Experimental Biotechnology
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Module No # 04
Lecture No # 17
Ion-Exchange Chromatography (Part 1)

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(Video Ends: 01:00)

Hello everyone doctor Vishal Trivedi from department of biosciences and bioengineering IIT Guwahati. And in our previous lecture what we were discussing? We were discussing about the basics of the separation techniques as well as then we started discussing about the different aspects related to the chromatography. And in that context we have also discussed about the protein purification system and then we have shown you a small demo how to operate a typical chromatography system.

And what are the different steps or the precaution you have to take and following that discussion today we are going to discuss about the chromatography techniques. So why and how we can exploit the different chromatography techniques because the protein what we are going to purify or what we are actually going to purify in this course is having the different properties. So let us see what are the different properties protein a typical protein molecule will have.

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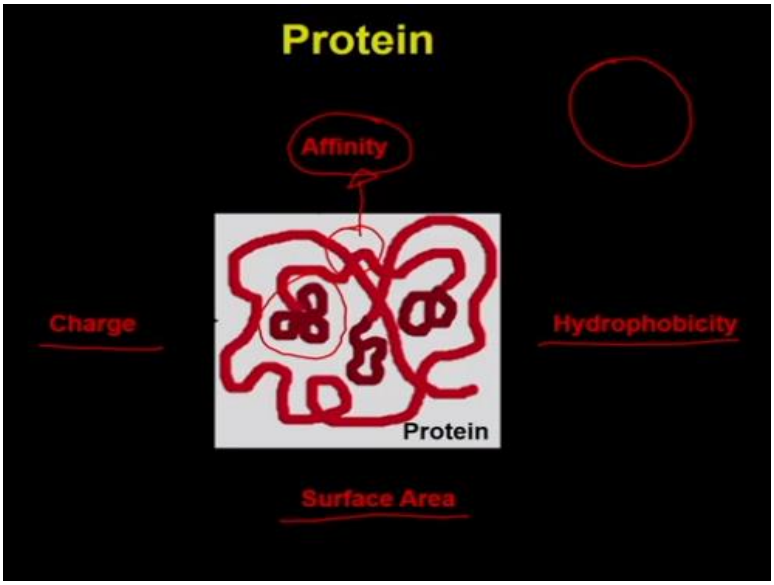

So if you see that the protein molecule so protein molecule is normally been synthesized as a linear chain of amino acids. And as soon as the protein is synthesized or a small polypeptide is been synthesized from the ribosome. It is start going through the process of folding this folding is always been governed by the intra molecular interaction between the side chains which are present on the amino acid.

And as well as there are additional forces which are actually playing crucial role in folding a protein into a property dimensional conformations. And once the protein is been folded properly into a 3 dimensional confirmation what you will see is that it is going to arrange all the amino acids in such a way, so that it is going to have the many different types of properties which can be exploited in different types of chromatography techniques.

So let us see what are these techniques what are these properties? So the protein which is going to be folded properly is going to secure its hydrophobic surfaces or hydrophobic amino acid groups inside the core whereas it is actually going to keep charge on the surfaces so you have the 2 properties one is the charge which you can exploit and then you can also exploit the presence of these hydrophobic patches.

Apart from that the protein is also maintaining a 3 dimensional confirmations and these 3 dimensional confirmations 3 dimensional arrangement of the amino acid is also forming a small ball like structure and these ball like structures have a definite surface area which is actually corresponding to the length of amino acid or indirectly to the molecular weight of the amino acid. So that surface area is also can be a property which can be exploited in a chromatography technique.

Apart from that the amino acids which are been arranged in the protein structures are also providing the specific 3 dimensional confirmations and these specific 3 dimensional confirmations are been utilized is providing the affinity or the biological affinity to the cognate receptor or the ligands. And that can be also exploited in a affinity chromatography so let us see what are the different chromatography techniques you can use to exploit these properties.

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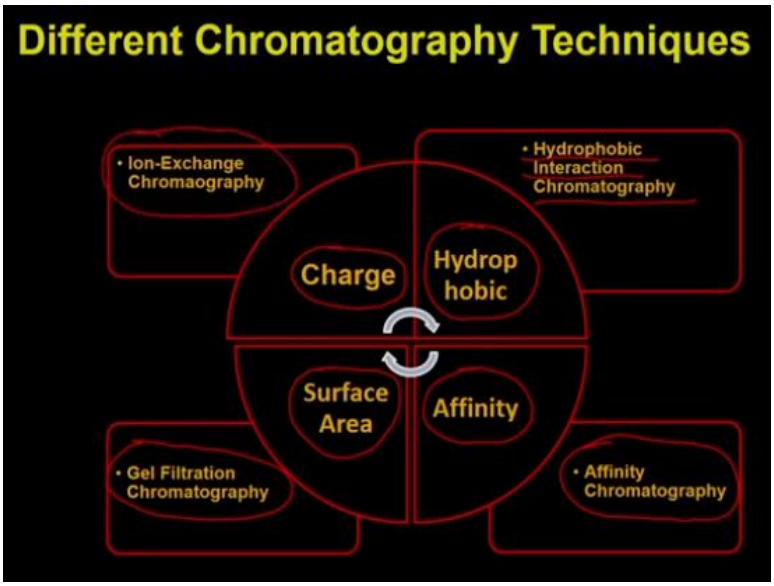

So as I said you have the hydrophobic core that hydrophobic core can be exploited in a technique called as the hydrophobic interaction. Chromatography similarly you have the affinity parameters or the affinity regions on the proteins and then we exploited in affinity chromatography. Then you have the surface area as the property and that surface as the property can be exploited in a gel filtration chromatography.

And lastly you have the charges different type of charges present on the protein either the positive charge or the negative charge and that can be exploited in a ion exchange chromatography. So today we are going to discuss about the ion exchange chromatography.

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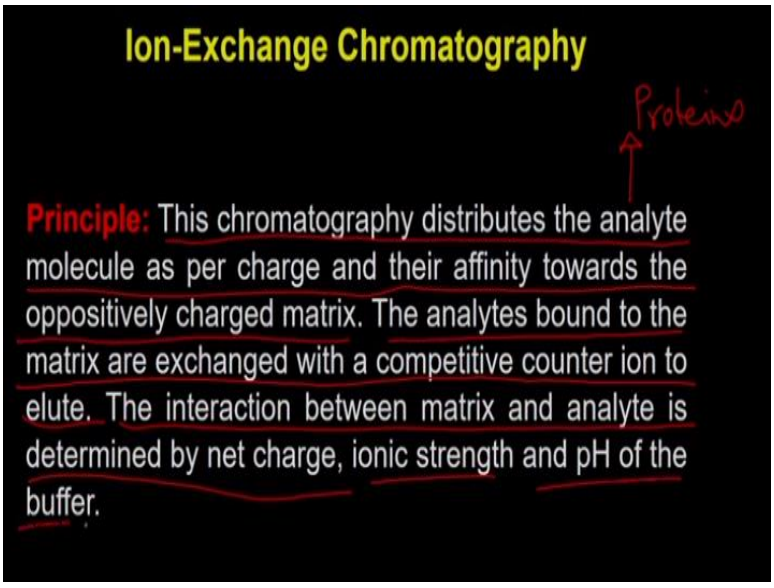

So what is the basic principal of the ion exchange chromatography this chromatography distributes the analytes which means the proteins. So different type of proteins as per the charge and; their affinity towards the positively charged matrix; the analyte's bound to the matrix are exchanged with a competitive counter ion to elute. The interaction between matrix and the analyte is determined by the net charge ionic strength and the pH of the buffer.

Which means this particular ion exchange chromatography is actually going to exploit the intrinsic charge whether it is positive or the negative present on the protein and it interaction with the opposite charges present on the matrix. So in a process what happen is if a positively charge protein is their it actually going to replace the counter ions and then counter ions are and the protein is going to bound. And this binding is going to be affected by many parameters such as the pH of the buffer, the ionic strength and all other parameters.

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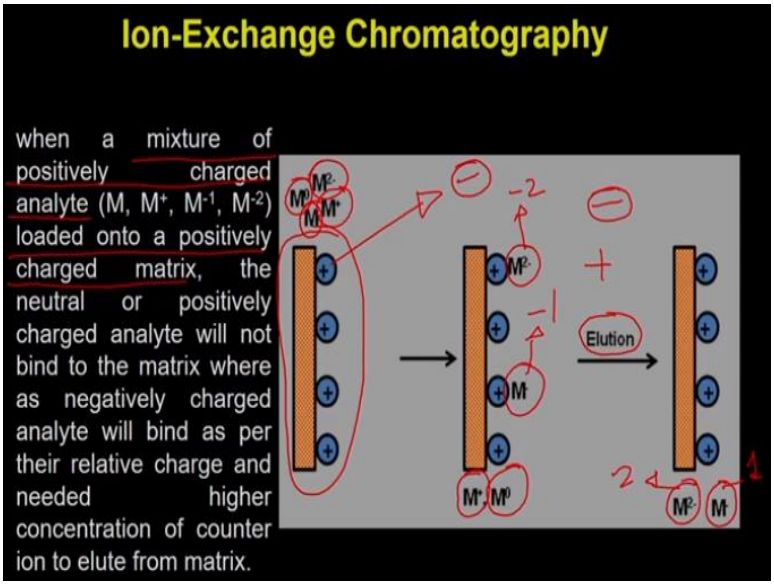

So let us see if you take a example let us see what we have is we have a positively charged matrix okay. So if you flow a mixture of the positively charge analyte's for example you have the 4 analyte's one is called M0, M-1, M+2 and M2-. So what you have is you have the 4 analyte's and you are flowing them on to a positively charge matrix which means this positively matrix is going to have the affinity for a negatively charged group.

So as soon as you flow this 4 molecules so you see that you have the M2-. M- and M0 so M0 means the protein which does not have the charge it is a neutral protein M+ means it is a protein

which as the basic charges and then M-1 or M2- is having the negative charges. So in this process what will happen is once you flow the this complex mixture the M2- as well as the M- is going to bind the matrix whereas the M+ which is actually going to ripple the bound charges and M0 does not have any charge.

So it will also not going to bind the matrix now ultimately what you can do is you can flow the negatively charged ions and that actually is going to displays these M- or M2- as ultimately you are going to see the separation of the 4 different types of protein 1 the 2 proteins are not going to bind in the matrix. Whereas the other 2 proteins which are binding to the matrix; is having the differential affinity.

Because this particular protein as the -2 charge whereas this particular protein as the -1 charge so the when you going to elute with the help of the negatively charged counter ions. The M-1 is going to be elute first so this one is going to be elute first whereas this one is going to be elute later on. Because both of these have the differential charges and they will going to have the differential affinity matrix.

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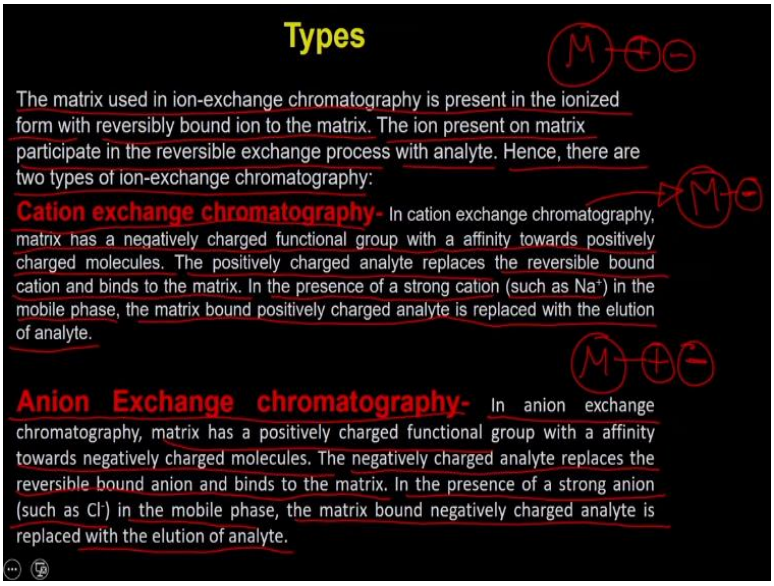

Now what are the different types of matrix which are available for performing the ion exchange chromatography. The matrix used in ion exchange chromatography is present in the ionized form with the reversibly bound ion to the matrix. Which means; if you have a matrix it is actually

having the positive or the negative charge so it is going to have a positively charged and then it is going to have the counter ion which is negative ion.

The ions present on the matrix participate in the reversible exchange process with the analyte hence there are 2 different types of ion exchange chromatography one is called cation exchange chromatography the other one is called as the anion exchange chromatography. As the name suggest the cation exchange chromatography is the chromatography where matrix as a negatively charged functional group with a affinity towards the positively charges molecule.

Which means the cation exchange chromatography; the matrix is going to be collected to a negatively charged group. And then it is going to have the bound positively charged counter ions. The positively charged analyte replaces the reversibly bound cation on the to the and binds to the matrix which means when you flow the positively charged proteins it is actually going to displays the reversibly bound ions which means the cation in the case and it will bind.

And then ultimately what you going to do you are going to flow a strong cation such as the Na+ in the mobile phase and as a result, what will happen the matrix bound positively charged analyte which is the protein is going to be displaced and that is how the analyte is going to be eluted. Now in the anion exchange chromatography it is actually going to be completely reverse so in the anion exchange chromatography the matrix has a positively charged functional group.

Which means the matrix is going to be connected to a positively charged functional group which is actually having a counter ion of negatively charged anions. So it has affinity towards the negatively charged molecules the negatively charged analyte this replaces the reversibly bound anion and bound to the matrix. In the presence of a strong anion for example the chloride in the mobile phase matrix bound negatively charged analyte is going to replaced and that is how the analyte is going to be eluted from the matrix.

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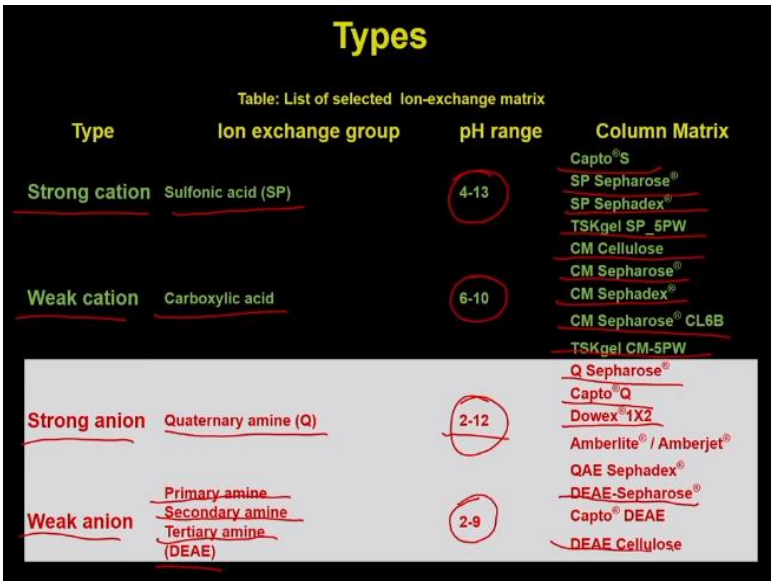

There are different types of the cation or the anion chromatography column or the matrix what you can use these are actually the either the week matrix or the strong matrix. The weakness or the strongness of a matrix is depends on the type of functional group or type of analyzable group present on the matrix. And that is how they are been classified either as the strong cation exchanges or the weak cation exchanges. So let us see what are these molecules so in the strong cation exchanges you have a function group which is called as the sulfuric acid or the SP.

The pH range where you can work with these particular type of cation exchange is 4 to 13 the examples are the SP sepharose, SP Sephadex all these. Similarly you have the weak cation is containing the carboxylic acid as the functional group which is present on to the matrix the group the pH range in which you can use this is 6 to 10. The example are this CM Cellulose, CM Sepharose or CM Sephadex and all this other kind of the column matrix which are been available from the different types of vendors.

Similarly you have the strong anion or the weak anion within the strong anion you have the quaternary amine which is actually going to have the positive charge and the range in which you can use this particular matrix is in the range of 2 to 12. And examples are Q supharose, Dowex and all that. Similarly you have the weak anions where you can have the primary amine or the secondary amine or the tertiary amine.

Another classical example's is the DAE and the range in which you can use this particular matrix is 2 to 9 and the examples are DA Supharose and DA Cellulose and all other matrix which are available from the different vendors. But the question comes how you are going to select the matrix how you are going to perform the ion exchange chromatography so let us discuss that.

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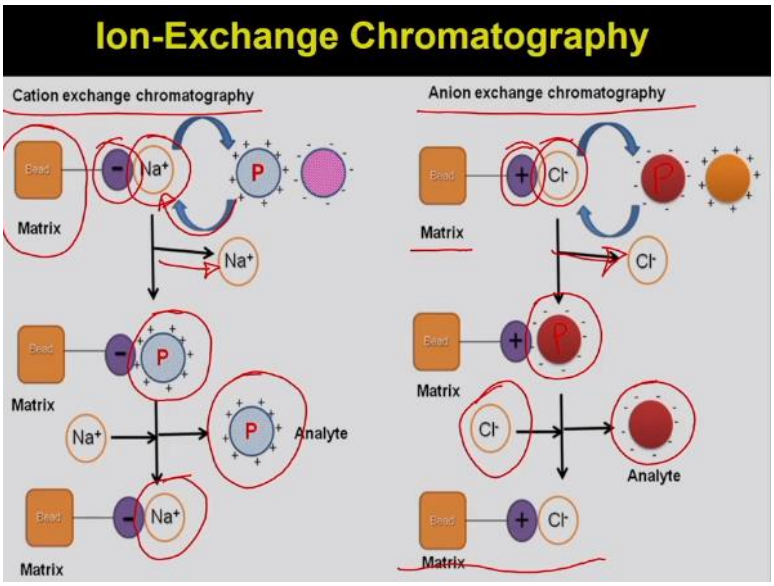

So this is the basic principle through which the cation or the anion exchange chromatography is working. So you can see that you have the cation exchange chromatography or the anion exchange chromatography where you have the beened bound chromatography matrix. So in this case you have a negatively charged group which is attached to the sodium. And the counter ion sodium is attached.

Now once you flow the positively charged protein is going to compitete for the counter and which will sodium and in this process what will happen is the sodium is going to be displaced from the matrix and the positively charged proteins is going to bound to the matrix. Now once you flow the sodium back which is means if you flow many high competition of the counter ion sodium.

The sodium is again going to bind to the matrix and it is going to elute the proteins similarly in a ion exchange chromatography you have the matrix where you the positively charged ions and then you have the counter ions. Once you add the negatively charged protein it is actually going

to go through with the exchange process in that process the chloride is going to be displaced from the matrix.

And the proteins is negatively charged protein is going to bind and then once you flow the high consultation of the counter and the protein is going to be displaced from the matrix, and it is going to be eluted and your matrix is again going to have the counter and bound and it is going to be ready to use. Now this brief discussion about the cation or the anion exchange chromatography.

When you start ion exchange chromatography the first question comes at which matrix I should use and at pH and under what conditions and how to perform the ion exchange chromatography. So let us discuss that.

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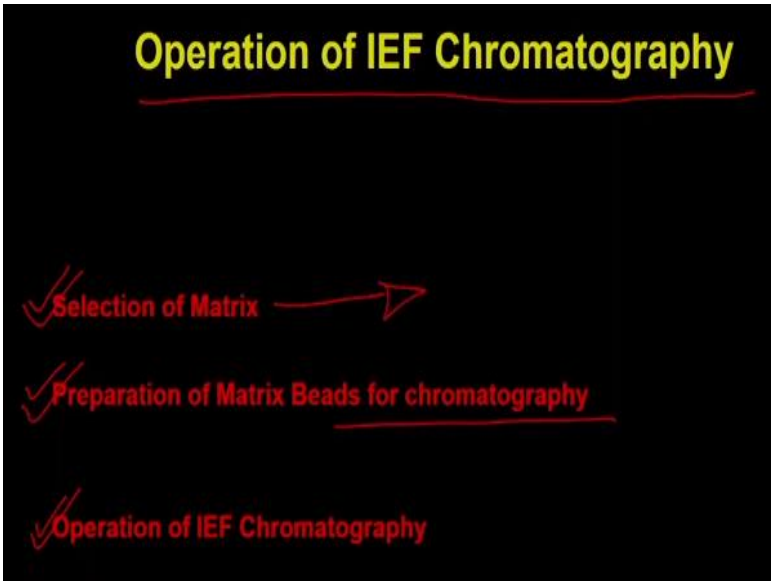

If you could like to run the ion exchange chromatography you have to consider the 3 different parameters one is you have to select the appropriate the matrix. Once, you select the appropriate matrix then you have to prepare the matrix for the chromatography, and then ultimately you are going to perform the chromatography by the following a set protocol. So let us discuss first how you are going to select the matrix.

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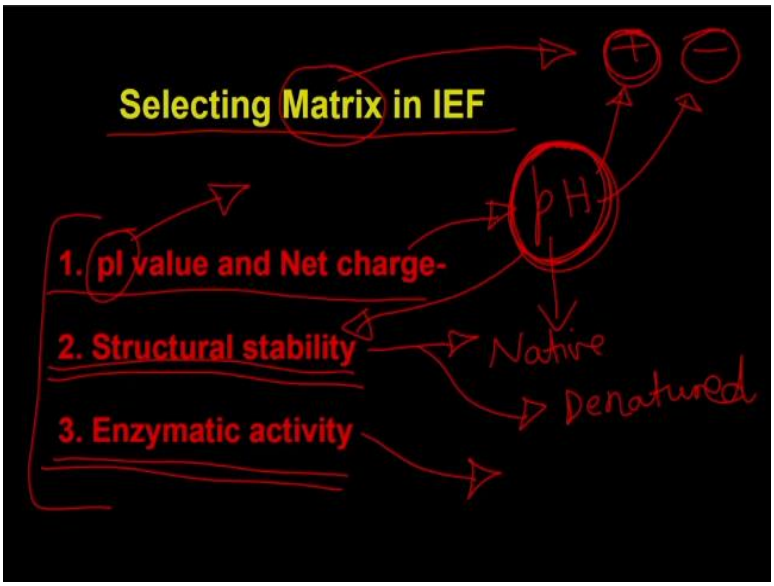

Now selection of the matrix is a very crucial parameter or crucial process if you could like to perform the ion exchange chromatography and the matrix is going to be selected based on whether the analyte is going to have the negative charge or whether it is going to have the positive charge. Now you know that the protein as the different type of charges whether it is the positively charge or the negative charge or some are the polar amino acids or the non-polar amino acids.

So if you take the protein to different pH the ionization of these side chains is always been changing. And as a result the effective charge on the protein is always vary as you go from 1 pH to another pH. And because of that it is important to calculate or it is important to know whether the protein as the positive charge or the negative charge. One of the parameter which actually is going to decide, whether protein is going to have the positive charge or the negative charge is the isoelectric point.

So the first thing what you have to know if you would like to purify or select the matrix is what will be the isoelectric point of the protein and what will be the net charge on a particular pH. Because then only you will be able to decide whether my protein is going to have the positive charge or the negative charge. The second thing is once you selected the pH you also have to decide whether this particular pH is not going to affect the structural stability.

Because the ultimate goal of any chromatography technique is to purify a protein which is 3 dimensionally stable which should have its native confirmations as long as possible or as much as possible. Because the ultimate goal of the chromatography technique; is to purify the functionally active 3 dimensionally native proteins. So you have once you selected the pH or once you decided that okay at this particular pH protein will going to have the positive charge I can use the particular ion exchange matrix.

But then you also have to consider whether the protein is structurally stable or not because if the protein is not structurally stable it is actually not going to give you the native protein then your protein is going to be denatured. And the purification of a denatured protein is not very desirable now once you decided that protein is having the structural stability it is also maintaining the native confirmation.

The third parameter what you have also see is if this protein is a enzyme whether the enzyme is also retaining the activity. Because you are going to perform the chromatography techniques for 2 application one you would like to isolate a structural proteins or you would like to determine isolate the protein in a native confirmations, and then you use them for the downstream applications or downstream process that could be that you can use this the proteins as a vaccine, or that you can use this protein for generating the antibodies.

But sometime you also isolate these proteins simply by performing the enzymatic proteins either these enzymatic reaction for the industrial applications or simply by doing some basic research. So that is also very important that you also should ensure that at particular this particular pH your protein is also having the retaining the enzymatic captivity. Once you done with this and these parameter is linked to prove the isoelectric point. Because that is the thing which is actually going to determine what will be the net charge at a particular pH.

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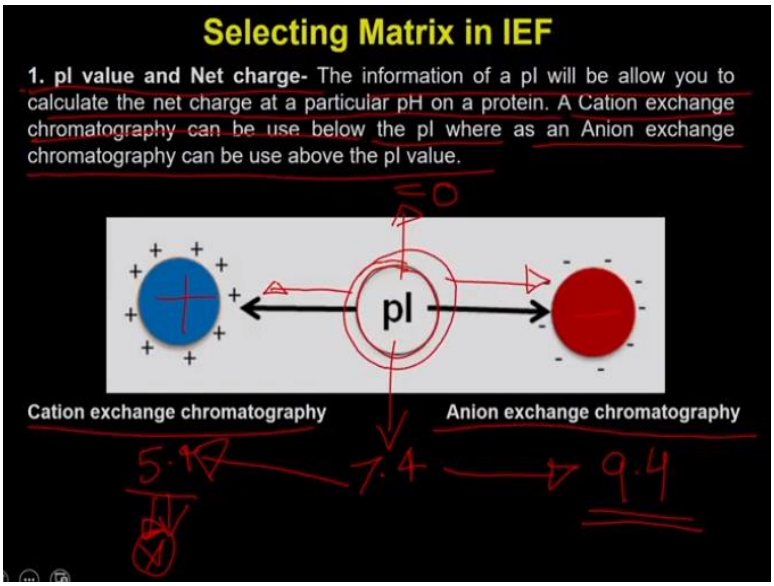

And you can see how the pI is going to decide the net charge the information of a isoelectric point will allow you to calculate the net charge at a particular pH on a protein at cation exchange chromatography can be used below the pI. Whereas anion exchange chromatography can be used above the pI value, which means at the pI value the protein is going to have the net charge 0. So if you go below the protein pI charge you are going to generate net positive charges on to the protein and that is how you can be able to use the cation exchange chromatography.

Similarly if you go to the above to the pI values you are going to have the negative charges and that is how you can be able to use the anion exchange chromatography. Let us take an example for example if you have the pI value of 7.4 now if I have to use and I have determined that the protein is having the no issue with the stability as well as the enzymatic activity. Then I can use the cation exchange chromatography at the pH 5.4 or I can use the anion exchange chromatography at a pH of 9.4.

Now whether I will use cation exchange chromatography or the anion exchange chromatography it will depend whether the enzyme or the protein what we are planning to purify utilizing the ion exchange chromatography is having the native confirmation at this pH as well as maintaining the enzymatic activity. If it is not maintaining the enzymatic activity or the 3 dimensional confirmation at this pH then we have to then we cannot perform the cation exchange chromatography instead we have to use the anion exchange chromatography.

And we also have to decide whether at that particular pH also it is maintaining the stability 3 dimensional confirmation as well as the enzymatic activity. So now the question is how you can be able to determine the pI of a particular protein or the isoelectric point of the protein.

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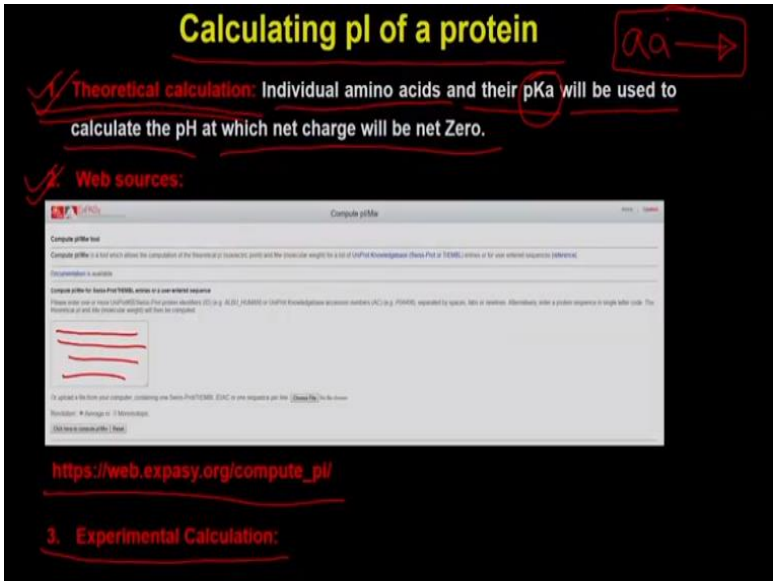

There are multiple ways in which by which you can be able to calculate the isoelectric point of a protein one is you can be able to use the theoretical calculations. Which means; if the amino acids sequence of a protein is known then what you can do is you take the individual amino acid and their pK values will be used to calculate the pH at which the net charge will be 0. So what I am saying is the net charge not the total charge actually.

So at the isoelectric charge the number of positive charges and the number on negative charges are going to be equal and that is how the net charge on a protein is going to be 0. So by theoretical for theoretical calculation one of the primary requirement is that you should know the amino acid sequences. So if you know the amino acid sequence you can be able to use the theoretical calculation or you can use some of the web services.

For example I have given you a link so that you can actually go and put some amino acids sequences and know what will be the isoelectric point of that particular protein. So once you go to this particular link what you have to do is you just copy paste our amino acid sequence into this box and then you ask the software or the webservers to calculate the pI values. It is not going to do anything else except it is actually going to take all the amino acids it will take up all their

pK values and then it will just going to give you the average number at to average pH at which the protein is going to have the 0 charge or the net 0 charge.

Now if you do know the amino acids sequence and it still you would like to calculate or want to calculate the isoelectric point then you can use the experimental calculation which means you are going to perform the experiments with the purified protein and you can calculate the isoelectric point. Let us see how we can calculate the isoelectric point using the experimental condition.

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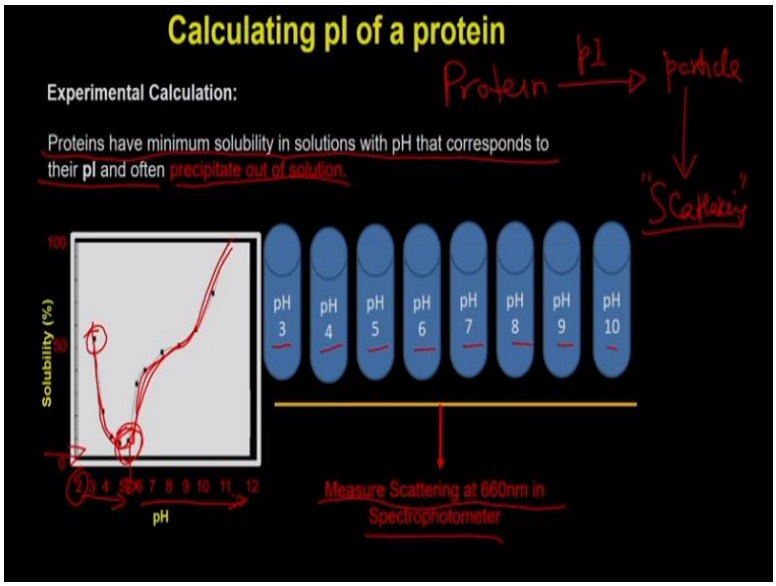

So for the experimental conditions the basic principle is that the protein is going to have the minimum solvability in a solution with the pH that corresponds to their pI and often precipitate out of the solution. So when the protein is going to precipitate it is actually going to it is start going to go form the particles. Which means it is start going to use the particles so the protein is when you will reach to the pI value it is actually going to start showing the insoluble particles and this is small particles are going to show you the scattering of the light.

So you can actually use this particular type of behavior of a protein in the aqueous solutions with different pH. So what you are supposed to do is you take the append of the different pH for example you take the pH3, 4, 5, 6, 7, 8, 9 and 10. And then what you do is you incubate the equal amount of the proteins and you measure the scattering of this particular solution at the 660 nanometer in the spectrophoto meter.

And when you plot this, what you find is that the protein is following a biphasic behavior where the solubility is going down and the solubility is going up. So what will happen is as you are going from the pH2 to pH5 the protein is actually the charges present on the protein is going neutralized. And as a result you are actually having the minimum solubility which means the maximum scattering.

So once it reaches to the minimum solubility then after that if you increase the pH again it is actually again regaining the charges and it is again becoming the soluble into the solutions. Which; means if you plot proteins pattern along the different pH and if you plot this scattering versus different pH. And if you consider the maximum scattering as the gio-solubility and the minimum scattering as the 100% solubility.

And so the protein at the place at which the protein is going to give you the maximum scattering or the 0 solubility that is the place where which is going to be considered as the isoelectric point. So if you follow this particular type of pattern the isoelectric point is somewhere between; the 5 to 6. But if you narrow down these pH conditions you can be able to determine the pI value of the particular protein very precisely.

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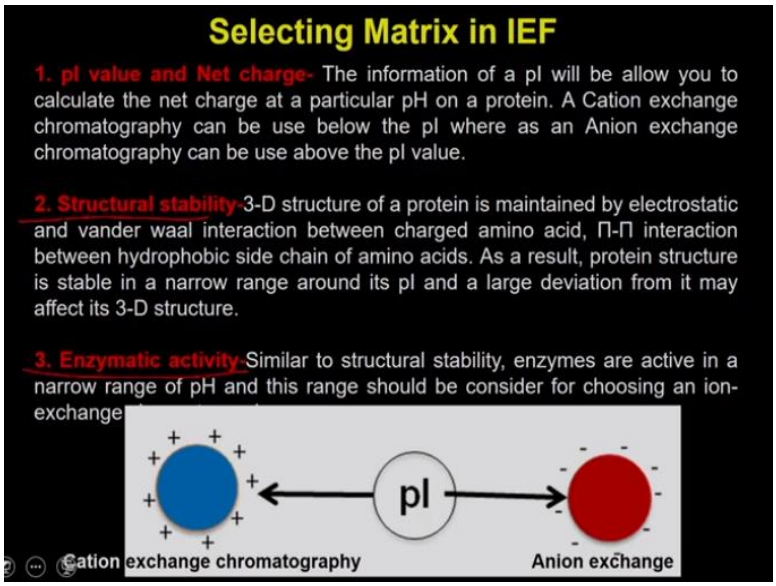

Now once you calculate the pI values you also have to consider these structural stability as well as the enzymatic activity that we have already discussed.

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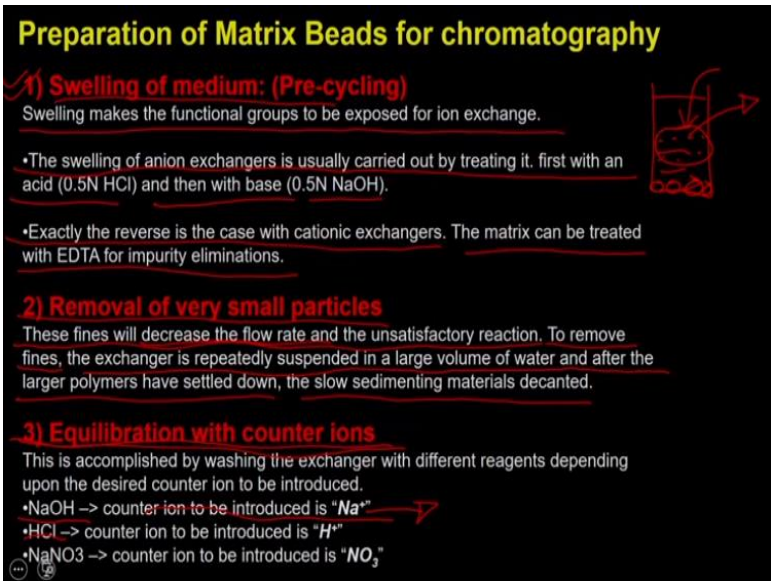

Now once you have decided whether you would like to use the cation exchange chromatography or the anion exchange chromatography you have to process this matrix for utilizing it into the ion exchange chromatography columns. Now in now a days most of the chromatography matrixes whether it is for the anion exchange chromatography or the ion exchange chromatography is always coming free of the any preprocessing.

For example it does not require any preprocessing it is always been coming in a ready to use mode. But in case some of these matrixes are not present in the ready to go mode. Then you might have to process them to make them for the useful matter okay. So what you are supposed to do you have to do 3 activity one you have to do the swelling of the medium, then you have to remove the very small particles which are actually going to affect the many parameters and then you have to equilibrate the matrix with the counter ion.

So that it will actually participate in the exchange process swelling of the process swelling makes the functional group to be exposed for ion exchange. So swelling of the medium means where you are actually going to swell the matrix so that it is actually going to take up the water from the medium. And then it is actually going to expose its functional group for participating into the ion exchange process.

The swelling protocol or the swelling process is different whether it is the anion exchange chromatography or the cation exchange chromatography for the anion exchange chromatography

is usually been carried out by treating it with a very mild acid. For example the 0.5 normal HCl and then with a 0.5 normal NAoH so with this treatment the functional group which are present on the medium is going to be exposed and they are ready for the exchange process.

Exactly the reverse is going to do with the cation exchangers the matrix can be treated with the EDTA for removing the impurities or the small metals because the metals are the cations. So you also want to ensure that there is no metal is present in the process then the next step is removal of the very small particles. These fine particles will decrease the flow rate and the it will actually going to participate in the unsatisfactory regions which means some time it is small particle itself is going to competete with the proteins.

And as a result it will actually going to interfere into the binding process also because there are fine impurities or fine particles they are actually going to decrease the flow rate. So and both are these processes are going to make the chromatography process very difficult. So to remove these fine particle the exchange is repeatedly suspended into the large volume of water and after the larger polymers have been settled down the slowing sedimention rate decanted.

Which means what you do is you take the matrix into a beaker okay and then you fill it with the large quantity of water. So what will happen is because this matrix are very heavy particles they will settle down first whereas the fine particles are going to be remain in the solutions. So what you do is you decant these small particle and you repeat this 4 or 5 times that actually is going to remove these find particles from the matrix and that is how our matrix is going to be more pure and more useful for downstream applications.

Then you have equilibrate the matrix with the counter ions this is important because you have to ensure that the whether it is a anion exchange matrix or a cation exchange matrix. It should have the bound counter ions you have to perform the these processes like NAoH you have to do to provide the counter ion which means when you treat the thing with the NAoH it is actually going to introduce the counter and the sodium and so on.

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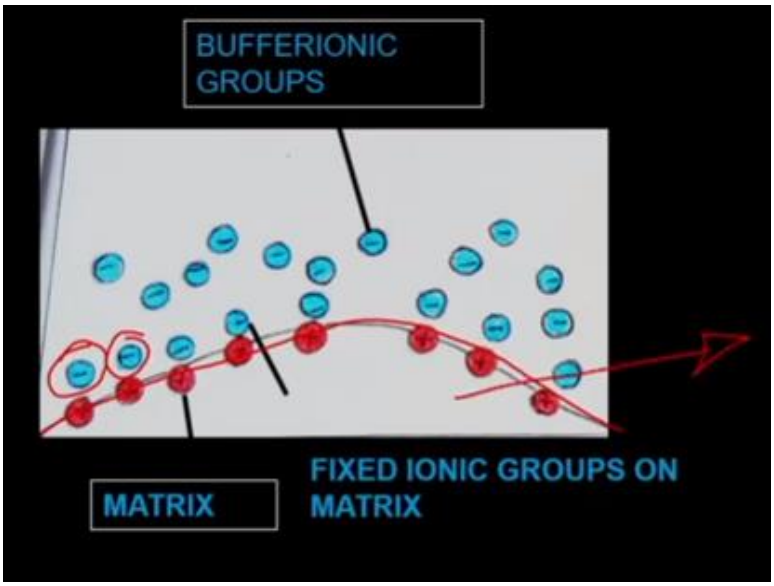

Once you produce a counter ions or once you perform these processes what will happen is the matrix which is actually containing the positively charge groups is going to bind the negatively charge counter. And now this matrix is ready to be used into the ion exchange chromatography process or the protocol.

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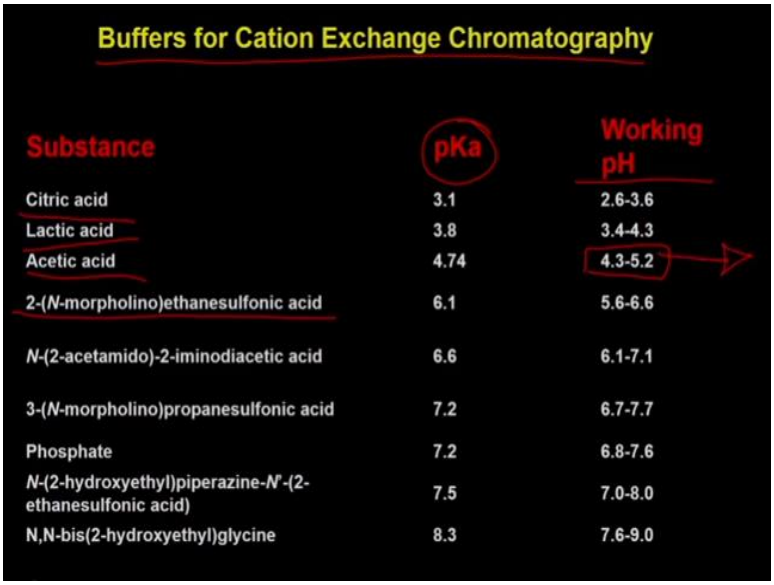

What are the buffers you can use so it is not that you can use any buffer of your choice you have to use the buffer considering that the buffer is going to maintain a stable pH and also going it is not going to interfere with the chromatography techniques, so these are the different buffers what you can use for cation exchange chromatography. You can use the citric buffer, Lactic buffer,

Acetic buffers you can use the all other these buffers what you have to consider is the pK values and as well as the working pH.

So depending on what pH you are interested you can choose the particular type of buffer because in this particular working pH only it is going to maintain a stable pH. So that it should not vary because as soon as the pH is going to vary it is actually going to change the charge present on the particular protein and as a result it is actually going to affect the overall illusion process. Because what you do not want is the illusion should be done simply by varying the charge on your protein itself.

What you want is that you can actually put the sodium or chloride as the competitive counter ion so that it is actually going to follow the basic principle of the ion exchange chromatography.

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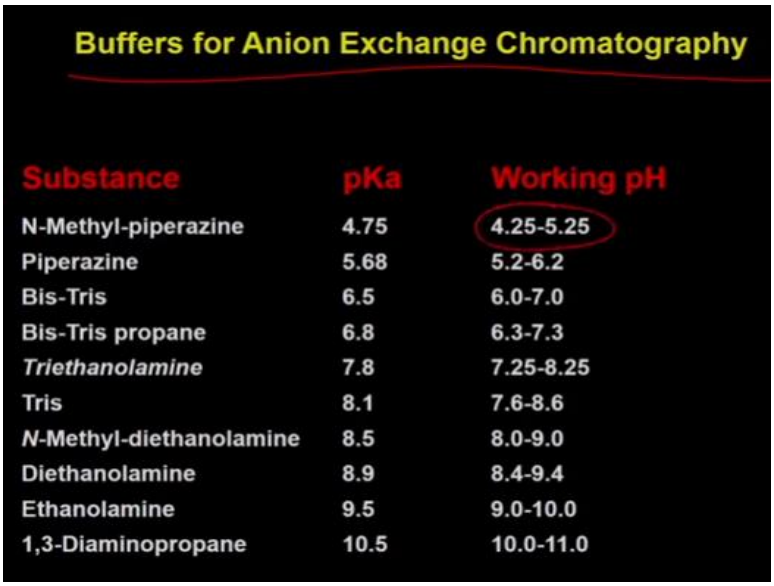

Similarly you have the different types of buffer for the anion exchange chromatography. So the only thing what you have to consider is what is the pH in arranging in which you are interested to work.

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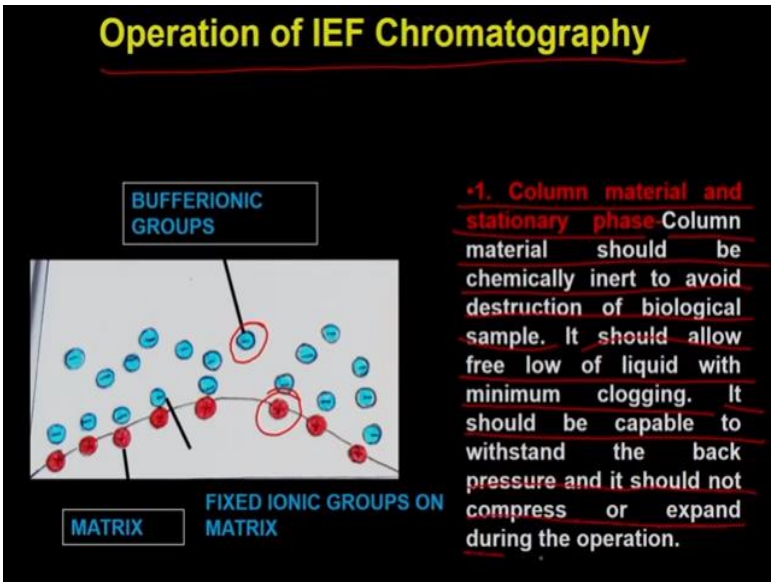

Now let us see how to do the operation of the ion exchange chromatography so the first thing is you have to equilibrate the column with a suitable mobile phase. The pH of the mobile phase is going to be decided based on the what is going to be the isoelectric point and once you do that the buffers the counter ion present on the buffer is going to bind to the functional group which is present on to the matrix and as a result now the column is going to be ready.

The column material so in this process of the equilibration of this stationary phase the column material should be chemically inert to avoid the destruction of the biological sample. Which means the matrix should not react with the biological samples it should allow the free flow of the liquid with minimum clogging it should be capable of withstanding the back pressure and should not compress or expand during the operation.

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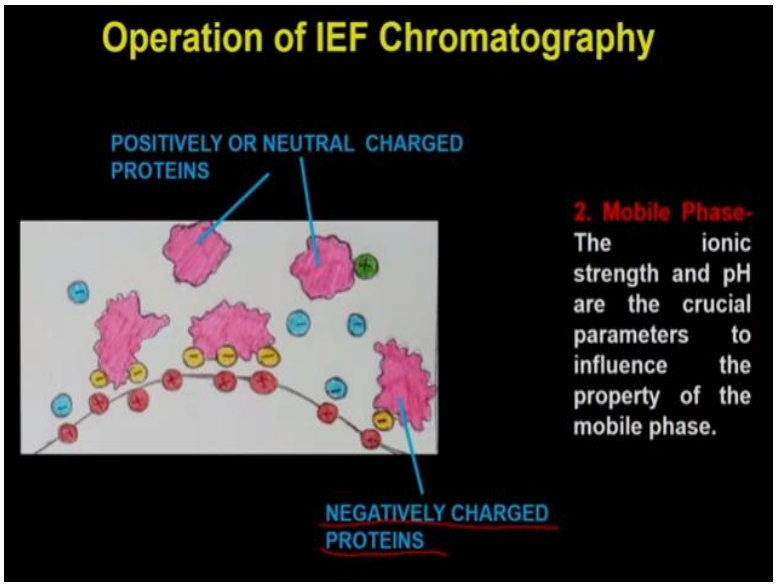

Now you are going to load the proteins to the matrix so in this case we are loading the positively charged. So the protein is having the positive or neutral charged protein so what will happen is and you have the negatively charged protein. So if you have a mixture of the positive charges positively charged proteins negatively charged proteins or the neutral proteins. It is actually going to flow to the matrix and as you can see this is actually this matrix is having the positive charges.

So it is actually going to show the affinity only for the negatively charged proteins and then that is how the protein is going to bind to the matrix or going to the bind to the ion exchange matrix.

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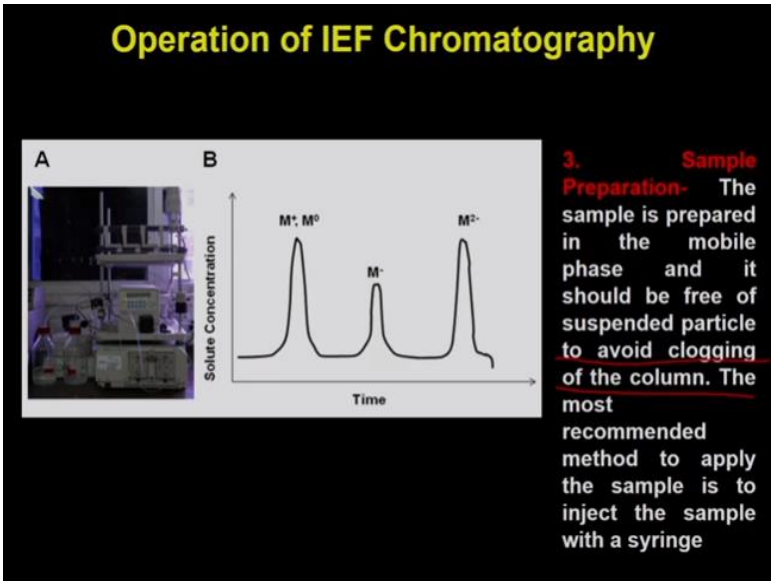

Then what you have to do is you have to flow the samples and the sample the only thing what you have to do the only precaution what you have to do is that the sample is free of the suspending particles which means the it should not having suspended particle to clog the column because that actually is going to reduce the flow rate.

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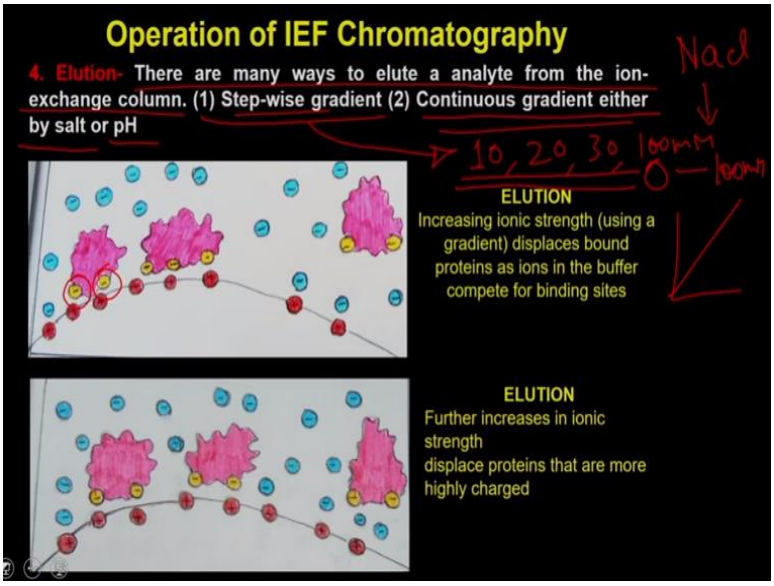

And then ultimately you are going to do the illusions so there are many ways to elute a analyte from the ion exchange chromatography columns. One is the step wise gradient and the number 2 is the continuous gradient either by the salt or the pH. So there are 2 modes either you do this step wise gradient. The step wise gradient means that you are going to for example if I am using the NaCl so that actually is I can use a linear gradient which means I can use from 0 to 100 milli molar.

So in this case it is actually going to be a linear for example it is going to be 0 milli molar, 1 milli molar, 2 milli molar like that. So it is going to be a linear tikkey the second is that if you want to use these step wise gradient then what I can do is I can take the 10 milli molar elute something then I take 20 then I take 30 and so on. Then ultimately I take the 100 milli molar so that is called as the step gradient.

Whereas the continuous gradient means I will going to follow a process so that it is actually going to maintain a continuous gradient which means after every micro liter what is going to be added into the system. Some amount of the sodium is going to be added and that is how it is

actually going to maintain the linear gradient. The step gradient is does not require any type of machine or something that can be done in a manual mode.

Whereas the linear gradient or the continuous gradient is require a gradient mixture this gradient can be made of either the salt or the pH which means you can make a linear gradient of pH, so that it is actually going to change the pH as it is going to change the pH it is actually going to change the charge on to the protein, and that is how it is actually going to come out. So once you flow the salt it is actually going to competete with the bound protein and that is how the protein is going to come out.

And as you remember we have discussed if the protein as the multiple charges is actually going to show more affinity that is how it require the more amount of salt or more amount of counter ions and the proteins which have a lesser amount of charges it is going to follow the lesser (()) (42:11).

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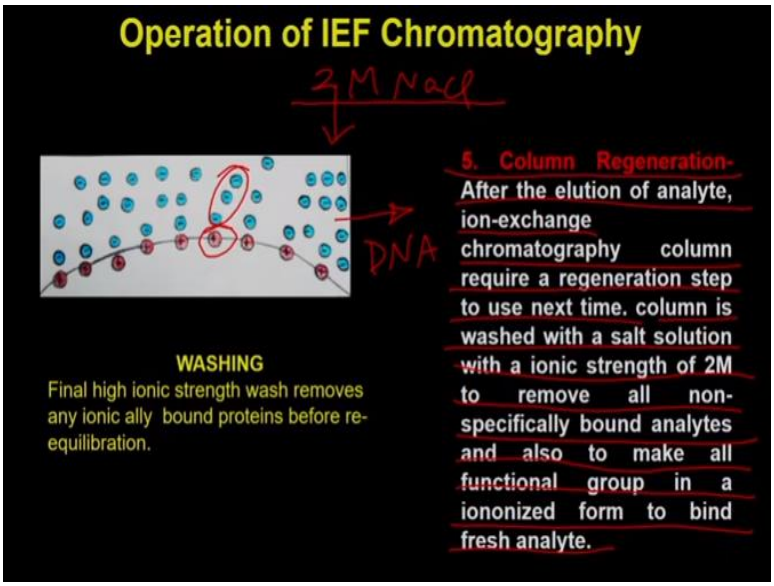

Now ultimately once you are done with the ion exchange chromatography you have to do a next process that is called as the column regeneration which means you are actually going to regenerate a column. So, that it is actually can be reused to the next chromatography technique after the illusion of the analyte the ion exchange chromatography column requires a regeneration steps to use the next type. The column is washed with the salt solution with ionic strength of 2 molar to remove the all noise specifically bound analyte.

And also to make all functional group in a iononized form to bind the fresh analyte which means what you are supposed to do is you are going to wash this with a 2 molar NaCl, what will happen? Once you wash it with the 2 molar naCl it is actually going to remove all the protein irrespect all the proteins. And as well as the other kind of the biological molecules which is not desirable but it is bound.

For example one of the things which going to bind to the most of the ion exchange chromatography is DNA. Because DNA is negatively charged so it will go and bind to this functional groups. But that is not what you want and because, these molecules will bind to the functional group this functional group will not be no longer available. So that is how you have to wash these columns with the very strong salt solution so that all the biological molecules are going to be removed.

And then these functional groups free to bind the buffer ions so that now the column is ready for the next round of purifications.

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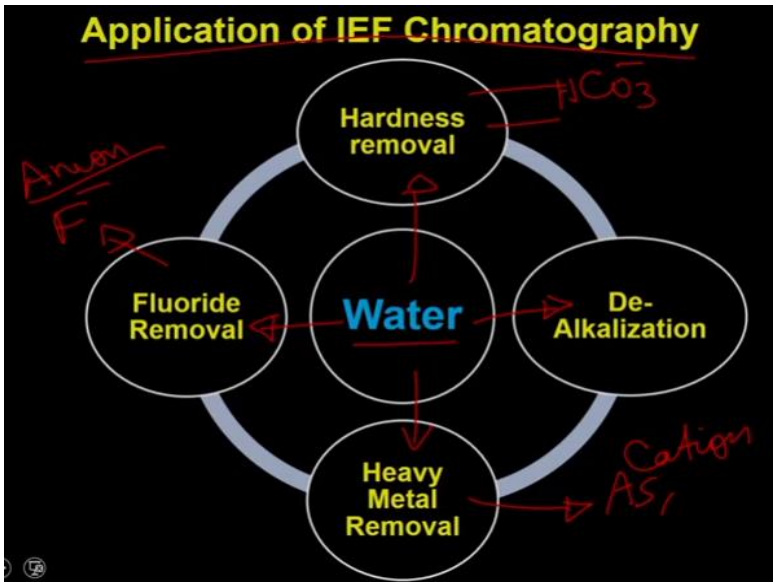

Now let us see the application of the ion exchange chromatography so one of the major application of ion or the major application of the ion exchange chromatography is that it can be used to purify the water. So water is having the different types of impurity for example it has the hardness it has the alkalization it has the different type of contamination. For example the

fluoride or the heavy metal and all these can be removed because the fluoride is negatively charged whereas the hardness is because of the different types of carbonates and other types of molecules.

And heavy metal is always been because or the arsenic or other types of heavy metals so this is actually a anion which is been present as the impurity into the water. Whereas the heavy metals are falling under the category of the cation and all other things are. So when you flow the water to the ion exchange matrix whether it is the anion exchange matrix or to the cation exchange matrix you can be able to purify or you can be able to remove this substances.

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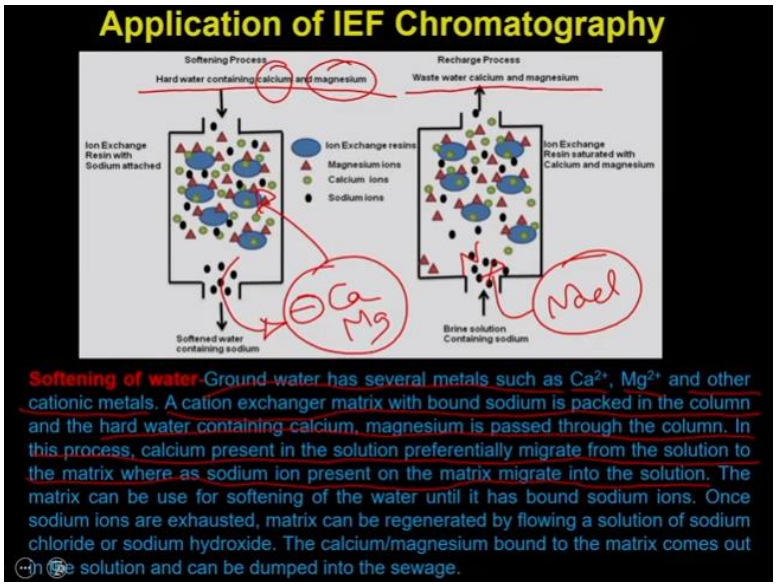

Let us take an example how you can actually be able to remove the hardness or you can able to make the water more soft. So the ground water as a several metal such as calcium magnesium and all other cationic metals. A cationic exchange matrix with the bound sodium is packed in the sodium for it is so you take a column where you have used the cation exchange chromatography. And this cation exchange chromatography is bound to the sodium now what you do is you flow the hot water containing the calcium and magnesium.

And once you flow them the calcium as well as the magnesium is going to displays this sodium from the bound matrix okay. And as a result the water whatever the water will come out is actually going to be divide of the calcium as well as the magnesium. Now packed in the column

and the hard water containing calcium magnesium is passed to the column in this process the calcium present in the solution preferentially migrates from the solution to the matrix.

Whereas sodium ion present on the matrix migrate into the solution. So this calcium and magnesium will remain trapped within the matrix, whereas the water will come out along with the sodium. Now once you are keep using this cartage so this cartage has the limited capacity so that it can be able to bind the calcium and the magnesium. And afterwards what you can do is you can just flow the solution of the NaCl.

And once you flow the solution of the NaCl what will happen is all the calcium and magnesium, which is been trapped from the water is going to be displaced from the matrix and it will come out and that can be throw into the suitable place. And then this cartage is again be ready to use so this cartage can be used on the several locations and that is how you can be able to remove the all type of the heavy metal as well as the all other transition metals.

And that is how you can be able to remove the hardness from the water. So this is just a simple application or simple description of how the cation or the anion exchange chromatography can be used to purify the water.

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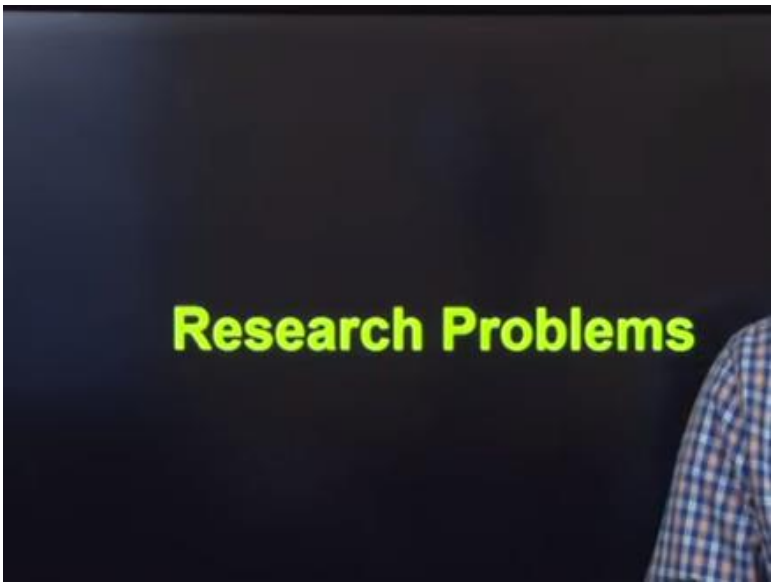

Now in our subsequent lecture we are actually going to discuss about the research problems where you can be able to utilize the ion exchange chromatography. And how; you can be able to

design the different types of experiments exploiting the ion exchange chromatography, thank you.