Quantitative Methods in Chemistry

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Lecture-37
Analytical Separations-Chromatography Part 02

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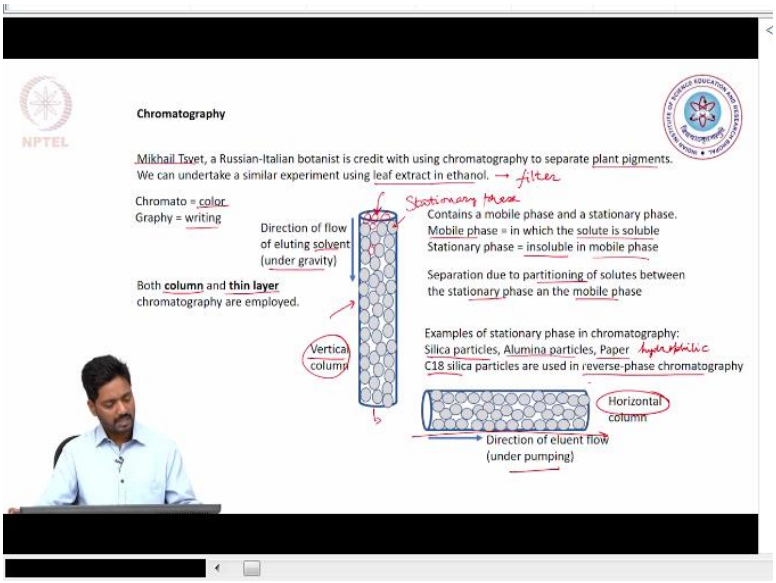

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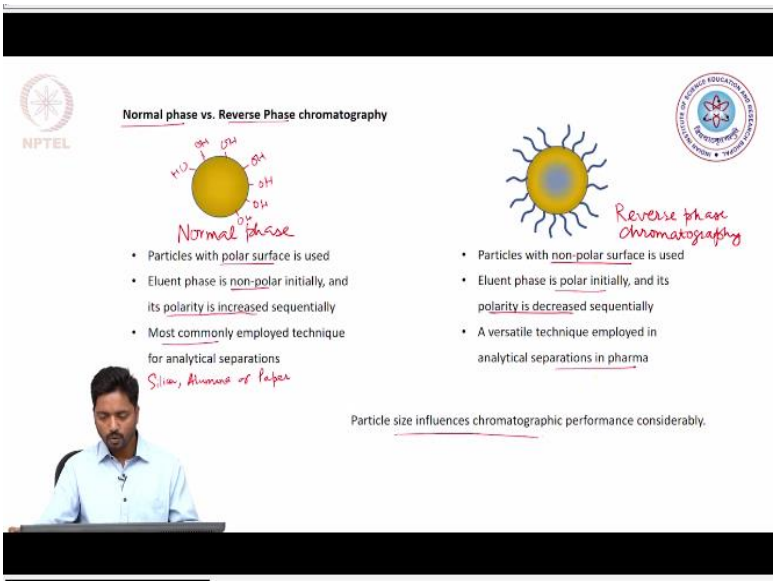

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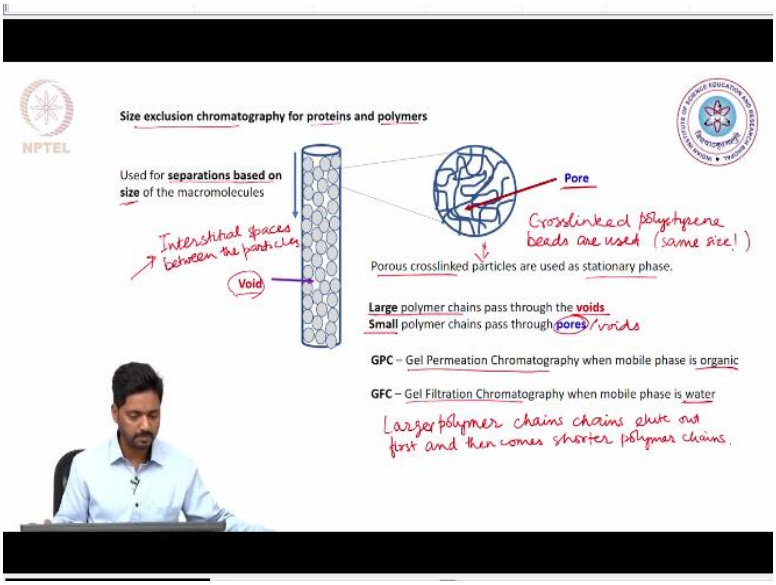

Let us go to the next topic, which is the use of size exclusion chromatography for use with proteins as well as polymers. So, size exclusion chromatography as the term clearly indicates, utilizes separations based on the size of the macromolecules or proteins of interest. So, in this case the only major difference is that the particles are made up of or the stationary phase is made up of porous particles.

So, we use porous cross linked particles as the stationary phase, typically cross linked polystyrene beads are used as the stationary phase and care is undertaken to ensure that these beads are of all same size. So, in these kinds of separations there are 2 important considerations to be kept in mind. One is that we have voids in between the particles. So, the voids are interstitial spaces between the particles.

However, the particle in itself is porous and we also have pores present in the particle within which the solvent as well as the solute can enter. So, what is observed in this case is that the large polymer chains prefer to pass through the voids that is the interstitial space while the small polymer chains pass through both pores and the voids. So, essentially the time spent by the smaller polymer chains inside the column of size exclusion chromatography is much larger.

And hence, we see that the large polymer chains elute out first compared to the smaller polymer chains, so the larger polymer chains elute out first and then come the shorter polymer chains. Now, the similar logic can be applied in purifying proteins in size exclusion

chromatography, larger proteins will travel mainly through the voids and will come out first and the smaller proteins will spend more times inside the pore and will come out much later.

Based on what mobile phase is used in this chromatographic technique, we call it either a gel permeation chromatography or a gel filtration chromatography. So, GPC or the gel permeation chromatography is the term used when the mobile phase is organic and if the mobile phases is aqueous, then we use the term as the gel filtration chromatography. Now, let us look at how the particle travels.

And what is the separation that can be achieved while using gel filtration chromatography or gel permeation chromatography. So, let us go to the board and try to understand this concept.

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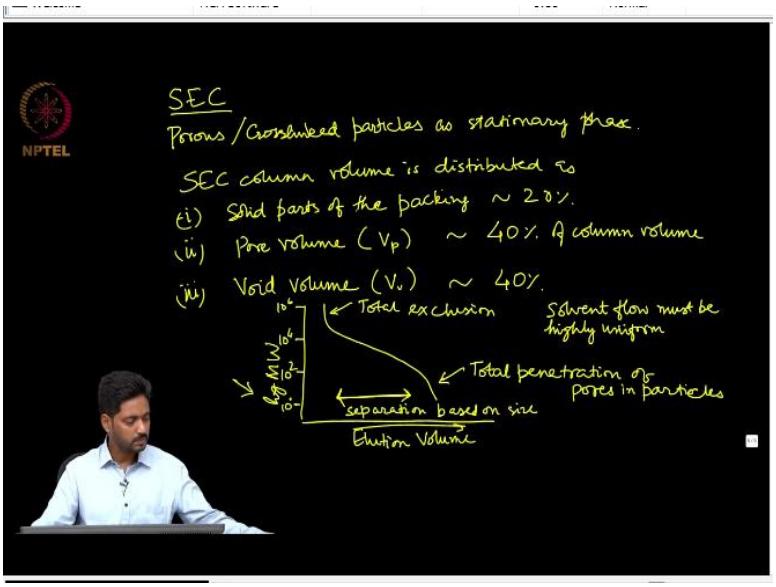

So, in size exclusion chromatography, we have porous cross linked particles as stationary phase. So, what people have realized is that the SEC column volume is distributed as in 3 types, the first one is the solid parts of the packing that occupies about 20% volume of the column. Then we have the pore and the void. So, the pores, the pore volume, which we denote as VP constitutes to about 40% of the column volume.

Finally, we have the void volume which we denote as V void that also constitutes another 40% of the column volume. So, we see that both the pore and the void are occupying large volume fractions in the size exclusion chromatography column and in the pores the large molecules will not enter, while in the voids, the large molecules or macromolecules will enter and will pass through the column more quickly.

So, when people plotted the elusion volume versus the log of molecular weight of the polymer, they observe a profile which looks something like this. And it is this regime, which is of interest to us, where actual separation based on size is happening in this regime. In the initial phase of the column, we have what is known as the total exclusion that means the polymer fraction passes only through the void volume and comes out very rapidly out of the column.

Similarly, in the other end, we have what is known as the total penetration, where the protein or the macro molecule is penetrating all the pores present in the particles. So, both these extremes are of no particular use to us in the size based exclusion technique and only the regime highlighted by the arrow is by the double sided arrows is of use to us where sort of a linear change in the molecular weight log molecular weight happens with the elution volume.

Now, it is also important to understand that we have a log scale in the y axis and a linear scale in the volume axis or the x axis. So, it is very important also to keep the solvent flow uniform. So, the solvent flow must be highly uniform while undertaking the size exclusion chromatography.

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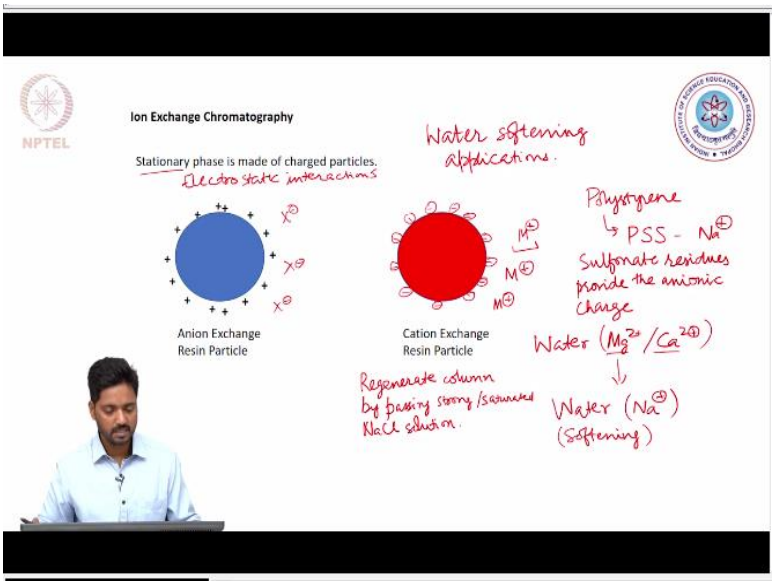

Now, what are the other techniques that are available to us for purification. One of the most commonly employed technique is also the ion exchange chromatography, where we deal with particles that are charged. So, in the anion exchange chromatography, inherently cationic and

then we have exchangeable anions that are attached to the particle and they undergo exchange with the analyte and this results in separation.

Similarly, the cation exchange resin has anionic charges on the surface and they have exchangeable cation present in them, which undergo exchanges. So, it is the ions that are exchanged between the analyte and the stationary phase. For example, the ion exchange chromatography is very widely used in water softening purposes. So, the stationary phase in this case will be based on again a polystyrene kind of a particle which is also having polystyrene sulfonate residues on the surface.

So, the sulfonate residues provide the anionic charge to the particles and we have the counter cation often as sodium. So, when we pass water that contains magnesium or calcium ions, which are often the source of the hardness because they can form the corresponding carbonates the magnesium or calcium and in water is exchanged in the column with the sodium ions and this results in the softening of the water.

And the advantage here is that when the column gets completely saturated with these magnesium and calcium ions, we can regenerate the column by passing strong or saturated sodium chloride solution through the column. So, this becomes a very convenient protocol for softening water, we can also separate proteins and analytes of interest based on the charge on the analyte.

Because the interaction between the stationary phase and the analyte will be based on the electrostatic interaction. So, an electrostatic interactions happens between the analyte and the stationary phase.

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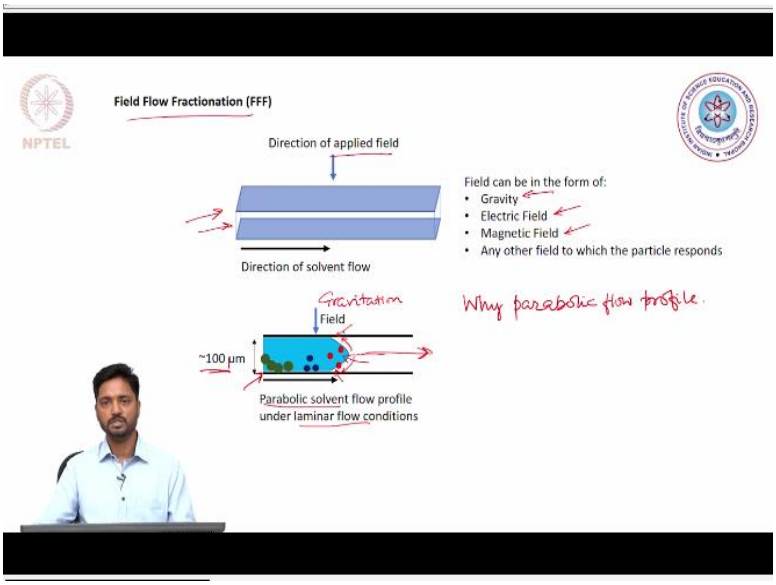

In this case now the final topic of this lecture is about the field flow fractionation which is abbreviated as FFF, here we have a thin strip of the material and through with the analyte is paused and a field is applied perpendicular to the direction of the solvent flow. And this results in separation of the analytes based on the how the analyte response to the applied field.

The field can be of wide varieties we can use gravity, we can use electric field, we can use magnetic field or one can choose any other field to which the particle responds to and what has been known is that inside such thin capillaries, the dimensions of this capillary or structure is about 100 microns. So, under these conditions, the flow of the solvent follows parabolic flow profile under the laminar flow conditions.

And if the field is say a gravitational field then we can see that the large particles respond most strongly to the gravitational field and they will be sticking closer to the walls of this capillary. However, as the particle size reduces, for example, the red particles here, they will start flowing more and more with the solvent flow or the solvent profile and will elute out first out of the column while the green particles which are the largest will elute out the last.

So, this becomes straightforward techniques to separate analytes of interest by applying appropriate fields. Now, I will just quickly tell you why the solvent takes a parabolic flow profile under laminar flow conditions. So, when solvent flows through capillaries, the walls of the capillaries attract the solvent and the solvent flow is negligible near the walls.

However, near the center of the flow, no such attractive forces present and hence the flow is maximum at the center of the capillary.

And the flow gradually increases as we move towards the capillary walls and as net result the profile that is generated under laminar air flow conditions is that offer parabolic solvent profile. Now, we will when we will discuss about electrophoresis we will see that under electro osmotic flow conditions, the solvent flow profile is very different and that aspect will be covered in the subsequent lectures.