**Circular Dichroism and Mossbauer Spectroscopy for Chemists**
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**Lecture – 61**
**Conclusion Section: CD Spectroscopy**

Hello and welcome to this final segment of CD Spectroscopy and Mossbauer Spectroscopy for Chemist. My name is Professor Arnab Dutta and I am an Associate Professor in the Department of Chemistry at IIT, Bombay. So, in this final segment we are going to follow up the major points of both CD Spectroscopy and Mossbauer Spectroscopy that we have covered throughout this particular course. So, first we will start with the CD spectroscopy.

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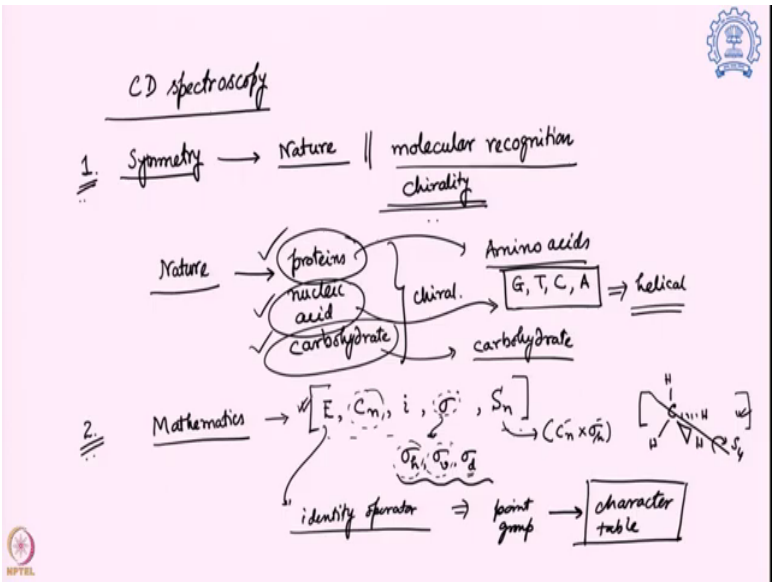


So, before going into the details of CD spectroscopy. What we have covered that why we need to understand chirality because we found the first point. Symmetry is a unique thing which is present in nature and that is present in all the different parts of the nature. Wherever you go and the different components of nature follows the symmetry and there are different portions of symmetry, it can be rotational, it can be reflection.

All this way we can see there is a connection between the different parts of the objects in the present in the nature and obviously, nature uses. This symmetry and one of the important aspect that is actually, used by nature is for molecular recognition because at the very down to that molecular level in natural system, you have to understand whether it is a molecule of it is interest or not and over there.

They use a symmetry as one of the tool to understand that in which particular symmetry they are utilized is the chirality and what is chirality? Chirality means that one part of that molecule is such that if you reflect that and then try to take that mirrored image and try to put that in the original molecule, they will not fit it there. They will not be indistinguishable, so that is why we call them chiral and this chirality becomes one of the important aspect of nature.

And if you look into the nature, the major portion of the nature which is bound with proteins bounds with nucleic acid bound with carbohydrate that themselves are chiral in nature. Why? Because proteins are made out of amino acids and these carbohydrates itself the molecules of glucose sucrose all this important aspect, in short from the carbohydrate molecules, they are chiral in nature.

So, obviously, when there is a secondary structure coming out of them, they are also becoming chiral. On the other hand, nucleic acids are typically made out of guanine, thymine, cytosine, adenosine all this particular basic setup, the nuclear tides and when they start forming a larger structure, they started becoming helical. And wants to become helical they also induce chirality.

So, you can find the major components of biological framework other chiral in nature and they uses this chirality for molecular recognition. So that is one of the point we have covered which is very crucial. Second, is how we can use mathematics to understand this chirality? So, mathematics is going to provide us a language when you are seeing this there is some matching of the different portions of a molecule to each other, how we can actually define them?

So, mathematics provide us, the language and we found in mathematics we can define all the symmetry elements in the form of identity operator, rotational axis around the symmetry. Then we also have centre of symmetry. We have reflection plane which can be of different kinds $\sigma_h$, $\sigma_v$ and $\sigma_d$. And this differentiation actually, comes into the place with respect to the relation of this reflection plane with this axis of rotation.

Whether the principal axis of rotation is perpendicular to that plane there is sigma horizontal or it contains it then sigma vertical and sometimes some of the sigma vertical planes we found. They are actually, bisecting 2 $C_2$ axis which is perpendicular to the principal axis and those are called the $\sigma_d$. or sigma dihedral and we also have improper axis of rotation ($S_n$) where we actually, do two operations next to each other one is $C_n$ followed by $\sigma_h$.

And over here this $C_n$ and $\sigma_h$ does not need to be present in the molecule in the beginning. So, they can be run individually and try to see if my molecule is actually, matching with its original structure or not. And for example, we have done the methane molecule and figure it out that methane molecule contains and $S_4$ axis present over here. So, first you do the $C_4$. It is not there. You do a $\sigma_h$ it is not there.

But if you do both of them, you see you are reaching to a molecular structure which is similar to this original one. So that is what we going through and that is the mathematics actually, bringing to us. What are the different symmetry elements can be present? And those are of these five kinds, so, E the identity operator it a unique one among them because this means that you are doing nothing but rotating the system of a 360°.

So, which is doing nothing and keeping the molecule as it is. So, why do we need this particular identity operator? Because when you put this operator, we can group this particular combination of the symmetry elements present in a particular molecule to a point group. So that generates the point group and each of the point group have something called character table which is following their name provide us all the information that molecule can follow belongs to that particular point group.

And then just looking into the structure, we can actually, estimate what will be the different properties of that molecule with respect to bonding with respect to stretching. So, these are very important for spectroscopy and bonding atmosphere. So that is what we covered in this particular segment that mathematics is providing us the language of following the symmetry**.**

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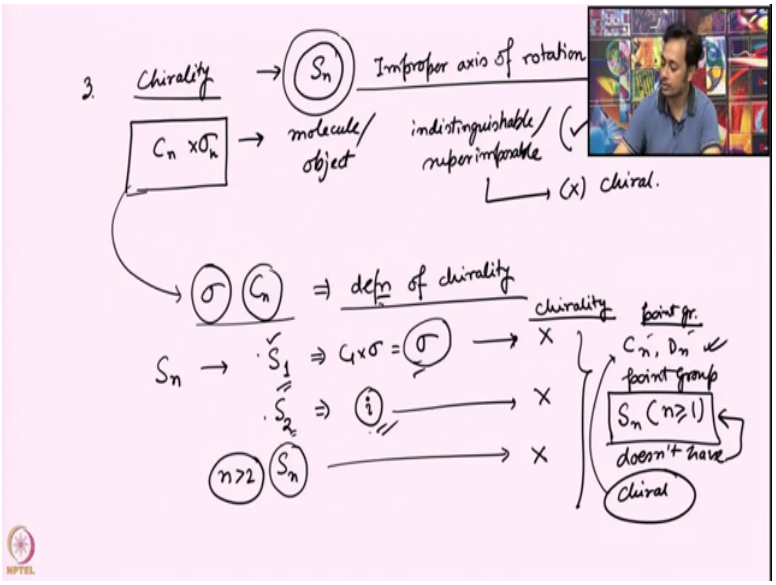


Next find out the chirality can be also followed with symmetry element and that symmetry element which actually allows us to do that is this $S_n$ axis or improper axis of rotation. So, what is the role of this $S_n$ axis over here? So, what it does is that you are doing a $C_n$ followed by $\sigma_h$ and try to see whether your molecule or object is indistinguishable and super imposable or not.

If it is then your molecule is not chiral so which is written as achiral and if it is not then you see your molecule is chiral. So, this is kind of the definition that we follow for chirality. You do a reflection that means you are doing a σ operation and then you try to fit that mirrored image that means you are doing a $C_n$ operation. So, both these things were already given in the definition of a chiral molecule but how mathematics help us.

We just like to find out whether I have a $S_n$ axis or not. If I have an S n axis in the form of S one which is nothing but $C_1$ followed by sigma which is nothing but a σ plane. So, if you have a σ plane that means you already have $S_1$. Your molecule cannot be chiral, if you have a $S_2$ this is nothing but a centre of symmetry (i), so that is also going to be a chiral. So, if your molecule have a σ symmetry, a plane of reflection or a centre of inversion (i) then your molecule cannot be chiral.

Or in certain molecules you can have higher form of $S_n$ axis which can also give you a chirality. So, either of this thing, a σ plane, a I which is nothing but case of $S_1$ and $S_2$ or any other form of $S_n$. Where n ≥2 is present a molecule cannot be chiral and which we find out

from the point group we find out which particular point group does not actually, have the $S_n$ axis and we found it is the $C_n$ and $D_n$ point group.

It does not have any $S_n$ axis where n $\geq$1. That means it covers σ and i also so, if you belongs to this particular two point group, $C_n$ or $D_n$ your molecule can not chiral. Sorry, your molecule cannot have an $S_n$ axis, so, your molecule can be chiral. So, just to figure it out whether my molecule is chiral or not what I need to do? Is just to simply find out it is point group and if your point group belongs to $C_n$ or $D_n$ your chiral.

If it does not chiral your achiral molecule. So that we found from the chirality and symmetry connection with respect to mathematics.

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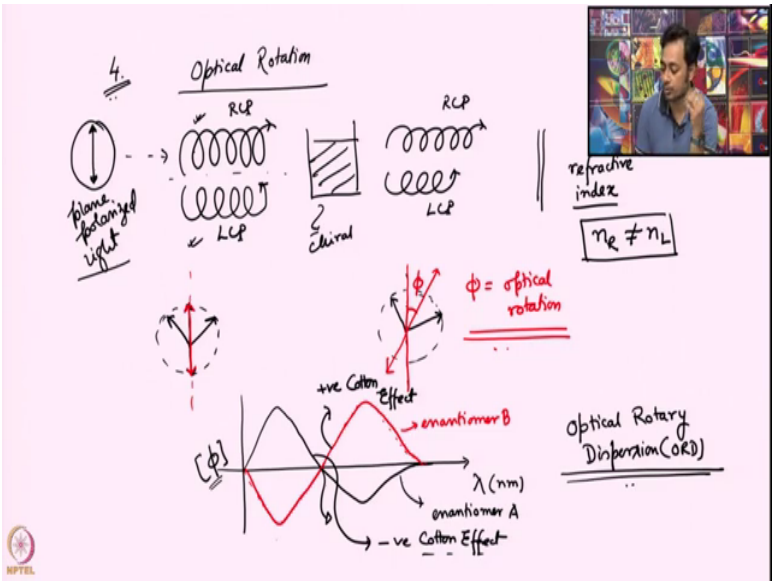


The next thing we find how we can monitor chirality, so, for that we have two options, one is optical rotation. So, for that what we do? So, we found out that our light, if we put a plane polarized light which actually have this electromagnetic radiation only in one particular axis that can be broken down in two different options. One is right hand circularly polarized light and one at the similar position but I am drawing it a little bit lower so that you can see it properly.

It is a left hand circularly polarized light. So once this two sets of right hand circularly and left hand circular polarized light which is actually, giving you two different helices. That means they are chiral to each other, so, they are actually, mirror image to each other but not

superimposable or indistinguishable. So, they are the generator of the two chirality and if it goes through a sample which contains chiral molecule.

It actually allows one of them to go faster compared to the other. So, one goes faster. So, in this case I am writing right hand circular polarized light going faster than the left hand one. So, what will be the effect? So, in the beginning, what we say that the both this one right hand and left hand circular polarized light is moving at the same speed. So, their resultant will be in this particular plane.

So, let me draw that with the red ink, so that is their resultant so that is over here. Now, after one of them passes faster than the other. So that means when I expose my chiral sample to this plane polarized light one of them is moving faster, so, the right hand circular polarized light compared to the left hand one. So, now the resultant will be in a different position. It will not be in the previous position that we actually, found over here.

It will not be in this particular position but it will be reflecting and where it will reflect somewhere around here. And over here you can see that it was previously and now I am over here. So, I have rotated a bit and that is known as the optical rotation and we can measure this for a particular chiral molecule and find whether the optical rotation is happening or not. And this optical rotation, when we actually figure it out, we can plot that optical rotation with respect to wavelength of a molecule.

And I found at the extent of optical rotation that will vary with respect to at which particular wavelength I am measuring and what I am going to get a structure like this. Typically which shows that it is showing at variation in the optical rotation and this is for one particular enantiomer, say like it is an enantiomer A and there will be another enantiomer of it which is the molecular reflection of it.

Which doesn't going to match so that is the enantiomer two and over here that band will be exactly a mirror image of the enantiomer A. And over here you can see, there is a point where there will be no optical rotation, it is 0 and that is typically happens when you have the maximum absorbance. Because the absorbance is so much there is no line of RCP or LCP will pass through that.

It is absorbing everything, so that is why we got to a 0 point but over here it is also crossing it over there. So, over here the red one is crossing from the negative to the positive region. So that is why this is called a positive Cotton effect. Cotton effect which is where we see this crossover of the value of the optical rotation from negative to positive and in other point I am seeing it.

It is coming from positive to negative and that is the negative Cotton effect. So, these are the typical change in optical rotation I can find with respect to wavelength and this is known as optical rotary dispersion or ORD and over there I want to mention that it is not always that the maximum effect of optical rotation is happening somewhere where It is absorbing because absorbance has nothing to do with this going faster or slower.

It is happening because there is a molecular property which is affecting this change in the speed and over here what is actually, happening. The refractive index is different for the right hand and left hand rotatory electromagnetic radiation and that is why we are seeing this difference. So, refractive index has nothing to do directly with the optical absorbers, so, you cannot really predict where I am going to see the best of my optical rotation.

And sometime we can see that optical rotation is happening in a position there is no absorbance at all. For example, sucrose actually, showcase a good optical rotation around the region of 580 nanometre, where there is not absorbance for sucrose at that particular condition. So that is why it is very difficult to predict where I am going to see it and also, at the same time, at the maximum absorbance point.

You might not get a optical rotation because it is absorbing so much that no light is passing through. So that is why optical rotation can be a good test to find out whether my molecule is chiral or not. But to do a qualitative to quantitative analysis and where I want to see like how much my sample is changing from one to the other is very difficult to do with optical rotation.

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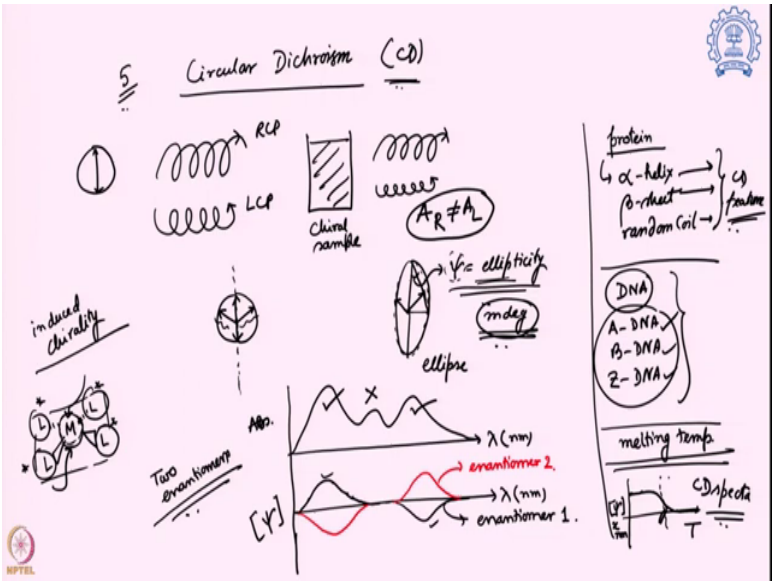


So, for that we need a better one and that is why we come to this circular dichroism and this is actually known as the CD and that is the basis of our CD spectroscopy and what we found over there. We also have this plane polarized light which is going through which actually, have this right hand circular polarized light and also the left hand circular polarized light and it is going through my sample.

And over here one of them is going to absorb more than the other. So, let us say, RCP is remaining as it is my left hand circular polarized (LCP) light it is getting absorbed. So, over here I am assuming there is no change in the speed so far, both of them moving at the same speed. But what I am seeing one of them is absorbing more than the other. So, what will be the effect of it? So, this is my chiral sample in the beginning.

So, before it hit the chiral sample, I am going to see a system like that because my right hand and left hand circular polarized light, are having the same extent of their amplitude and they are moving at the same speed, so, they are in the same phase. So, I am keeping my plane polarized light in this particular plane but after it goes through what happens? One of them absorbing more than the other.

And at the end, if this is actually, happening what I am going to get is not a circle anymore but an ellipse. So, here it is the maxima and here it is the minima. So, I am going to get an ellipse and over here this particular angle phi it will be the so, if I have already used so, like Ψ it will be the ellipticity. Because this angle is very small to be honest, it is not a very large ellipse. I am preparing this angle is comes in the region of milli degrees.

We can directly connect that this angle will be a ratio of the difference of absorbance of the right hand circular and left hand circular polarised light. So that is what we are seeing and the CD spectroscopy you can imagine it will be directly dependent where I am doing the absorbance, because unless I am doing the absorbance, I cannot separate between right hand and left hand circular polarised light.

And that is why, when we do an absorbance spectroscopy, so, this is the absorbance spectroscopy. This is the absorbance (A) versus the λ (wavelength, nm) and say I am seeing three bands like this and among them we say this particular band is chiral. This particular bind is chiral, this is not. So, how I am going to see that in the actual CD spectroscopy? Where I am plotting the Ψ versus the λ, I am going to see this band is say CD active.

That means I am going to see in non-zero phi value, psi value over here and say this band is also and this is negative over there. So, these two bands are active, so that will be I am be seeing for one of the enantiomers and it is counter but enantiomers will be the similar spectra but it will be changing the phase. So, it positive will become negative. Negative will become positive, it will be an enantiomer.

So that is what you are going to see in the CD spectroscopy and CD spectroscope is very easy to do because I know exactly where I am going to see the bands. It will be one of the absorbance band. If you have the molecule chiral, you will see at least one of the bands will be chirally active. Otherwise, if molecule is achiral, you will see a blank line. No difference in the CD spectroscopy with respect to it is chirality.

So that is why CD spectroscopy give us a better idea where I am going to see the chirality. So, these are a very good system to differentiate to enantiomers because it will, let me know like which of them is present because I can do that and find out for each of them if it is 100% what is the Ψ I am finding or expecting? And then do that for a unknown sample and find out if it is 10% or 20% or 50% of the enantiomer present.

And what is the enantiomeric excess? If it is 0 that means they will cancel each other out. We will see like a blank line in CD or we can get the 100% line that we expect because it is a 50% enantiomeric excess. So that is what we actually, going to do that in CD spectroscopy.

And CD spectroscopy is specifically important because all the protein structure can have different secondary structure like α −helix, β −sheet, also random coil.

So, different structures it can have and each of them has particular CD feature. So, you can just run a CD spectra and find out whether my protein has a CD spectra of this kind that means I have this much of alpha helix, this much of beta sheet and this much of random coil. So, I can have a very good idea of what is the secondary structure looks like for that particular protein. So that we can gain the knowledge from this particular CD spectroscopy.

And the other one we can also do that for DNA'S and DNA can have different versions that it can be A-DNA. It can be B-DNA, it can be Z-DNA. All these structures we can figure it out and CD spectroscopy, can give us again different kinds of features. By that I can follow and figure it out whether my molecule is actually, having A-DNA, B-DNA or Z-DNA configuration. So that we can also follow it from here and the other thing we can do is the stability we find for melting temperature.

So, each of the protein or DNA that we actually going through it can have a particular absorbance such that I am getting a particular ellipicity for that molecule at a particular λ say like X nm. And then I am looking that at particular temperature variation. So, say this temperature it is remaining same at one point of time it is going to change structure. Once you start changing structure, the Ψ value will change and you will get a different values.

So, over there I can follow that up and find out at which particular temperature it is actually breaking down and that is known as the melting temperature of the protein or the DNS that we have also covered earlier. And by using a CD spectroscopy we can follow that where my molecule is stable and where it is not. And by that we can figure it out if I want to develop an experiment where we should do that that is one.

And the last part is the induced chirality. So, sometime what happens that I have a metal complex which is actually coordinated to a totally symmetric ligation environment which is a chiral in nature but my ligands I am actually using their chiral. So, what happens? Their chirality is getting induced to my molecule and this my metal centre, although it is archival in beginning but it can have some induced chirality which will make some of the absorbance band chirally active.

And this is very much useful for molecular complexes which you are showing some metal based transitions, d-d transition MLCT or LMCT transition which are typically not a chiral in a chiral in nature. But because of this induced quality, they become spiral and show some bands, even in the CD spectroscopy. And they are very important because by that we can actually, follow whether by molecule is bound to a protein or DNA or not.

And if it is binding what is there overall structure and their effect on the temperature. So, all those things we can follow again from CD spectroscopy.

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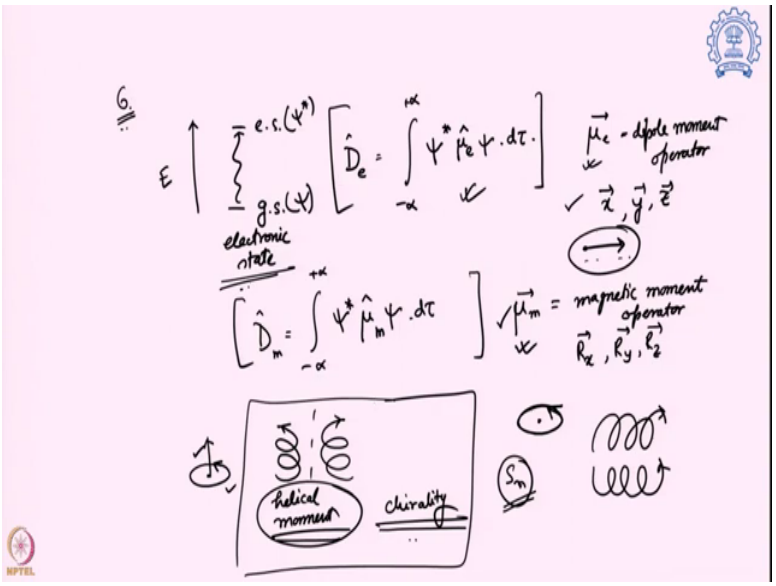


And the final point we want to cover why a molecule becomes chiral. So, we know a molecule when it is absorbing. It is going through big different state change from ground state to excited state and this is actually happening for an absorbance when it is changing this electronic state. So, the electronic distribution changes from the ground state to the excited state and when it is actually happening, it actually needs a change and this is actually defined it.

The probability of this change it is defined from $-\infty$ to $+\infty$. The state it is changing, so, it is the excited state ($\Psi^*$) and it is the ground state $\Psi$ and this ground state represented by $\Psi$ and it requires a dipole moment to change it. So, dipole moment change actually changes this electronic distribution over there it is affecting the electrical field. So, $\mu_e$ is actually, the

dipole moment operator and that actually goes in the x axis y axis or z axis because these are linear in nature.

So that is how the dipole moment actually, looks like a linear in nature. And that is actually responsible for a change in the electrical field and that is how an optical absorbance happens? This is the probability but if there is another probability that I can have, this is the electrical that my magnetic moment can be also coming into handy. So, this can also help to change from $\Psi$ to $\Psi^*$.

There is a magnetic moment operator ($\vec{\mu}_m$) that can help. Now, $\vec{\mu}_m$ has two issues one this is actually much weaker. $\vec{\mu}_m$ is quite weaker in nature because it is magnitude is actually much smaller compared to the electrical field and this magnetic moment how it looks like? It looks like a rotational axis along $\vec{R}_y$, $\vec{R}_x$ $\vec{R}_z$.

So, it looks like a rotational motion like this. So that is how it looks like for magnetic moment now say a magnetic moment operator and dipole moment operator both are active then what I am going to do? So, I am going to do a rotation, I am going to do and motion in this x axis. So, all together if I do this translation and rotation together, what I am going to get is a helical moment and once we go to a helical moment.

Now, I am chiral because this can have other motion also which is mirror image to each other but not superimposable and indistinguishable. So, this origination of helical movement is very important to have chirality in my molecule and that is what is actually happening in a molecule. So, a molecule which is asymmetric in nature which does not have any S n axis. So, in that molecule we are going to have this helical moment induced.

Because it will be magnetic moment operator active and electrical dipole moment operator active. And that is actually originates the chirality in a molecule and that is why this molecule interacts with the right hand circularly polarized and left hand circularly polarized light differently, either through optical rotation or ellipicity. But it does it because it has this intrinsic property of helical moment generating in that molecule.

So that is why we expect that now we have explained you, the basics of the city, the different applications of the city and you will be able to use CD spectroscopy in your future research or future job, wherever it is needed to understand the property of a chiral molecule or optical active molecule and find out it is different applications. So, thank you. Thank you very much.