Quantitative Methods in Chemistry
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Lecture-40
Basic Chromatography Part 02

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Now let us get introduced to the concept of selectivity factor. The selectivity factor is the term that comes into consideration when we are considering 2 analytes or more. So, for example, here I have shown you the chromatographic profile of 2 analytes analyte 1 and analyte 2 that are eluting out of the chromatography column and the time that the analyte 2 spends inside the column is much larger or analyte 2 is retained longer within the column than analyte 1.

So, based on this information, we can describe something called a selectivity factor, which is denoted by the symbol alpha and that is nothing but the ratio of the distribution constants of analyte 2 and analyte 1, this is denoted here in the equation as K c2 divided by K c1. The selectivity factor can also be described in terms of the retention factor and it is nothing but the ratio of the retention factors for the analyte 2.

And analyte 1 and this again can be written in terms of the retention times and void times in the equation that is given below, where the retention time 2 minus the void time is divided by the retention time for analyte 1 minus the void time for the column. Now, please note here

that alpha is always described as being greater than 1. So, on the numerator we have the analyte which is retained longer on the column.

So, far we have got introduced to the concept of distribution constant, selectivity factor and the retention factor.

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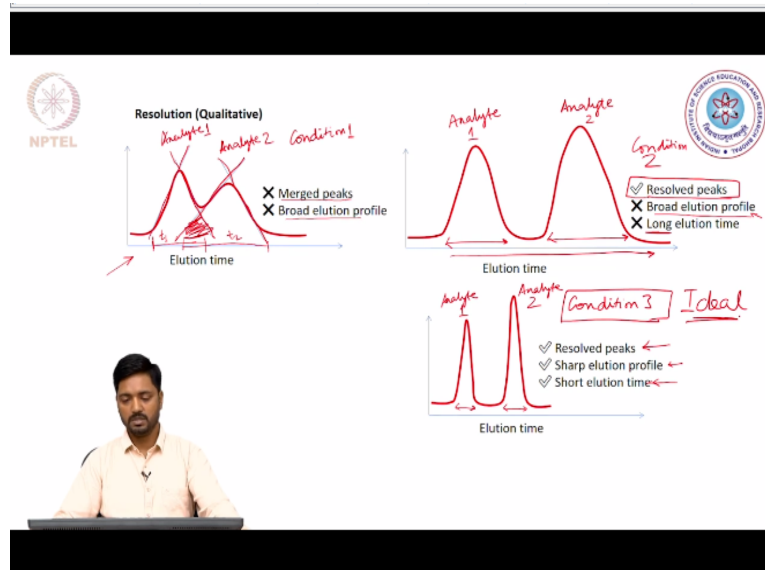

Now, let us move to the concept of resolution. So, resolution is a very important concept in chromatography and I will first describe to you resolution in a qualitative manner and then we will see how we can quantitate this resolution from the chromatographic profile or chromatogram that we have generated for our analytes. So, again here, what we can see is a chromatographic profile, where 2 analytes say analyte 1.

And analyte 2 are eluting out of the column. Now, what we see in this profile is that, these analytes are giving merged peaks in this chromatographic profile alongside what we observe is that there is a broad elution profile. That means, the times that the analytes take to come out of the column are significantly spread out, which now we denote as t 1 and t 2 and what I have also drawn are these tangents to show you how there is merging of peaks.

So, this region we have a significant mixing of the 2 analytes and I have shaded that region for you. And this obviously is not a very good scenario, because we are not able to separate out the analytes that we intended to separate, a very simple solution to this would be to either reduce the flow rate or increase the column length. When we do that, we can reach a condition something like this, where now the 2 peaks are decently resolved.

That means we will be able to separate out analyte 1 from the analyte 2. However, there are 2 major problems here, which are still existing. One is that these profiles are still pretty broad. And we can see that we have increased the elution time. And as already described, if an analyte stays too long within the column, it can start undergoing degradation and we start losing out on the resolution because the peak starts spreading out.

So, and moreover, we will not be able to carry out our chromatography in a fast paced manner. So as we moved from condition 1 to condition 2, we have seen that we achieved success in terms of having a well resolved peak. However, we are still facing the problem of having a broad elution profile and we require a very long elution time to achieve this separation.

Now, the best case scenario is shown here, where the 2 analytes are giving well resolved peaks. And they also have a very sharp pollution profile. That means, this spread here is very small and we also have a short elution times. So that we get a much better resolution. So, ideally, our aim is to achieve condition 3 in our chromatography. So, this is our ideal case scenario and chromatography people strive to achieve chromatograms that are sharp, well resolved and still require short elution times.

Now, after attaining this qualitative understanding of the resolution, let us try to see if we can have a quantitative understanding of the resolution.

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So, before that I would also want to quickly highlight to you that sometimes as chemists, we utilize certain unique interactions between the analyte and the stationary phase to improve the resolution of our columns. So, in this particular example, people have taken silica particles and have impregnated them with metal ions such as silver ions, which have some interaction with the pi electron cloud of alkenes.

And this allows the alkenes to be retained somewhat longer within the column and allows us to achieve an efficient separation of isomeric alkenes. If we are not using silver impregnated silica particles, the resolution is much poorer and we improve the resolution by exploiting the specific interactions between the stationary phase which is the silver impregnated silica and the analyte, which are our isomeric alkenes.

Now, let us look at what would be the quantitative analysis of a chromatogram. So, I have shown here, the detector response with the elution time for the 2 analytes the blue and the red analyte which having retention times the tR 1 and tR 2 and the width at the base of the peak is given as w 1 and w 2. So, by resolution we would want to inform the reader or the researchers as to how far apart the analyte bands are with respect to the width of the bands at the baseline.

So, this is shown here in terms of the retention times, differences between the analyte 2 and the analyte 1 divided by the sum of the widths of the peaks or the bands at the baseline and this whole is divided by 2. So the resolution can be very straightforwardly calculated from the chromatograms if we know tR 1, tR 2 the widths 1 and widths 2. So, these of course, are very readily available from the chromatographic profiles.

So, it would be straightforward to calculate the resolution for the peaks of our interest. Now, when we do that, we observe that the resolution value of 1.0 indicates that these bands are just about resolved, but they still contain about 4% of the contaminant in them. So, that means, we will be talking about chromatography profile where we have something like this and if we extend this, this regime here will contain about 4% of the other analyte.

On the contrary, if we have the resolution value as 1.5, we are talking about a profile that would look something like this and this indicates that there is negligible amount of the contaminant present in the 2 peaks. That means, these bands are well resolved at a resolution

value of 1.5 and they contain only about 0.3% contaminant at this resolution value. So, depending upon the extent of purified material that we need, we should choose our elution conditions.

So, that well resolved peaks can be generated for the analytes that we want to separate. Now, the resolution value can also be expressed in terms of the selectivity factor.

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And the retention factor and that equation is shown on the board as R s equal to square root n by 4 into alpha minus 1 divided by alpha and this multiplied by the retention factor. So, alpha here is our selectivity factor which is nothing but the retention time for the analyte 2 minus the void time divided by the retention time for analyte 1 minus the void time. Similarly, K B is or K A is our let us put this as K.

Because we have been studying this as K A instead of K B. So, K A is our retention factor and that is nothing but the ratio of the time the analyte spends on the stationary phase divided by the dead time or the void time. So, here the term N is a new term and that is the number of theoretical plates present in the column. In the next lecture, we will be talking about the concept of theoretical plates. How do we calculate the number of theoretical plates and the plate height count for chromatographic separations. Thank you.