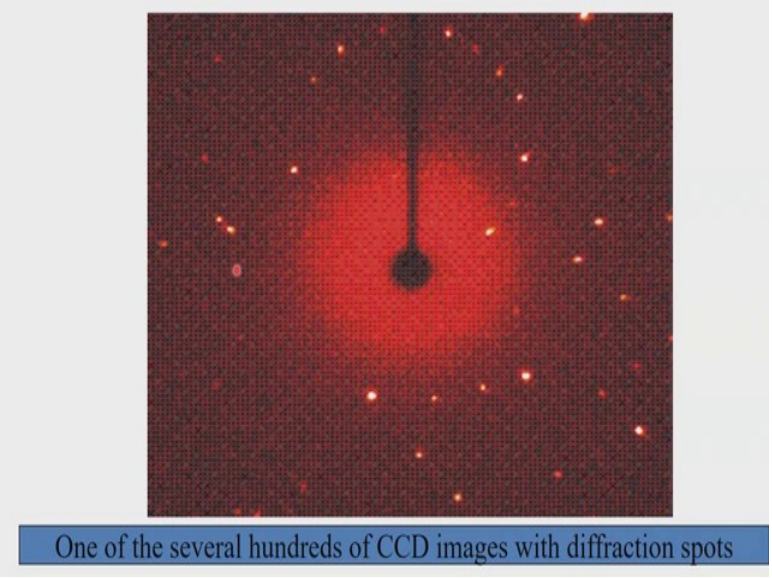

So, when we put a crystal and collect the data we get these reciprocal spots.

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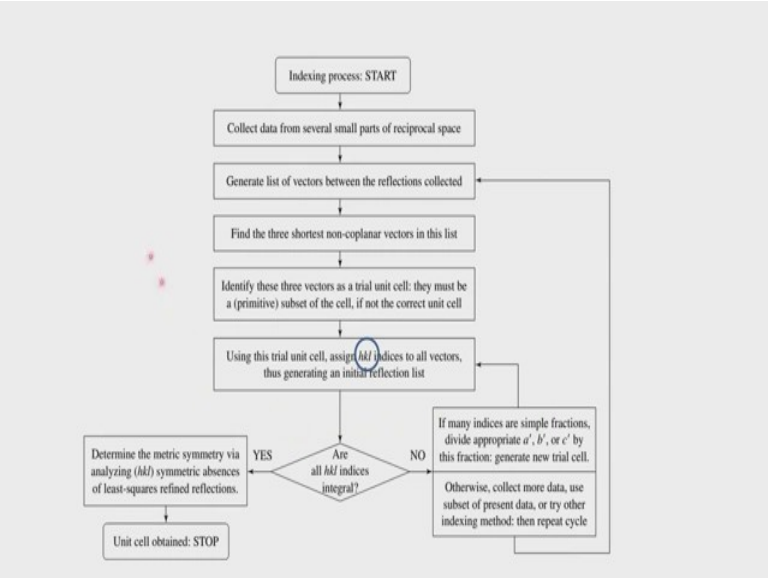

These reciprocal spots now can be indexed and we determine the all possible hkl values from which we get the unit cell dimensions.

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Crystal System	$1/d^2_{hkl}$
Triclinic	$(1-\cos^2\alpha-\cos^2\beta-\cos^2\gamma+2\cos\alpha\cos\beta\cos\gamma)^{-1}\left(\frac{h^2}{a^2}\sin^2\alpha+\frac{k^2}{b^2}\sin^2\beta+\frac{l^2}{c^2}\sin^2\gamma+\frac{2kl}{bc}(\cos\beta\cos\gamma-\cos\alpha)+\frac{2lh}{ca}(\cos\gamma\cos\alpha-\cos\beta)+\frac{2hk}{ab}(\cos\alpha\cos\beta-\cos\gamma)\right)$
Monoclinic	$\frac{h^2}{a^2\sin^2\beta}+\frac{k^2}{b^2}+\frac{l^2}{c^2\sin^2\beta}-\frac{2hl\cos\beta}{ac\sin^2\beta}$
Trigonal (R)	$\frac{1}{a^2}\left(\frac{(h^2+k^2+l^2)\sin^2\alpha+2(hk+hl+kl)(\cos^2\alpha-\cos\alpha)}{1+2\cos^3\alpha-3\cos^2\alpha}\right)$
Hexagonal/Trigonal (P)	$\frac{4}{3a^2}(h^2+k^2+hk)+\frac{l^2}{c^2}$
Orthorhombic	$\frac{h^2}{a^2}+\frac{k^2}{b^2}+\frac{l^2}{c^2}$
Tetragonal	$\frac{h^2+k^2}{a^2}+\frac{l^2}{c^2}$
Cubic	$(h^2+k^2+l^2)/a^2$

a=b=c
α=β=γ=90°

And one once we have the unit cell dimensions we can use this $1/d^2_{hkl}$ formula to determine the crystal system based on these and therefore, get the a, b, c, α, β, γ value.

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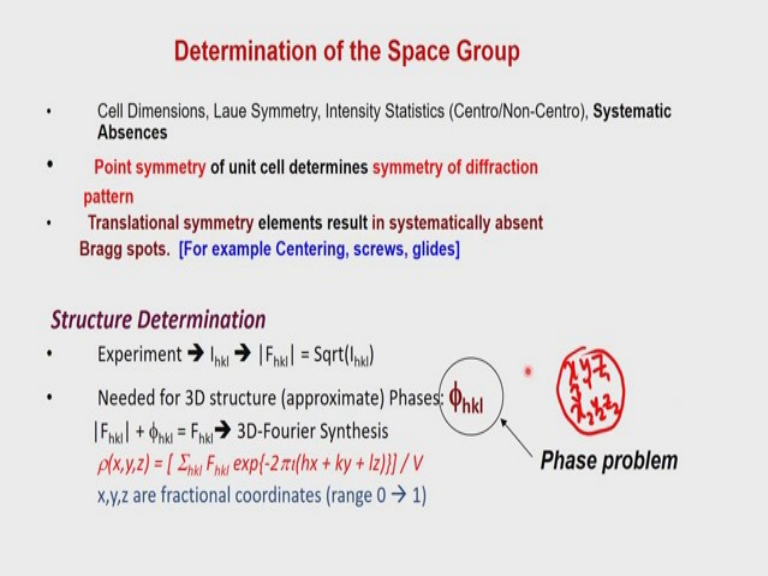

And then we determine the space group by looking at the systematic absences in their structure, we can also get the cell dimensions and then of course, Laue symmetries there already and the analysis of the intensity statistics, which will tell you the difference between centro symmetric and non centric systems. We will also be possible to use this methodology to determine the crystal system whether it is a centro symmetric or a non-centric system.

Particularly in cases where systematic absences are not helping and then we of course, we will go to the structure determination we follow this protocol, determine the electron density get them the starting model the starting model is now put back into this calculation. And therefore, we solve the phase this is the major problem in the phase problem.

So, we get only the estimate of the phases. So, we get the starting model, the starting model electron density is analyzed atom positions are identified and the F_{calc} is made with respect to that then we take the difference between the F_{obs} and F_{calc} and that delta F analysis will give us the rest of the atoms. We go through this protocol until we do not get any more of better value of the so called R factor. And at that stage we will now this is the set of refinement procedures which we will follow and that is where we stop and say refinement is complete and make a representation of that in an R type plot.

So, that is the overall protocol and then of course, we analyze the geometry the structure validation of course, is very crucial. And this is the overall experimental procedure. Now having seen the overall experimental procedure and the technical problems that are involved, we will now go into the details of how we take the data, how we treat the data and how do we reduce the data into an absolute scale before we subject it for structure determination protocol.

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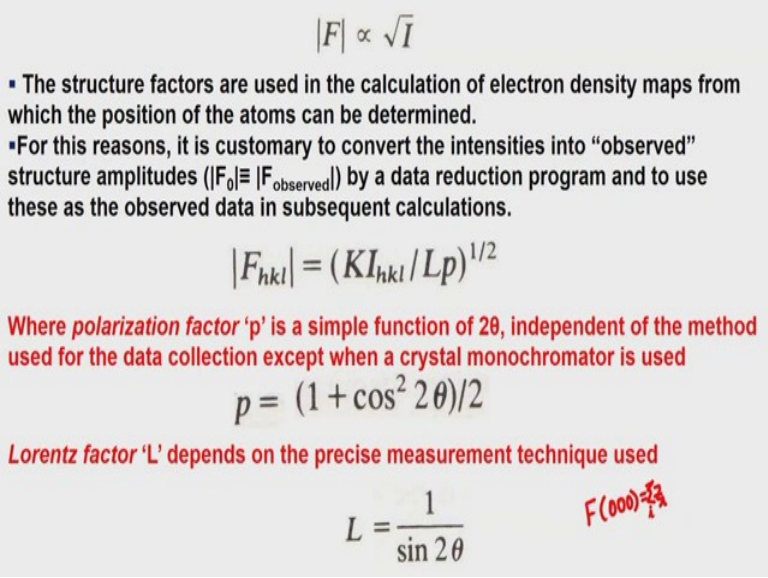

And this is the one which follows here. We have already learnt some of these things, but I will go through it in such a way that we get a hang of what we have to do. This is again an experimental data.

So, experimental data will give us the intensity measurements, we have of course, hkl identified. So, if the modulus of structure factor is proportional to root of I. So, it is used in the calculation of the electron density map. This is I have written it again just for your continued remembrance and also as a continuity. I will not be explaining it again and again because you know what we have what we have written here. So, we convert the intensities into observed structure amplitudes modulus of F_0 by a data reduction program.

Now, this is where I told you that we can collect the data on any part of the surface of the earth on any instrument, maybe we can even correct data in space it does not matter, but what instrument you are using is deciding what intensities you will get because it depends upon the input radiation, the scattered radiation, the efficiency of the detector

and so on. So, one once you have that data you have only a relative data, relative intensities therefore, you get a modulus of F which we call as the observed structure amplitude.

Now, you have to treat this data or what we call as a data reduction methodology, such that we correct for all systematic errors that might occur on this particular value. And one of the rigorous procedures which must be followed before we go any further is to treat this F_{hkl} by this expression. The expression tells us that the F_{hkl} modulus of course, it is I_{hkl} to the power of half as it shown here and that proportionality constant is replaced by K/Lp capital K by capital L and small p. Now, the quantity p is called a polarization factor and the quantity L is called the Lorentz factor.

The polarization factor depends upon the incoming radiation and the scattered radiation and is a simple function of 2 theta; so we can correct this p as equal to $(1 + \cos^2 2\theta)/2$. So, independent of the method used for data collection except when a crystal monochromator is used we will not go into the detail of it except to tell that this polarization comes up due to the fact that even the x ray radiation is coming they are all circularly polarized. That means, the electromagnetic waves are uniformly oscillating in all directions. And, when the impinge on the on the diffracting plane or the reflecting plane on reflection it. So, happens that they go in such a way that if they are partially polarized.

So, when we are measuring the intensities of partially polarized light and p is the factor which corrects for that partial polarization. The factor L depends upon the way in which we measure the data, remember we have this problem of the reciprocal lattice rotating with the crystal. And the origin of the crystal is somewhere at let us say some central point and as you go in the reciprocal space to higher and higher angles. The amount of time that is spent by a reflecting geometry sphere; that means, the way in which the diffraction occurs is that that particular point will intersect with the Ewald sphere. Whenever the reciprocal lattice point intersects with the Ewalds sphere then we get diffraction.

Now, the amount of time that is spent by that particular diffracting point will be different depending upon the orientation and the distance of that point with respect to the origin. And this is where we have a issue. So, the issue is in terms of L which is called the

Lorentz factor and this is dependent upon (1/sin θ). So, we use this L and p this is independent of the method of data collection we have used it is independent of the instrument we have used where the L and p is a constant factors. So, we calculate these L into p and put it in this expression K is the overall scale factor, with which we have to now put the F_{hkl} on take the I_{hkl} take the square root and K is the proportionality constant. Lp correction is made on the every intensity which we observed.

So, the values of L and p are listed in international tables again in a different volume not in the volume where we find the space group information, but in the second volume of the international table. So, one can get the values of L and p as you see they depend only on the scattering angle. And therefore since they depend on the scattering angle they depend upon at what angle this scattering is coming, at what angle the h k l reflection is coming rather than anything else.

So, therefore, it is possible to get the Lp corrections uniformly organized for every given wavelength, it is going to depend on the wavelength; obviously. So, it is a wavelength dependent correction Lp, but it is available on the international table.

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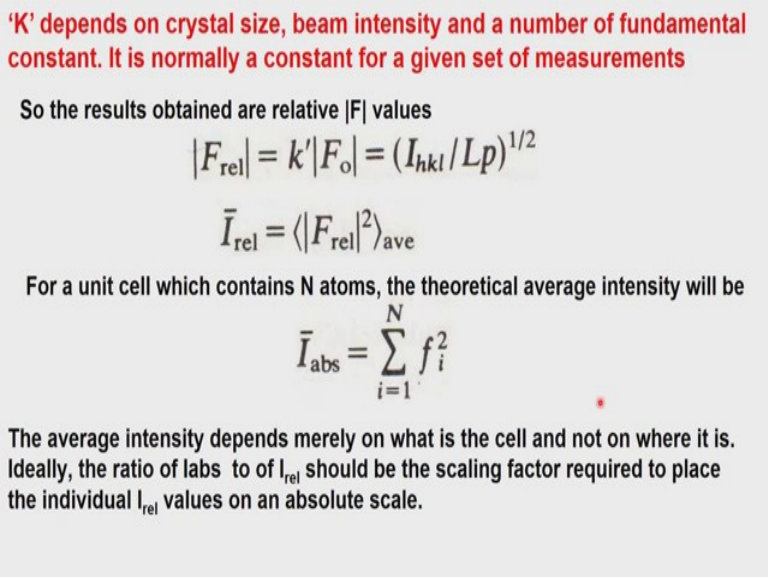

Now, K is depends on the crystal size the beam intensity and a number of fundamental constant it is normally a constant for a given set. That is the proportionality K we are talking about which depends upon the way in which we collect the data.

So, effectively we have to treat the intensities and make the corrections associated with the Lorentz and polarization factors. Before we process it further this whole processing of the data depends upon various issues, it also depends upon the way in which the amount of X-ray gets observed inside the crystal and way in which the total internal reflections can take place depending on what we call is extinction factor and so on. These are all parameters, these are all factors which affect the diffraction condition. And these factors which affect the diffraction conditions obviously, are parameters.

So, we can refine these parameters overall in our structure determination protocol. So, the absorption correction can be applied we can refine the absorption correction in the way in which we would like to do. And also the case of the extinction we can apply the extinction correction. So, therefore, the data has to be manufactured in such a way that this particular data which we are going to use finally, is put on an absolute scale.

So, in order to put the date on an absolute scale we have a protocol on that protocol we will have to depend upon the fact that we have already made the structure factors into a proper position and a proper scaling. So, the amount of information which we have is enormous, we have a very large number of data points and those data points now has been treated for this data reduction protocol.

So, we now have a collection of F_{hkl} this F_{hkl} is now divide of all systematic errors as far as possible. The only problem is it is still dependent upon the way in which you have collected the data the instrument that is used the way in which the detector has been place. So, it depends whether you have used a photographic film or you have used an imaging plate or you have used a CCD detector or a CMOS detector. So, it all depends upon the power with which you can convert the number of photons which are falling on the detector to the electrical signals which eventually measures the intensity.

So, the intensity measurement therefore, is a totally relative phenomena and. So, we have to therefore, put this intensity measured on an absolute scale. And the measured intensity to be put on an absolute scale essentially comes from the fact that we need to have them on the scale which counts the total number of electrons. So, what the logic of this whole thing is that we have a crystal and in the crystal, we have electron density and the electrons are all over the place it is distributed all over the place.

Of course, they are atom centered, but anyway for all practical purposes it is not the position of the atom which matters now as far as these the scattering overall is concerned it essentially matters that we have the total number of electrons. So, what we can do is we can normalize all the structure factors with respect to the total number of electrons. So, in other words the quantity F_{000}which is our standard quantity. So, the quantity F_{000} is equal to the total number of electrons. So, we can call it sum over all the z values of every atom.

So, if you have i atoms in the structure i runs from 1 to n. So, sum over Z_i. So, suppose you have 20 carbons, 30 oxygens and so on. So, 20 time 6 plus 30 oxygens times 8 that summation will be now the F_{000}. So, that gives us the overall content of the of the unit cell. So, the overall content of the unit cell is sum over z of i which we have to determine this includes the symmetry related atoms as well. So, that provide us the required scaling and that required scaling is the one which we will have to worry about. And how do we find that scaling factor is something which we will discuss in the next class.