Quantitative Methods in Chemistry

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Lecture-36
Analytical Separations-Chromatography Part 01

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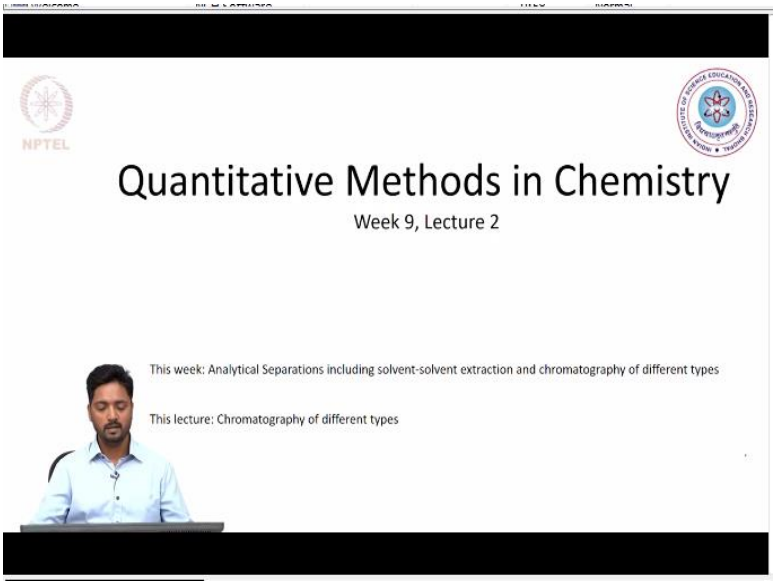

Hello, and welcome back to lecture 2 of week 9 for this course quantitative methods in chemistry. This week our focus has been on understanding how analytical separations are undertaken in chemistry and we have already dealt upon in the last lectures on the solvent-solvent extraction. So, this lecture our focus will be on understanding chromatography of different types.

So, before that, I would like to quickly tell why there is a need of undertaking chromatographic techniques or why did researchers develop chromatographic techniques when separation techniques like solvent-solvent extraction, distillation or precipitation were already available to us. So, what people realized in the early stages already is that distillation process is both tedious. And it is restricted to only solutes or analytes which are easily vaporizable.

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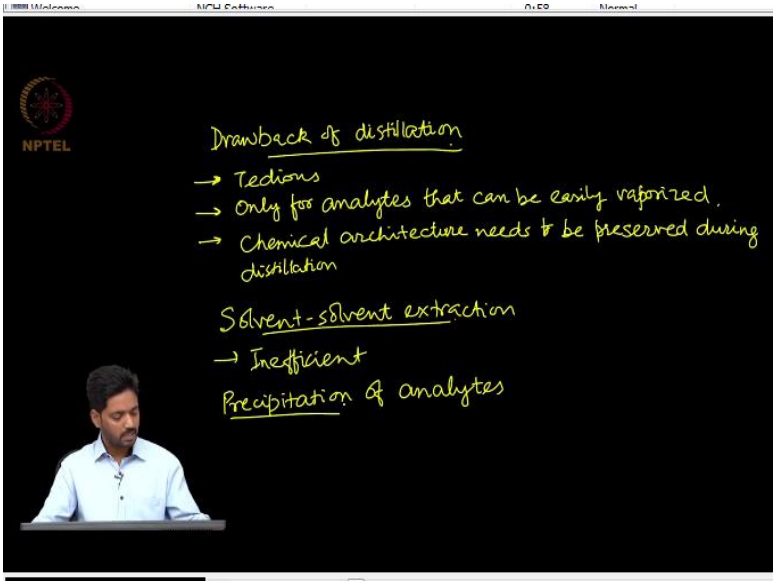

So, to mention the drawback of distillation we observe that it is of course a tedious process and is amenable only for solids or analytes that can be easily vaporized. Now, it is of course true that not very many organic compounds can be easily vaporized while ensuring the chemical architecture. So, the chemical architecture needs to be preserved during distillation and that started becoming a challenging process for a large number of solids.

Similarly, solvent-solvent extraction was often found to be inefficient in separating the analytes, which have similar properties and it also is considered to be somewhat tedious. So and the same goes for precipitation process that it is also inefficient in separating the many of the analytes. So, researchers very quickly realized that these techniques of distillation, solvent-solvent extraction or precipitation of analyte good for certain compounds but not for all.

So, people started looking for alternatives and that is how various chromatographic techniques developed.

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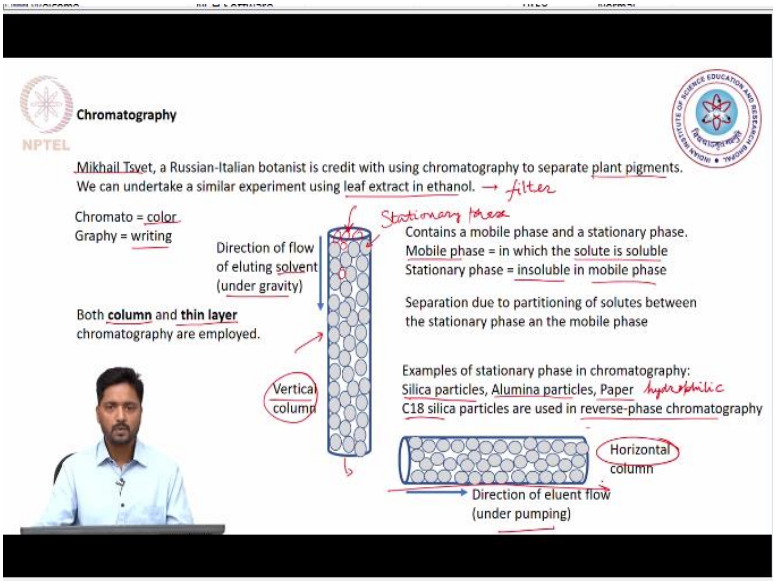

So, let us go to the slide and understand what do we mean by chromatography. So, chromatography as the term indicates it is literally meaning chromatic which is color and graphy which is writing. So it is some sort of mark or writing that occurs when analytes are separated especially analytes, which are colored and Mikhail Tsvet is Russian Italian botanist who is credited with having used chromatographic techniques to separate plant pigments.

And in fact, this is one of the very common chromatographic techniques that are demonstrated in undergraduate laboratories where we take leaf extract in ethanol and then undertake its chromatographic separation on something like filter paper. So, filter paper is used and the leaf extract is chromatograph on the substrate and this results in separation of the various pigments that are present in the leaf.

And these can then be later isolated and characterized if there is a need of that. So, basically in chromatography which can be either a column based chromatography which is what we have shown here, where there is a cylindrical column, which in this case is oriented vertically and the column is filled with these particles which are shown here, which we call as the stationary phase.

So, these stationary phase are materials which are insoluble in a phase which is moving, which is known as the mobile phase. So, for example, we can use an organic solvent to elute out or to move out our solute or analyte of interest from this column. So, the compound will be the mixture will be put on the top and the product extracted out at the bottom in a analytically pure manner.

Since, in this case, the mobile phase is moving under gravity. This is also known as the gravity chromatography. And we choose our mobile phase in this case, based on the condition that the solid needs to be soluble in this mobile phase. So, often for organic compounds, the mobile phase is an organic solvent such as hexane or ethyl acetate or in certain conditions stalling.

And here in chromatography, we can have the column chromatography which I already explained or a thin layer chromatography which is what we often use in laboratory for demonstrating for example, the separation of leaf pigments, where the stationary phase is in the form of a thin layer. For example, filter paper will be considered a thin layer of stationary phase and the analyte is separated on it.

Now, stationary phases can be of wide varieties, they can be silica particles and this is the most commonly utilized stationary phase in column chromatographies. So, particles of different mesh size are used. So, they are packed into this column very tightly. So, they are packed here very tightly and the solute is then passed through this column and separated we can also use alumina particles that is aluminium oxide.

And both of these particles whether it is silica or alumina, they are considered to be hydrophilic. And the same goes true for paper. And all of these examples are stationary phases that are considered hydrophilic. We will discuss this aspect in the next slide as well. And we can also use what is known as the C 18 silica particles and we will understand what this is in the next slide in what is known as a reverse phase chromatography.

Now, typically, we will be using the column in a vertical position. However, under pumping conditions, we can even keep the column in a horizontal position and pump the element from say, left to the right of the column. In this case, we are not getting any benefit from the gravity so we would require a certain pumping action to push the solvent through the particles present in the chromatographic column.

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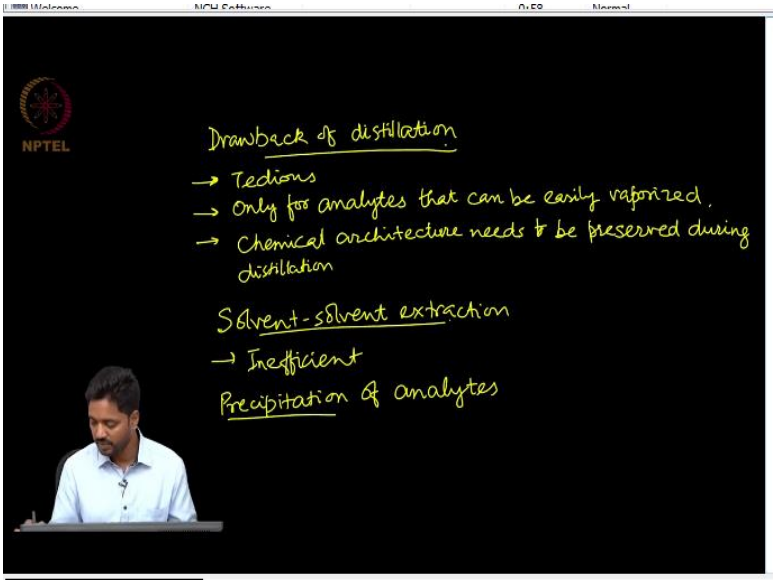

So, chromatography can also be segregated into what is known as the normal phase chromatography versus the reverse phase chromatography and let us try to understand what are the differences in the stationary phase in these 2 cases. So, in normal phase chromatography, so, which is described here and here we are talking about the reverse phase chromatography.

So, in the normal phase of chromatography we take particles which have a polar surface for example, silica or alumina, because these particles will have hydroxyl groups on their surfaces and these hydroxyl groups are what gave these particles the hydrophilic property and the analyte or the solute in this case is simply absorbed on the particle surface as it moves through the column.

So, the partitioning of the analyte between this solid particle and the element or the mobile phase is used for achieving separation. Now, what we use here is an element phase which is nonpolar and the polarity of the mobile phase or the earlier phases increased in a sequential manner. For example, in laboratory, we can use silica particles as the polar surface, pack them in a glass column.

And then pass a non polar solvent such as hexagons pack the column initially and later we can start increasing the polarity of the solvent by adding some polar solvents such as ethyl acetate or even acetone or in some cases, chloroform and methanol are also used, where the methanol obviously is the polar solvent whose volume fraction is increased in the mobile phase to achieve extraction of the analyte from the column.

So, what we have observed is that this normal phase chromatography, whether we are using silica alumina or paper all of these are hydrophilic surfaces used in the normal phase chromatography and it is hence, the most commonly employed technique for analytical separations. Now, a completely reverse of that happens, when we are undertaking what is known as the reverse phase chromatography.

Hence, the name also is reverse phase chromatography. Here, we have consciously or by design converted our stationary phase into having non polar surface and how that is done is discussed a little later. And what we do here is to use the eluent phase as polar initially typically often water is used as an eluent phase or methanol can be used as an eluent phase and the polarity is now decreased sequentially to elute out our analyte of interest.

This of course has gained wide popularity in pharmaceutical industry as a versatile technique for achieving analytical separations. So, before I discuss how particle size influence influences chromatographic separations, let me quickly describe to you how the particles of silica are converted from a normal phase particle to a reverse phase particle by undertaking chemical reaction.

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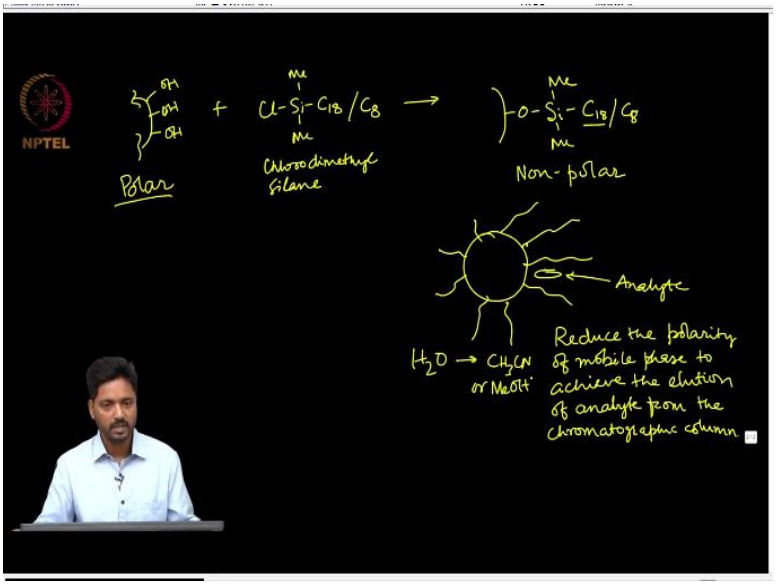

So, what we do here is, we have these particles which have hydroxyl residues on the surface and we undertake their reaction with chlorosilane usually chlorodimethylsilane, and here we will attach a long chain such as C 18 or C 8 based on what we want to create or attach on our

particle. Silane is reacted with the silica particle, we undertake reaction that happens between the 2.

And essentially, the chlorodimethylsilane is anchored covalently onto the particle surface. This completely changes the chemical character of the particle. So initially before the day revitalization the particle was polar. However, after we have added our alkyl silane, we create a particle which is very non polar. And what happens in this case is that you have these alkyl tails that hang out of the particle and this is where the analyte gets partitioned.

So, if the analyte is also non polar, it would like to partition well into this non polar phase and will not elute out. So, we need to reduce the polarity of the mobile phase to achieve the elusion of analyte from the chromatographic column. So, this obviously requires the mobile phase typically, we start with mobile phase as water and then we add either acetone nitride or methanol in various fractions to reduce the polarity of the medium and achieve effective elation out of the analyte of interest.

Now, let us go back to the presentation and understand how particle size influences chromatographic performance. So, we have already realized that, in the chromatographic separation, we have the analyte partition between the stationary phase and the mobile phase and the analyte usually absorbs onto the stationary phase and that becomes a way to achieve the separation of the analytes. Now, as we reduce the particle size, we increase the surface area of our particles.

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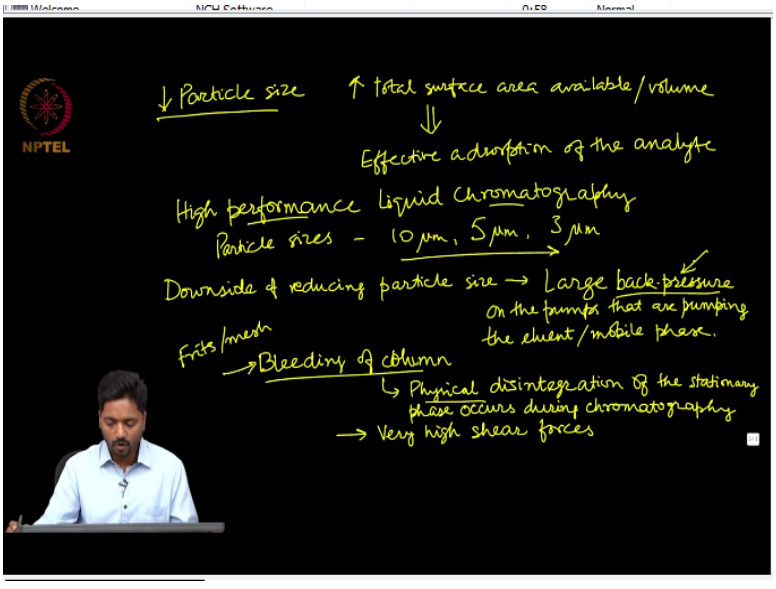

So, reduction of the particle size increases the total surface area available per unit volume and that allows effective adsorption of the analyte. So obviously, the interest of chemists has been to achieve reduced particle sizes for undertaking chromatographic separations, and this indeed forms the basis of what is known as the high performance, liquid chromatography were particle sizes of 10 micron or 5 micron or even 3 microns are routinely used.

So, as we decrease the particle size, our efficiency of separation improves or in other cases, the performance of the chromatographic technique improves. So, we will be requiring a much shorter column to achieve the same separation. However, there is a downside of reducing particle size and the downside relates to the fact that we create large backpressure on the pumps that are pumping the eluent or the mobile phase.

So, this becomes a very significant technological challenge or and is also important parameter to be considered in terms of the cost of the equipment because as we keep reducing the particle size, we will require high efficiency pumps and high performance pumps whose cost becomes quite significant consideration in undertaking the purchase of the equipment.

So, while there is a very clear interest in reducing the particle size, the backpressure becomes a very important problem. Another problem that comes here is that of the bleeding of column. Now, this is where the physical disintegration of the stationary phase occurs during chromatography. So, you need to understand that when the mobile phases pumped into the column which is tightly packed with the stationary phase.

What we observe is that this moving of the solvent or the mobile phase generates very high shear forces. Now, these high shear forces act adversely on the stationary phase and often result in physical disintegration of the stationary phase and then these degraded stationary phase starts coming out of the column and this is known as the bleeding of the column. One common way to avoid this bleeding of the column is by using frits or mesh of appropriate size.

So that only the mobile phase and dissolved analyte comes out of it and no or minimal stationary phase comes out of the column. However, this also results in generation of the backpressure which is discussed previously.

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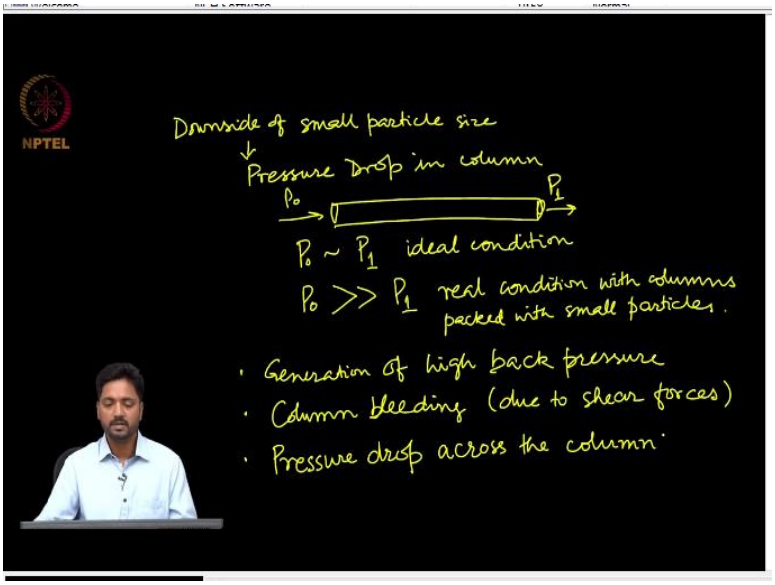

Another very important downside of having small particle size is that we create what is known as the pressure drop in the column. So, if we are talking about a long column and we are applying pressure P 0 here and we are measuring the pressure that is at the other end of the column let us call it as pressure P 1, what we would ideally want is that the P 0 is similar to P 1.

However, if we are using particles which are very tiny and which restrict the flow of the solvent then the ideal condition of P 0 by is similar to P 1 does not hold, and what we observe is often that the initial pressure at the column is much greater than the final pressure at the end of the column. So, this becomes real condition with columns packed with small particles.

So, the 3 major downsides that we saw with regards to using small particles is that the there is a generation of high back pressure then we have column bleeding that happens due to the shear forces that are generated by the movement of the solvent. And finally, we saw the case of pressure drop across the column. And all of these needs to be taken into consideration when utilizing smaller particles for packing chromatographic columns.

And one needs to be very careful that the pressure rating on the pumps are never exceeded. Otherwise, our pumps that pump the solvent or the mobile phase into the column will start undergoing rapid performance decrease.