

Course: Adsorption Science and Technology: Fundamentals and Applications

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Week 07

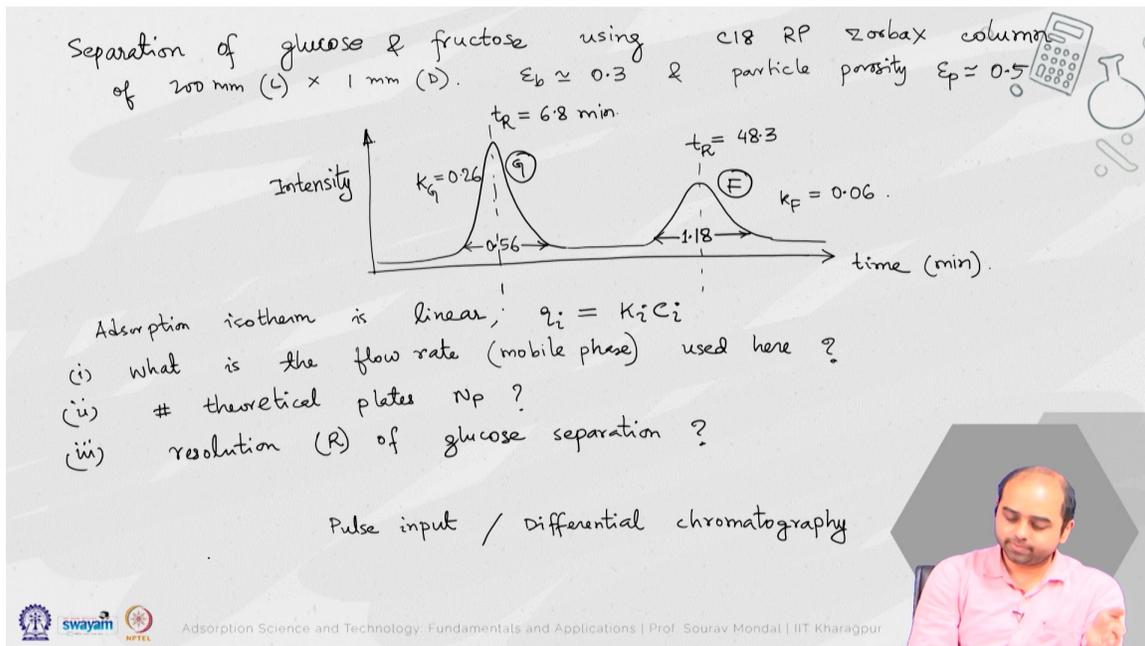
Lecture 34 | Chromatography: Illustrative Problems 1

Hello everyone, welcome to this class on the chromatographic separation. As you know we have been discussing on different principles of chromatography and its use in you know practical applications particularly for separation of very high purity compounds with extremely high selectivity. In fact, chromatography is one such technique where you can separate isomers of a compound with very high selectivity and purity and that is why it is used for enrichment of certain products for purification for separation particularly which is essential in the pharmaceutical industry. Now, today we are going to talk about in this class is related to illustrative problems I mean we will work out some problems and see how this concepts whichever we have discussed. So, far in this week can be used for the study or in practical applications in analyzing chromatograms or in designing chromatography columns. So, let us talk about the first problem which is related to the separation of glucose and fructose.

So, this is one particular example where you see both glucose and fructose has almost as a same molecular weight and their chemical structure is almost same except some subtle differences, but and these are essentially stereo isomers, but they can be separated with high degree of you know selectivity using a chromatography technique. So this the problem is on separation of glucose and fructose using C 18 reverse phase you know Zorbax column. So, this is a chemical sorry commercial name C 18 stands for carbon you know column and RP stands for reverse phase. These are all chemical structure of the adsorbent which is related to it and these are very standardized and they have certain degree of their pore size their particle size and everything.

So, depending on that so these generally have very low particle sizes. So, that the separation peak can be really sharp and there are several such columns which are you

know prepared keeping in mind for separation of certain class of compounds and certain over different range of pH and other parameters or ionic strength etcetera. 200 mm that's like the length cross 1 mm that's the diameter of the column bed porosity is 0.3 30 percent and particle porosity is 0.5. Of course, these parameters you know does affect a lot and we will see that you know what are the information that is needed, but this is like the general specification of this column not necessarily all of them could be needed for solving a particular problem, but these are the general specifications of the column. The chromatogram in this case looks something like this. So the first peak is for glucose and the second peak is for fructose. This is time and this is like some sort of intensity or the you know optical signal from the equipment. So this is for glucose this is for fructose the yes mean residence time here is 48.3 so everything is in minutes and here the time is 6.8 minutes. The adsorption isotherms are linear and it follows the relationship this. So K for glucose, I write it as k_G is 0.26, k for fructose is given as 0.06. The base width of this one is 0.56 minutes and this is slightly wider or broadened 1.18. So please note that this diagram is not up to the scale so you can realize that whatever this i am showing on writing down the numbers particularly in the time scale and the time in axis is not related to scale, so the question that is asked that what is the flow rate that is the mobile phase or the element used here. Next is number of theoretical plates N_p .



The third is the resolution, are of glucose separation which gives us the, a metric on how good is this separation efficiency. Now coming to the first part of the problem, this, as you realize the the chromatographic process output is given through this chromatogram and all of you realize that this is a problem of pulse input or differential chromatography so this is a problem related to pulse input or differential chromatography. Now the

profiles these are like the parts to the solution. The profile the chromatogram profile is Gaussian in nature, with solute mean residence time this t_R is L by this solute wave front velocity. So, this is the solute wave front velocity.

velocity. Now we understand that since there are two residence time in this problem the solute wave front velocity for the case of glucose and the case of fructose would be different. And these essentially you know if you if you work out the separately what is the solute wave front velocity for both of these material it should correspond to the residence time that we see in this case. Now, this solute wave front velocity is given by this you know v_i or u_i the interstitial velocity divided by $1 + 1 - \epsilon$ this bed porosity and this is sorry bed porosity and dQ_i by dC_i . So, for linear isotherms this converts to simply K_i . Now this is the solute wave front velocity the the velocity that we need to use in this case would be the the superficial velocity.

But from the residence time analysis we can work out what would be our solute wave front velocity and from there you can work out what would be the superficial velocity. So, the superficial superficial solution velocity U_s is given by the bed porosity to U_i right. So, it is now time to use some numbers. So, using the so for part 1 using the glucose peak information we know that T_r for glucose is 6.8 minutes, the L for this column is given as 200 mm, the bed porosity is given as 0.3, the k of glucose is given as 0.26. So, from here one can work out what would be the velocity u_i . So, u_i is, sorry, sorry u_c . that is 200 by 6.8 minutes so that gives almost 29.4 mm per minute or 2.94 centimeter per minute now u_i is u_c multiplied with $1 + 1 - \epsilon$ by ϵ is 2.94 , $1 + 1 - 0.3$ by 0.3 . 4.72 centimeters per minute. So, from here one can work out the superficial velocity. So, the superficial velocity would be u_s that is this quantity and this turns out to be 1.417 centimeter per minutes. Therefore, the volumetric u would be πd^2 by 4 that is a column cross section multiplied with the superficial linear superficial velocity.

Solution. Chromatogram profile is Gaussian in nature, with solute mean residence time,

$$t_R = \frac{L}{u_c}$$

← solute wave front velocity

$$u_c = \frac{u_i}{1 + \frac{1-\epsilon_b}{\epsilon_b} \frac{\partial q_i}{\partial c_i}} = \frac{u_i}{1 + \frac{1-\epsilon_b}{\epsilon_b} K_i}$$

Superficial solution velocity, $u_s = \epsilon_b u_i$

Using the glucose peak information, $t_R = 6.8 \text{ min}$ $L = 200 \text{ mm}$ $\epsilon_b \approx 0.3$ $K_A = 0.26$.

$$u_c = L/t_R = 200/6.8 = 29.4 \text{ mm/min (or } 2.94 \text{ cm/min)}$$

Now, $u_i = u_c \left[1 + \left(\frac{1-\epsilon_b}{\epsilon_b} \right) K_i \right] = 2.94 \left[1 + \frac{1-0.3}{0.3} (0.26) \right]$

$$= 4.72 \text{ cm/min.}$$



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So, if you put the numbers this will come out to be I am writing all this in centimeter square. So, this will turn out to be 0.01 ml per minute. So, in this case you can see that we using this information of the superficial velocity you can work out the volumetric flow rate. Of course, in this part we have used the information for the glucose you know this calculations are for the glucose peak information, but if you work out for fructose you should land up with the same value of the volumetric flow rate or at least close to this value.

Now, coming to the next part where we have to find out the number of the theoretical plates first thing one needs to calculate is the standard deviation. This is sigma square is given as $t_R H$ by u_c where of course, H is the height of the theoretical plate. So, for Gaussian curve this is something we have discussed in the previous class. This peak base width is 4 times the standard deviation. So, from here this we can work out let us call that peak base width as P_b .

So, you know that we already have this formula for the number of theoretical plates as $16 t_R^2$ by P_b^2 which gives us the value that 16×6.8^2 square by. So, base width for glucose is 0.56 square and roughly this would give us a number of 2359. So these are the number of theoretical plates that is needed for this separation of this glucose.

Coming to this resolution, resolution R of 2 solutes is defined as the ratio of the difference let us say for glucose minus the glucose and we take the modulus divided by this peak width of at the base width of glucose plus base width of glucose the mean base width divided by 2. So, if you use the numbers and minus 48.3 modulus of this one. The difference is important in absolute sense. Then we take the average of the base width.

Superficial velocity, $u_s = \epsilon_b u_i = 0.3(4.72) = 1.417 \text{ cm/min}$
 \therefore Volumetric flowrate, $Q = \frac{\pi b^2}{4} u_s = 1.417 \left(\frac{\pi (0.1)^2}{4} \right) \approx 0.01 \text{ mL/min}$

(b) Standard deviation, $\sigma^2 = \frac{t_R H}{u_c}$ ← height of the theoretical plate
 For gaussian curve, $\text{peak base width} = 4\sigma$
 So, $N_p = 16 \left(\frac{t_R^2}{t_B^2} \right) = 16 \left(\frac{6.8^2}{0.562^2} \right) \approx 2359$

(c) Resolution R of two solutes is defined.
 $R = \frac{|t_{R|G} - t_{R|F}|}{(t_{B|G} + t_{B|F})/2} = \frac{|6.8 - 48.3|}{(0.56 + 1.18)/2} = 47.7$

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So in doing so, this gives us a number of 47.7. So, please note that the resolution in this case is actually very high any resolution above 10 is actually very good for separation and in this case we have achieved almost 47.7 and primarily because of the fact that the difference between the residence time or the retention time is very large. Now, let us look into one more problem based on similar lines, but with slightly different information.

So, the second problem is related to again you know separation of two compounds are binary mixtures. So, separation of a binary mix of A and B using a 30 centimeter long almost a foot long chromatography column the eluent or the elution rate is 0.15 centimeters per second. This is like the linear velocity superficial velocity of the mobile phase and the capacity factors defined as k_A is 9.0 and AB as 6.67. So, please note that this capacity factors relates to the inverse. This you know the fraction of the compound that is present in the elution phase with respect to the solute phase. So, k_A can be related as k_A can be related in this case this is not the equilibrium isotherm constant k in this case. This relates to the capacity factor and that is related to the ratio of how much is the solute that is present in the you know the solution phase with respect to how much is the solute that is present in the fractional amount of the solutes. Essentially this for linear you

know this isotherms this can be like dq by dc , but essentially this is a different terminology and something that we have already defined as $1/w$ in the previous lecture. So the first part that we have to calculate that what is the difference with this information difference in retention time of the compounds A and B and selectivity.

So for finding out the difference in the retention time the first thing we have to find out is the dead time. So dead time for the mobile phase we can consider this to be t_m as L by V . So, you can consider that the dead time is actually the amount of the time that something related to the the residence time of the mobile phase. So, how much time does it take to elute the entire bed is something like the dead time and after this only the solute wave front will start to enter is like 30 centimeter by 0.15 centimeters per second this gives a value of 200 second.

Now in this case the capacity factor can be related to the residence time. So, essentially we know that K' can be related to the the ratio like this. So, t_{RA} minus t_{RB} can be related as k' of A minus k' of B into t_m right. So, if you write down the values of k' for 2 different components.

Separation of binary mix of A & B, using 30 cm long chromatography column. The elution rate is 0.15 cm/s and the capacity factors $k'_A = 9.0$ & $k'_B = 6.67$.

(a) Difference in retention times of the compounds & selectivity.

Dead time for the mobile phase, $t_m = L/v = 30 \text{ cm} / (0.15 \text{ cm/s}) = 200 \text{ s}$.

$$k'_A = \frac{t_{RA} - t_m}{t_m} \quad k'_B = \frac{(t_{RB} - t_m)}{t_m}$$

So, $(t_{RA} - t_{RB}) = (k'_A - k'_B)t_m = (9 - 6.67)(200 \text{ s}) \approx 7.8 \text{ min}$.

Selectivity (of A over B) $S_{AB} = \frac{t_{RA} - t_m}{t_{RB} - t_m} = \frac{k'_A}{k'_B} = \frac{9}{6.67} = 1.35$

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So, this is like k_A . So, we write t_{RA} similarly I can write also k_B as t_{RB} minus the dead time by the dead time. So, if you put the numbers in this case this is like 9 minus 6.67 multiplied with 200 seconds. So, this gives us a roughly a number of 7.8 minutes.

Selectivity of A over B is defined by $S_{A/B} = \frac{t_{RA} - t_D}{t_{RB} - t_D}$ is t_{RA} minus the dead time divided by t_{RB} minus the dead time. Please note the dead time is very important because in this problem you are starting your calculation time with respect to the you know the point when you are injecting your eluent into the systems the understanding of the dead time is very important. and if you work out these numbers I mean this is nothing but the ratio of k'_A to k'_B this will come out to 9 by 6.67 and this is around 1.35. So, this suggests that the selectivity of this compound A over B is greater than 1. So, it is possible to separate these two you know compounds within this chromatographic column with this eluent rate or the elution rate. The next is to calculate the the height equivalent of each theoretical plate. if the peak width that is t_B of compound A is 150 seconds right. So, to calculate this we will once again use this formula for the number of plates and the height equivalent plates, but first it is important to quantify the retention time of or the mean retention time of the compound A.

So, that would be $k' + t_m$. So, this is 9 plus 1 multiplied with 200 and this obviously gives us a value of 2000 seconds as you can see. Now, peak with t_B is given as 150 seconds. So, number of theoretical plates N_p from the formula itself we know $16 \times t_{RA} / t_B^2$. So, in this case that is $16 \times (2000 / 150)$ squared squared. So, this comes to around 2840, and the height equivalent of each theoretical plate H_{ETP} or also known as this H that we have written down is length divided by the number of theoretical plates in this case we are not since we do not know the information of the chromatogram I cannot use the technique for analyzing the you know the standard deviation and everything what is the peak height etcetera from that we cannot bring in those relations here instead I can use a simple correlation that the total length of the column is nothing but the height equivalent of each plate with the number of plates give the total height so using this relation you can easily work out the value so that would be like 30 centimeter by 2840 or that is which is 1.06 mm right of course you can use those other the other formula for finding out the height equivalent but the simpler way is to use this correlation that number of theoretical plates times the height gives the total length of the column the third part is to calculate the resolution calculate resolution between the peaks So, since we do not know the information of the peak width of the compound you know this b or what is the width of that compound you know this other compound b , we cannot use this technique of difference of the residence time divided by the mean peak width. Instead what we are going to use here the formula is this selectivity relation and here we are using the capacity relation. Now please note in this case the capacity factor that we are going to use k' would be like the average of the two capacity factors. and if you put the numbers this turns out to be 7.84 right for this case because this is 9 this is 6.67 this turns out to be 7.14. So, with this information and selectivity we have already found out as 1.35 and this is 2840. So, we will use this numbers.

(b) Calculate HETP, if the peak width (t_B) of compound A is 150 s.

$$t_{RA} = (K'_A + 1) t_m = (9 + 1)(200) \approx 2000 \text{ s.}$$

$$t_B = 150 \text{ s.}$$

$$N_p = 16 \left(\frac{t_{RA}^2}{t_B^2} \right) = 16 \left(\frac{2000^2}{150^2} \right) = 2840$$

$$\text{HETP (H)} = \frac{L}{N_p} = \frac{30 \text{ cm}}{2840} = 1.06 \text{ mm}$$

(c) Calculate resolution between the peaks

$$R' = \left(\frac{\alpha - 1}{4} \right) \left(\frac{k'}{1 + k'} \right) \sqrt{N_p}$$

$\alpha = \frac{K'_A}{K'_B} = \frac{9}{6.67} = 1.35$
 $k' = \frac{K'_A + K'_B}{2} = \frac{9 + 6.67}{2} = 7.84$

$$R' = \frac{1.35 - 1}{4} \left(\frac{7.84}{1 + 7.84} \right) \sqrt{2840} = 4.14$$


So, this resolution comes out to be 4.14. So, we see that the resolution is still higher than 1, but it is not very high as in the previous problem in this case we have seen it to be like around 47 or so. In this case it is around 4. So, which suggest that this is possible, but there is a scope of improvement particularly if the number of you know plates can be increased or if the you know mean residence time can be increased for compound a then the separation between these two peak will have a better degree of resolution. So, I think all of you followed the two problems and you found some information useful information on how to handle chromatographic systems. In the next class we are going to talk about a problem using three components and how that can be used for you know in that case of column chromatography for production of you know high purity or separation of high purity products into three distinct steps thank you and see you in the next class.