Essential of Biomolecules: Nucleic Acid, Peptides and Carbohydrates

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Lecture -29
Spectrospic Techniques -II and Purification Technique-I of Biomolecules

Everybody and welcome back. So, we are discussing module 8 on some modern techniques that are being used in Chemical Biology. And in the last lecture, I have been talking about the spectroscopic techniques such as UV, fluorescence, IR and so on. So, I had given an overview about the principles of this spectroscopic techniques. What is the principle behind UV spectroscopy that it is for an electronic transition where the electron goes from the ground electronic state to the higher excited electronic state. On the other hand, if you consider fluorescence that is kind of an opposite to UV visible.

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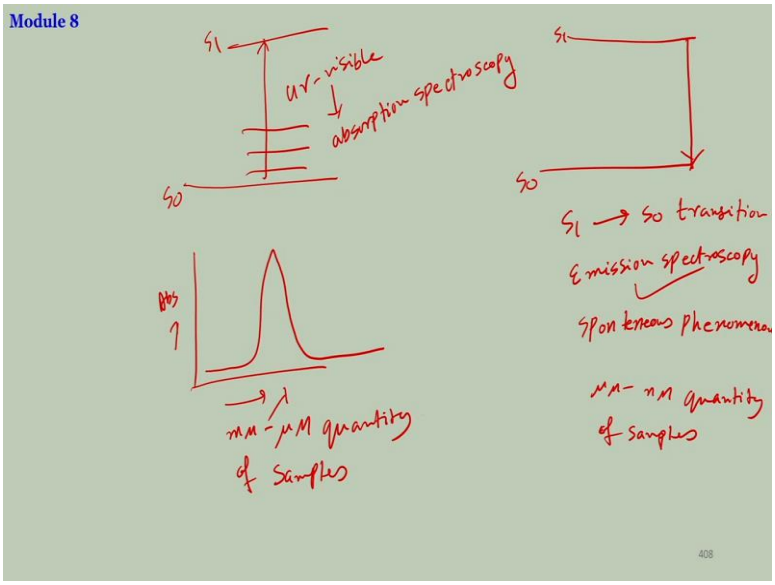

Because UV visible is the absorption that goes from ground state to the higher excited states, so this is for the UV visible spectroscopic, so it is basically which means the molecules are or the electrons are absorbing the light and going using that to reach the excited state. So it is basically an absorption spectroscopy. UV visible is an absorption spectroscopy. Fluorescence on the other hand is the opposite where it is an emission spectroscopy, electrons are coming down from the excited state.

So it is the excited state to ground state S 1 to S 0 transition. So, therefore, this is basically an emission spectroscopy. Since this is coming down that means the molecule will release

energy all are emit radiation and that radiation, you can see in fluorescence spectroscopy. And obviously, when something is coming down, it is a very favorable process, natural process. So, therefore, it is a spontaneous process.

Phenomenon said spontaneous phenomenon and since this is a spontaneous process very favorable process the signal intensity is very high that means the signal that you will receive from a fluorescence or from a fluorescent molecule would be much higher compared to the molecule which gives to the UV spectroscopy. In other words, you have to use very very small quantity of your material.

Very small amount of sample would be required to study or to see a good fluorescence signal. relatively larger amount would be required to see the UV visible signal and this is very important for bio chemical biology or biology. Because the amount of samples that you get for example, the DNA samples that you isolated the protein samples that you isolate, and you tag the other biomolecules.

The sales that you collect from the body or from some organisms, their concentration is very, very low and you want to spare minimum amount of it. So, as less as you can afford would be better for your experiments and they are comes that is why people prefer to go for fluorescence spectroscopy because it takes really less amount of samples in the range of even micromolar to nano molar quantity.

Quantity of of samples would be required, UV visible is also very sensitive, because you can see that since this is an electronic transition, all transitions are allowed no selection rules. So, therefore, the probability of transition in quantum mechanical terms the probability of transition is pretty high. So, therefore, this is also quite a sensitive technique and you can use millimolar to micromolar quantity of samples.

Very small amount of samples would be really very small amount of samples would be required to see a good UV signal also. This is the lambda value, this is the absorption. Now, this is about absorption spectrascopy and here is your emission spectroscopy. If you look usually if you have seen the UV spectra, you will always notice that it is usually a broad spectrum that you get you usually, the molecule absorbs a certain radiation.

So, therefore, it should have been quite a narrow spectra the peak area should have been less, but usually you see a broad peak area for UV signal the reason being that when you are getting electronic transition along with that, of course, I have not explained the Franck Condon principle that you can study so, all electronics transitions would be associated also with vibrational transition as well because they are all vibrational levels that are present within an electronic level.

So, along with electronic transitions you always have vibrational transition because they take lesser energy. So, this the specter that you get here is actually a coupled of the electron transition, coupled with some vibrational transitions that are usually called a vibranic coupling. So, that is reason to get a little bit larger area peak area. So, absorption spectroscopy emission spectroscopy. Now, coming to IR.

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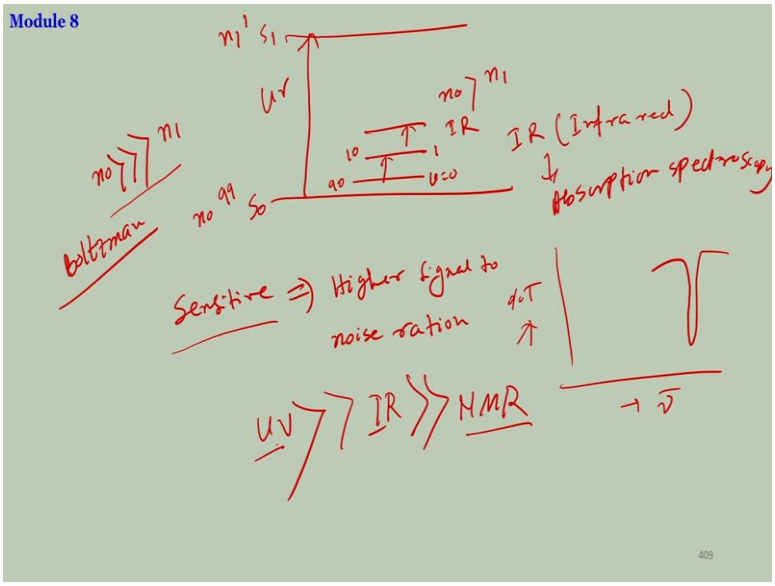

Infrared I have talked that infrared spectra is about the vibrational transitions that happened within the same electronic level. So, therefore, the energy is much lesser compared to an electronic transition if that is show and obviously, since this is a transition from lower level to higher level, this is also this is higher infrared this is also an absorption spectroscopy. The energy gap is much smaller compared to an electronic transition.

The spectra that you see for infrared spectroscopy is also a little bit spread up much less compared to an UV usually, but that is also is little bit usually studied the transmission spectroscopy so something like that, something like this you will get for the IR, here is the

percentage transmission and this is usually the wave number. So, that is in being again, that vibrational transition is associated with the rotational transitions.

So, vibrational transitions always will be associated with some rotational motions of the molecule that is the region for the larger peak area of that you see in IR spectroscopy. Now, I have a tricky question. If you compare the electronic transition and the vibrational transition, which one would require more sample, which one would be more sensitive. Sensitive means, higher signal to noise ratio.

The higher the signal to noise ratio is, in other terms, higher the signal intensity is you require less amount of sample and that would be a better technique that you want to afford, because you just a little bit of sample or just a little bit of irradiation will give you a very good signal that you can analyze properly. So, sensitivity means how sensitive the techniques are little bit push.

Or little bit amount of material will give you a good signal that is what the definition of sensitivity is? So, if you compare the electronic transition here the UV and if you consider the IR the question is which one would be more sensitive? You think of it that has to do with the basic principles. So, the energy gap for the electronic transition is pretty high which means the molecular distribution would be mostly here. If the n 0, if this is n 1 for example, then n 0 would be much higher than n 1.

According to Boltzmann distribution law. So, your homework is finding about the Boltzmann equation. There, you will find that if your energy gap is high, then the number of molecules that would be present in the higher excited state would be very negligible. In other words, the number of molecules present in the ground state would be much higher compared to almost nil excited state molecules.

And if there is no molecule in the excited state, obviously, this transition would be fragile favorable compared to here, the energy gap is much less which means the number of molecules present in the ground state would be highered compared to the number of molecules present in the excited state, but the gap is much less compared to this, which means there is a fair number of molecules that will exist in the excited state.

If there are 90 molecules present in the ground state at least there will be 10 molecules present in the excited state. On the other hand, here, maybe 99 number of molecules would be in the ground state and 1 or almost 0 number of molecules with the excited state. So, population distribution is much less here, more here. So, there are a fair number of molecules that are existing in the excited state.

So, the transition probability or intensity of transition would be less if the higher excited state is already populated, the transition will not be much favorable. Therefore, the sensitivity of IR would be much less compared to UV. For UV is much more sensitive compared to IR. Similarly, if you look in NMR, in NMR energy gap is very, very small. If you see in terms of the energy, they have a very small difference of energy in the ground NMR state and the excited on spin state.

And therefore, in NMR is wildly sensitive, you need much more amount of sample to study on NMR, on the other hand, you need very, very small amount to sample for UV relatively larger amount of sample for IR, so, this is about spectroscopic techniques.

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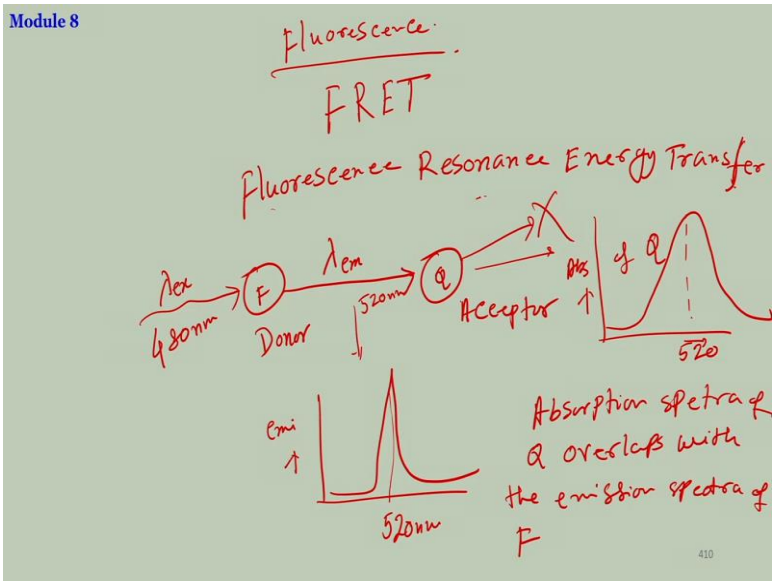

Now, I will move on to some separation techniques. One thing is left over that I should mention about the spectroscopy that I told that fluorescence spectroscopy is being widely used in chemical biology to study what is happening if you want to even see in real time something where bio molecule is getting in into the cell or in which molecule it is going and interacting with, you can study by tacking a fluorophore or a fluorescent molecule to your target molecule.

Now, there is one important aspect that is known as FRET which is very oftenly used in Chemical Biology FRET means Fluorescence Resonance Energy Transfer which basically means the fluorescence energy that it will come out from a fluorescent molecule will be transferred to another molecule. So, therefore, there will be shifting or there will be further absorption of the fluorescent energy by the second molecule.

I will explain and this has been a method to study many biological interactions. So, if you have a fluorescent molecule here, which we call would term as donor D If you have another molecule that is called the acceptor or that is called quancher which is actually an acceptor, donor if it is a fluorescent molecule what you will do? You will excite with a certain wavelength that we call the excitation lambda ex is a fixed wavelength that you know that this molecule would be excited to the higher electronic state.

And then it usually would emit radiation that is the fluorescence radiation, fluorescence emission which is known as lambda emission. So, I had given an example that, if this is flourishing for example, then you can excite it with 480 nanometer light and then you will see a spectra hear which is this maximum is about 520 nanometers, this is emission. So, this would be roughly about 520 nanometer would be the emission energy.

And you should see a bright fluorescence coming out of this molecule usually a green light green color this molecule will show now, that is the property of the fluorescent molecule or the fluorophore. Now, FRET means, you have a second molecule placed somewhere very close to this molecule and this molecule has an absorption spectra which is very close to 520. So, this is an absorption spectra of Q, UV visible spectra.

And UV visible spectra shows kind of a maximum or it falls into the idea where 520 nanometer light is there which means if this light it is this molecule, it is the quencher molecule or the acceptor molecule, then this energy would be absorbed by it, because it can absorb your absorption shows that it can absorb this radiation. So, the emitted light from the fluorophore would be absorbed by the acceptor molecule.

Then what will happen? now, if this is not a fluorophore, if the quencher is not a fluorophore then what will happen? you will not see any light after you excited the fluorophore this has

emitted, but that light has further been absorbed by the Q. Therefore, nothing is coming out of it. So, when these molecules are placed together very close in proximity, then you cannot see the fluorescence from the fluorophore.

That is called the fluorosense energy has been transferred to the acceptor. Why is it called the resonance energy because, of course, the absorption spectra of the Q overlaps with the emission spectra of F. So, the emission spectra of F will overlap with the absorption spectra of Q. Therefore, all the emission energy would be in resonance with the absorption energy with the absorption spectra of Q and it will be transferred to Q and Q does not emit anything. So, ultimately you see a dark thing. So, how is it applied in biology? For example.

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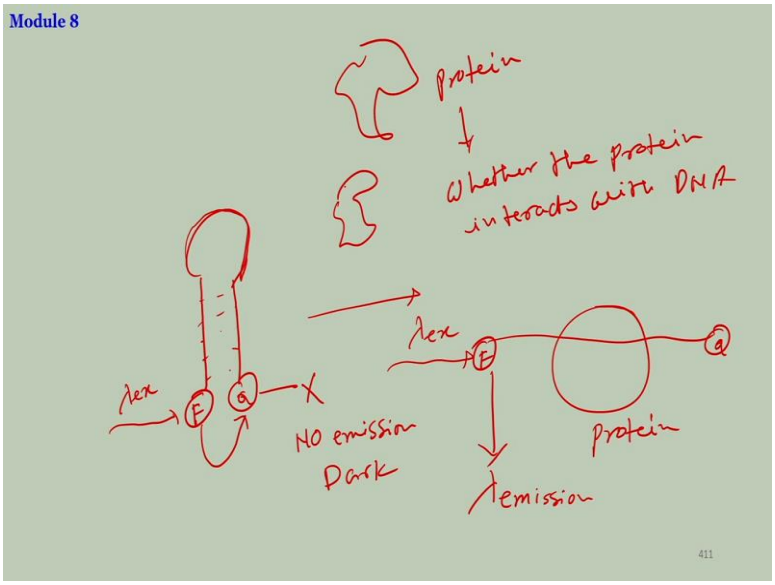

You have a protein and you want to see how this protein interacts with the DNA, whether this protein interacts whether the simplest case the protein interacts with DNA. One way to know it by FRET is if you take a DNA having the hairpin shape, self complimentary that we have seen before and this part contains the sequence where the protein is supposed to be bound. I attach a fluorophore here, I attach quencher here.

So, what will happen? When I have not added any protein, when this molecule is existing all alone, then what will happen? If you excite this the fluorescence energy would be transferred to the quencher and there will be no emission or in other words, dark, nothing will come out if you study a spectra to study a fluorescent spectra, there will be nothing there, because all emission have been absorbed by this.

Now, if you add the protein, and if the protein interacts with the DNA here, what will happen protein in order to interact, it needs to bind with the DNA. If this is the sequence it binds, then it will force because the protein DNA binding protein binding is usually pretty strong binding. So, that binding force would be enough to cleave the hydrogen bonding base pairs here, because protein has to dock into this it needs space, so it will force the hairpin to unwind.

So, your DNA would be unwind to a chain, here would be your fluorophore. Now, here would be your quancher now, and here would be your protein. Now, what will happen? This fluorophore and quencher are far away from each other. And therefore, if we excite now, as you have done before the energy cannot be transferred, it is too far. And you see, you can see now emission spectra very well. So, you can see the fluorescence now.

So, if you do a time dependent study, after the addition of the protein, you will see as slowly your fluorescence is increasing. Because the over time the protein is interacting more and more with the DNA that forces the DNA to unwind that separates the fluorophore and the quencher far away. And therefore, you see a fluorsense signal coming in. So, that is what is the use of FRET. FRET is used based on the separation between the donor and the acceptor. There are many varieties that you can use this thing about. So this is one of the example.

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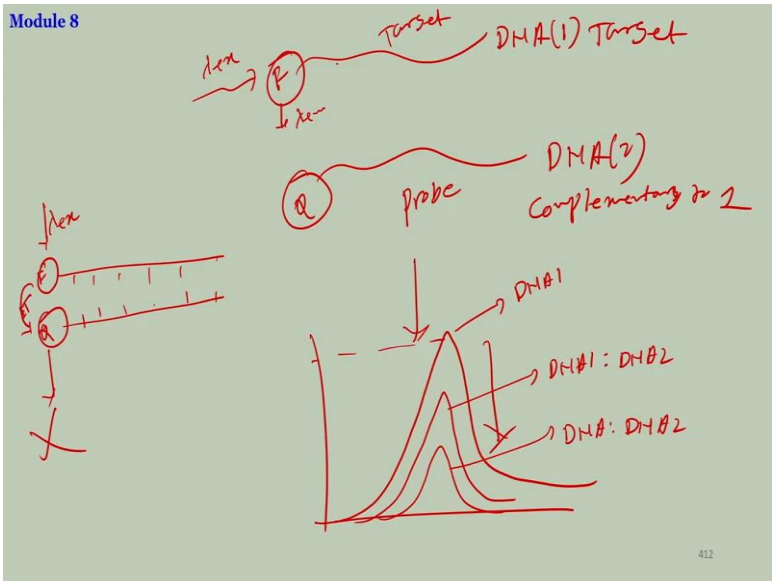

Moreover let us say you have a DNA and you want to see whether this DNA would be paired of with another DNA. DNA 1 DNA 2 whether they have mismatch or whether for example, this DNA is supposed to be complemented with other DNA. You want to find out whether

you have any mutation or not less this is your target DNA sample that you have isolated from a disease sample and you want to see what is the cause of the disease.

Whether it is from any mutation that has been present in the DNA. So, your original sequence of the DNA is known and you fabricate and other DNA which is supposed to be fully complimentary to the target. So, this is complimentary to DNA 1. Therefore, if you mix them together, they are supposed to form a double helix perfect double helix. Now, how would you read that?

Whether a double helix has been formed or whether the double helix has not been formed? How would you read that, how would you analyze it? How would you see it, how would you detect it? If there is any mismatch, then it will not form the perfect double helix there would be some weak interactions therefore, the helix may not be formed under your suitable experimental condition. So, one way to study that you attach a fluorophore here in your target, there are ways to do it.

Similarly, you attach a quencher because this you are fabricating, this is your probe. This is the target. If you want to study the target, you need a probe that can detect it. So, this is your probe. Now, before addition of your probe, if you only study the fluorescence of your target DNA, you should see emission because this is a fluorophore, so it is should show you fluorescence. So, let us say you have fluorescence. This is DNA 1 only. Now you add your probe with this.

If they are perfectly complimentary, what will happen? They will form double strand, here will be your fluorophore for here would be your quancher. So, if the double strand is formed perfectly, the fluorophore and the quencher are now in close proximity. Therefore, your emission spectra or emission energy, this is called energy transfer would be transferred to the quencher quancher absorbs everything and emits nothing. So, there will be no emission ultimately.

So, if they are perfectly complimentary, then over time, you will see the reduction of fluorescence this is DNA 1 : DNA 2, this is also DNA 1 : DNA 2 time dependent after some time you will see that everything is vanished there is no fluorescence, then you can say that

the probe this DNA is perfectly complimentary to this and there is no mutation in it. If there is a mutation here, then the perfect hybridization perfect double helix will not be formed.

And even if you mix them together, you will see still see this and these are just existing in your solution like 2 different molecules and there will be no interactions between the fluorescence and the quencher and the fluorescence will not be reduced. Then you can say that there is mutation in it. So, like that there are many different ways to study, there are varieties of ways you can also develop on your own. So, FRET based studies are very popular in Chemical Biology.

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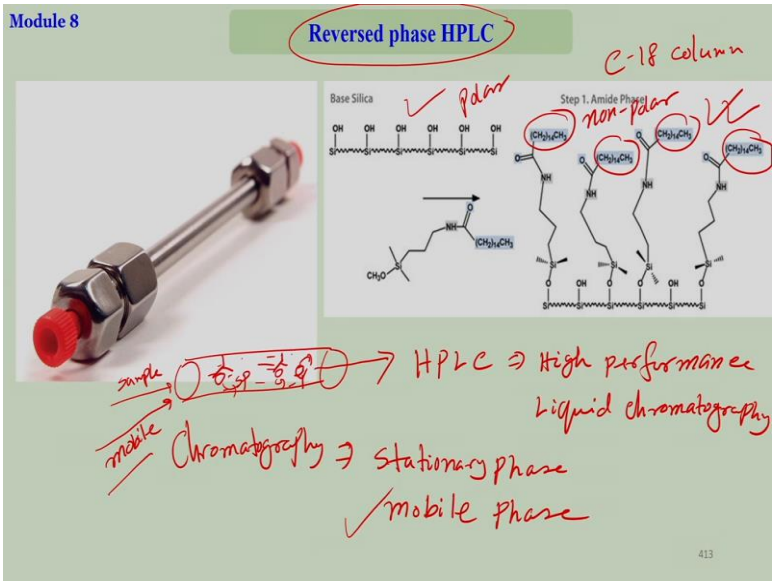

Now, we will discuss some separation techniques. So, spectroscopic techniques, we have talked about, now, some purification and separation techniques that are used in if you have large biological macromolecules such as proteins such as DNA, such as carbohydrates or even the smaller biological molecules, then how can you separate them or how can you purify them, they of course, these are very, very important.

Because the normal organic chemistry separation tools many times you cannot use like column chromatography that you do usually in organic chemistry laboratory cannot be done here. Number 1 is your molecule is pretty large in this case. Secondly, the amount of sample that you have is pretty small. So, you need something a little bit sensitive techniques or sensitive separation techniques for such cases.

So, one of these is reversed phase HPLC or in other terms, the first the HPLC high performance liquid chromatography. HPLC are known as high performance liquid chromatography is basically a chromatographic technique and a chromatography means the basic principle of chromatography is that it has 2 phase apart from your sample the basic structure or the operator setup requires 2 phases.

One is called the stationary phase that is present in all chromatographic techniques, including the column chromatography that you do in organic chemistry laboratory, stationary phase. And then of course, you have a mobile phase the idea being that sample when you are injecting into the chromatographic tube it interacts with the stationary phase and then it gets stuck your molecule or the sample gets stuck to the stationary phase.

And this interaction varies from molecule to molecule. So, if you have multiple molecules with different physical properties, their strength of interactions or their binding property or their binding strength with the stationary phase would be different. So, but nonetheless, so, the idea basic idea is the molecule will interact with the stationary phase and be bounded there. Then, obviously, you do not get back your molecule because it is bound to the stationary phase.

Then you required the mobile phase. The idea is the mobile phase when you push through the column then the mobile phase will break that interaction between your molecule and the stationary phase and the mobile phase, if the mobile phase breaks the interaction, then your molecule comes down again. So, you get back at the end of the chromatography. If you have a column here.

This is full of stationary phase SP which does not move solid thing that is packed inside. Then you have your sample first injected and sample would be bound. This is your sample, this is your stationary phase sample is bound. This is your stationary phase, your sample is circle bound to the stationary phase weak interactions. Now, you push the mobile phase some liquid through these to the column and this liquid has the nature that it will break this interactions.

So, slowly your molecule will come out of it molecule will come out your molecule will come out and they will ultimately eluted be eluted out of the column and you can collect. So,

if you have impurity, if you have mixture of molecules, their physical properties would be different and their degrees are binding would be different. So, their binding will be broken also in different times or different strength of the mobile phase.

So, you will see that some molecules, the molecules which are strongly bound will come later, the molecule which have very weak interaction will come fast. That is how you could separate into molecules such as the HPLC Only thing is that it works with very less quantity of material and it works the time required for this to use this choromotography technique can be varied, it can be very short time to you can make it even lengthy and the performance is high.

So, there are many types of HPLC techniques, one of the most leveled HPLC technique is called the reversed phase HPLC. So, as have said like all chromatographic technique, it has a stationary phase, this is a column typical column or a typical reverse phase chromatography column that it looks like this and inside this column the solid material is packed and this is the solid material is a silica kind of compound polymeric chain.

That is for the normal phase reversed phase is that this has been modified. This is actually the material that is packed here. If you see the terminal it has a long carbon chain, this is 14, 16, 17 and including this is 18. So, it is a basically C 18 column. There are variation of chain length. One typically whose column is called C 18 column. So, long carbon chains are at the end of the material. So that is packed inside this column.

So, what is the property that if you have a long carbon chain, that means the stationary phase is hydrophobic is highly hydrophobic in nature. Why is it called reversed phase? That is why actually it is called reversed phase. The basic stationary phase originally was the silica and silica has hydroxyl groups. So, this is polar and you have modified the silica to make it non polar. So, you have changed your phase from polar phase to the non polar phase. So, that is why it is called the reversed phase. So, ideally now, your reversed phase HPLC column has a hydrophobic side are it inside it then what will happen?

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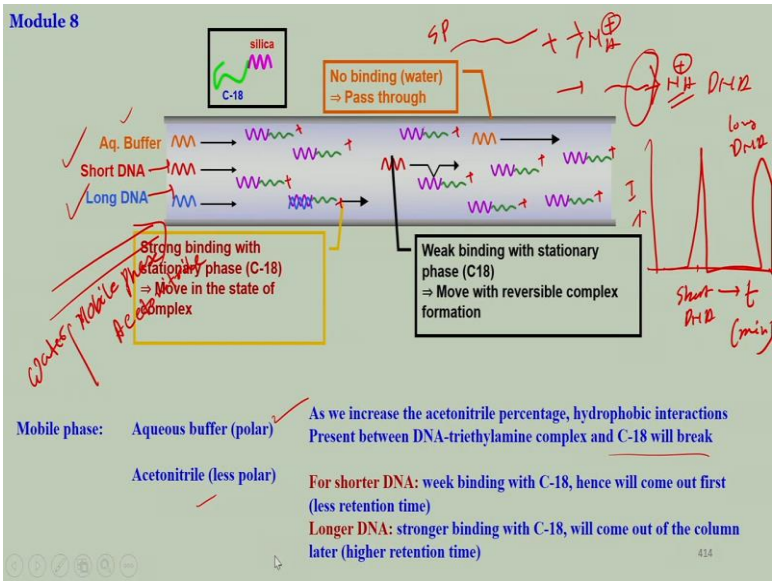

Now, this is your stationary phase, this is a silica this is the chain carbon chain, carbon chain, carbon chain. Now, usually what happens so, it is the hydrophobic one you have a hydrophobic chain carbon chain that is your stationary phase. Now, you use a certain liquid triethylamine usually and that has been made into positive charge. If this is your column you first inject with triethylamine into the column, then what will happen? This tool interact and will give you.

So, hydrophobic will interact with the hydrophobic part you will get this. So, now, at the end of your stationary phase you have positive charge NH+ NH+, you have positive charge So, all these are now positive charged. Now, if you want to purify for example DNA, here I have given the example of DNA, you have a short DNA, you have a long DNA and as a control you just have nothing, only the buffer water maybe.

So what is the difference between the short DNA and long DNA? Short DNA has less number of negatively charges phosphates. Long DNA has more number of negatively charged phosphates. Negatively charged which means when we inject the short DNA and when you inject the long DNA what will happen? Short DNA has less number of negative charge. So they will interact with the positive charge through a weak interaction.

On the other hand, long DNA has more number of negative charge. So the interaction with this positive charged would be stronger and that is the difference and the buffered will not interact at all. Now, so, when you inject your sample mixture of sample which has 2

components, one is short DNA, one is long DNA, 3 actually and you have also have the water the buffer you send it through the column.

And they will interact with your stationary phase short DNA would be stuck but weakly stuck, longer DNA would also bind, but strongly bind. Now, they are inside this nothing is coming out. Now, we have to take them out. Now, you inject the mobile phase and the nature of the mobile phase has to be such that it will break those interactions. If you use this kind of scenario, then acetonitrile is such kind of solvent.

Usually use a mixture of water versus acetonitrile, water is polar acetonitrile is nonpolar, a little bit less polar. So, it will try to break that interactions. Now see what happens? Buffer is this is. So, your interaction is basically here. If you want to detach your DNA, you basically have to cleave the hydrophobic interaction only then, your molecule your DNA which was bound here with this would be detached from the stationary phase.

Cleaving this bond will not matter much cleaving this bond here or this not bond this interaction here would take everything out. So, your idea is to break the hydrophobic interaction that is present in this case or nonpolar interaction, water is polar. If you inject water, nothing will come out. Now, if you use a little bit nonpolar solvent, now, it will start breaking the interaction. It will look like this.

Buffer no interaction comes very fast, shorter DNA with less interaction will interact. Then unwind, bind, unwind, bind unwind, it will move fast, relatively slower. It will be slower than that than the buffer but faster than the long DNA. Long DNA on the other hand would be bound strongly therefore it moves slowly. So, that is the basis of the revest phase HPLC stationary phase I have shown you mobile phase usually the aqueous buffer.

And then acetonitrile as we increase the acetonitrile percentage, hydrophobic interactions present between DNA and triethylamine complex and the C 18 will break for shorter DNA weak binding with C 18. Hence, it will come out first it is called the retention time. So, if you make a plot you will see. So, here is your short DNA this is the time this is the intensity, this time t in minute. It will come out first.

So less time and long DNA would come out or it out later or with higher retention time. So that HPLC is one way of purifying biological macromolecules. So, long DNA long protein chains can be separated or even carbohydrates can be separated using the HPLC. Smaller molecules also can be separated by using HPLC. We mostly use them for purification, because most of the times for bio logical chemistry.

If you want to study those certain phenomena or any experiment that you want to do in Chemical Biology, they are the samples or they are the probes, whatever you call them, has to be utterly pure. Otherwise you get lots of ambiguity their. results. So, in order to make sure that your compounds are purified, your molecules are highly pure HPLC is one of the best technique to make the compounds pure.

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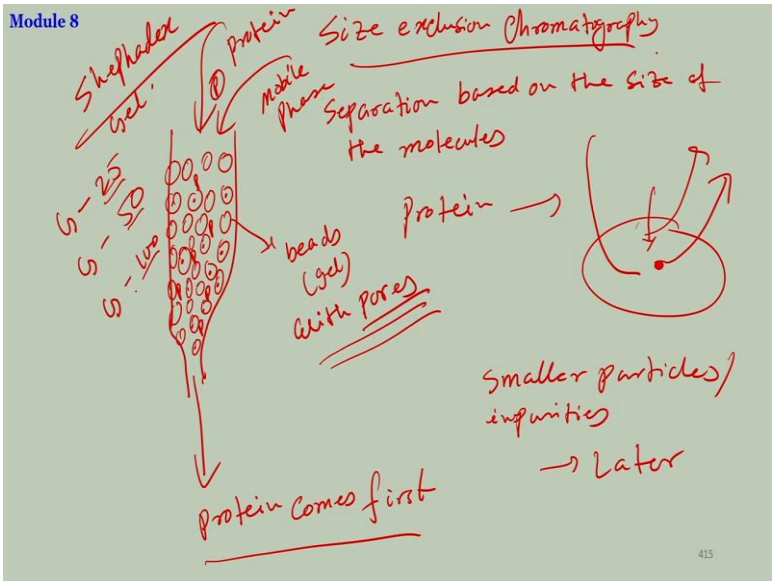

Second one of purification or separation is called size exclusion chromatography. Size exclusion chromatography is also a chromatographic technique where you separate the molecules based on their size. So, HPLC was used to separate the polarity that the principal was that molecules with different polarity can be separated. In this case, the molecules with different sizes can be separated based on the size of the molecules.

This is mostly used to purify the macromolecules large polymeric molecules such as proteins. If you have protein, protein usually have very large size and very high molecular weight 60 70 80 100 200 kilod dalton molecular weight very large proteins. And when you isolate proteins, they are usually associated with a lot of non protineous molecules which are small molecules or sometimes even the fragmented short proteins that you do not desire.

You want to separate you want to purify your protein of interest which has a large size and in that in those cases size exclusion chromatography is highly useful. So, this is a column and you pack the column with beads. columns are packed with beads or materials, usually they are gel materials and since they are beads or they are gels, they have pores with pores which means they have cavity like molecular sieve you know, molecular sieve has pores inside it.

So, they can trap materials. Similarly, these kinds of beads have pores in them. So, they can trap materials inside them and you can get them in different sizes predetermine sizes, know what happens, you add just simply think of that you are trying to purify a protein from its impurities there are small molecules there, which has maybe 50 dalton molecular weight which is very small from the 200 kilo daltan molecular weight which is very high.

So, you inject your sample in it, what will happen if you inject your sample then the small molecules which are there would be trapped inside those pores because the pores are small in size. So, the smaller molecules can get into the pores the larger molecules are too large to be accommodated in the pores. So, they will not get into the pores. So, the protein I call it P now, would be here, not into the beads just outside the beads.

So, that is their interaction pattern. Now, you inject certain mobile phase, mobile phase means solvent and solvent is large in quantity to push through what will happen whatever was not inside the P will come out immediately if you push something they will flash out basically. So, will protein comes first because they were not trapped inside the smaller particles or impurities they are inside the force.

So, now when the solvent is getting in, solvent has even smaller size almost if you consider water if you consider acitonitrile their molecular weight is very, very small and they are large in quantity. So, they will get into the pores, if this is the bead this molecule support solvent are coming in, they will knock it out over time. So your protein will come out first, and then eventually you solvent we will go and we will knock the material which was inside out.

So it will come later much later and you do not care if it is impurity you do not care because you do not want them. You have your protein first at the beginning. Now, if you have various sizes of proteins that you want to purify, that can be done here itself because, according to

sizes, they would be trapped. The smaller sizes would get deep inside the bead little bit larger. We will go to some extent but may not get into fully.

So, whenever your solvent is coming in, the one which was not attached properly, will go first and then the smaller size will come out. So, it will be a size distribution. So, your separation would be based on the size higher size will come out first, laser size will come out later that is a very good way to separate the macromolecules which have large sizes usually and depending upon your requirement, you can choose which pores you want for your use.

One typical material that is used for size exclusion chromatography gel is called sephadex. You can look up it is structure, it is a shephadex gel and it comes in different pore sizes G 25 G 50 G 100 these are means that they are I guess it this angstrom but I am not writing I am not pretty sure these are the pore sizes actually. So, 100 means probably 100 angstrom. So, you can use that for larger molecules.

If it is 25 then that means you want to use it for relatively smaller molecules something like that. So, according to your need or according to the samples that you have, you want to purify you can choose which one would be proper for them. So, this is about the chromatographic techniques that you use for the separation.

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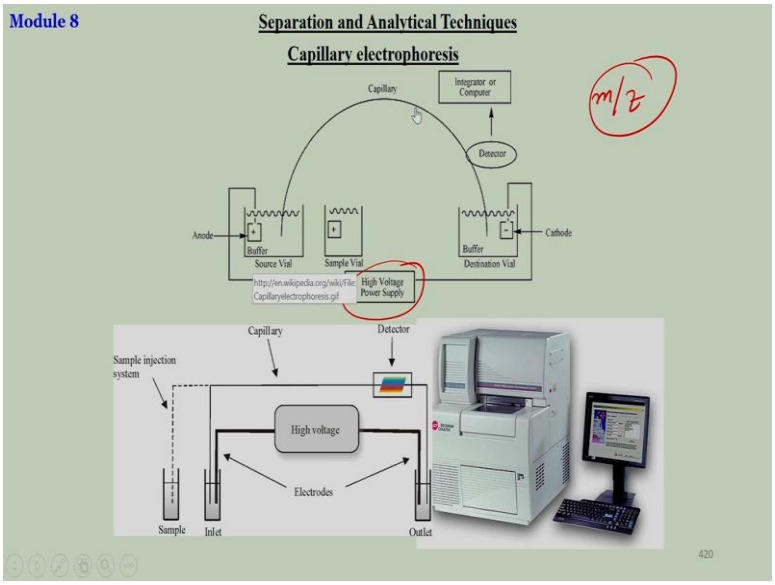

Another days very good way to separate compounds is the capillary electrophoresis I think you have heard the name. Capillary electrophoresis is also a separation techniques, but based on the charged species. So it works In terms of m / Z value charged species and you can

induce the voltage across the capillary. So, basically you make 2 electrodes one is anode own is cathode, anode and cathode are nothing but your buffers in it according to their ionic salt.

So, you can make it one positively charged, one negatively charged basically they are the buffers and depending upon their concentrations and the charge, you can make them different and you dip a capillary into them and end of the capillaries there are electrodes in it. And so, one electrode would be positive depth here that will give you the positive charged. Other one would be the negative charged and here is your sample. So, you first fill your capillary with the buffer So, it will be full of the charged particles and then you inject it into your sample Then you place a voltage across this capillary, this is a typical instrument.

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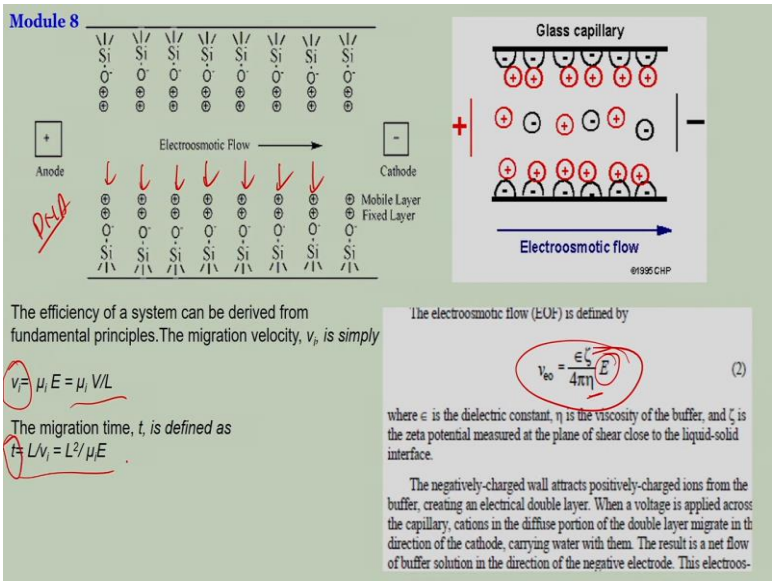

What will happen? So, this is the capillary wall is made up of silica, that is o – o – o – o - this is the other one. Now, you inject your buffer, buffer is basically salt. So, they are positive charge, they will form a complex system here. So, basically after you inject your buffer, your capillary is filled and it has positive chaged in its surface. Now, you inject your sample for example, DNA. DNA has negative which has phosphate.

So, they will be bound here, they will interact here. And again if you have a mixture of DNA, shorter DNA will have less number of phosphate groups say they will interaction would be weaker, longer DNA will have stronger interaction. Now, if you create a voltage, if you send a voltage across the capillary, then everything will start moving electro osmotic flow plus positive charge to the negative.

Or it can be other way around also depending upon what you want, you can make it reverse you can make it plus you can make it minus also. So, everything now is moving stronger the interaction will move slowly, we can interactions will move first. So, that is basically the point of it. This is the equation where you can calculate the electroosmotic flow, this is the zeta potential, this is the viscosity this is the electric field which means basically the voltage that you have supplied.

The velocity electroosmotic flow is associated with the ionic mobility or ionic velocity which component will move in which speed of the molecule that would be moving across the capillary, velocity would be governed by this equation, which comes from here. So, you can ultimately create an equation in terms of time. So, basically, the longer DNA will come out later or will take will be will have higher retention time, smaller DNA will have lower retention time mostly for the charged samples. So, such as DNA.

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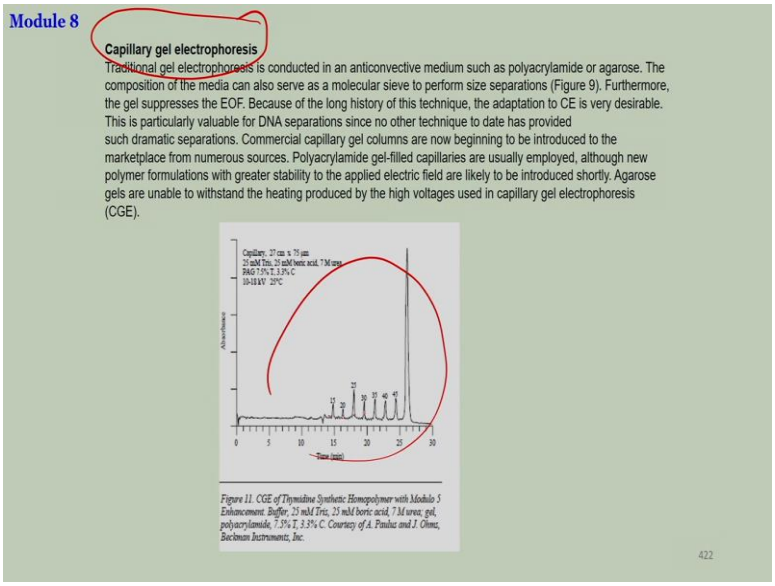

This is a variation of capillary electrophoresis which is called capillary gel electrophoresis where, instead of the simple buffer you fill the capillary with gel. Of course, the gel has to be conductive gel, such as the agarose is a conductive gel because it has to take their flow the current inside it, why gel, because gel makes the mobility slope. So, you can get a better separation technique that is the difference between the normal capillary and the capillary gel electrophoresis.

Here you can see using the gel electrophoresis you can separate Thymidine Synthetic Homopolymer, a polymer of t only for thymine can be separated this is 15 molar length, 20

molar length, 25 molar length, 30 molar length and so on. So, very small difference in their in DNA length can be separated very nicely using the capillary gel electrophoresis. So, this is all about capillary gel electrophoresis and with this I will conclude today is lecture. Thank you.