Course: Adsorption Science and Technology: Fundamentals and Applications

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Week 07

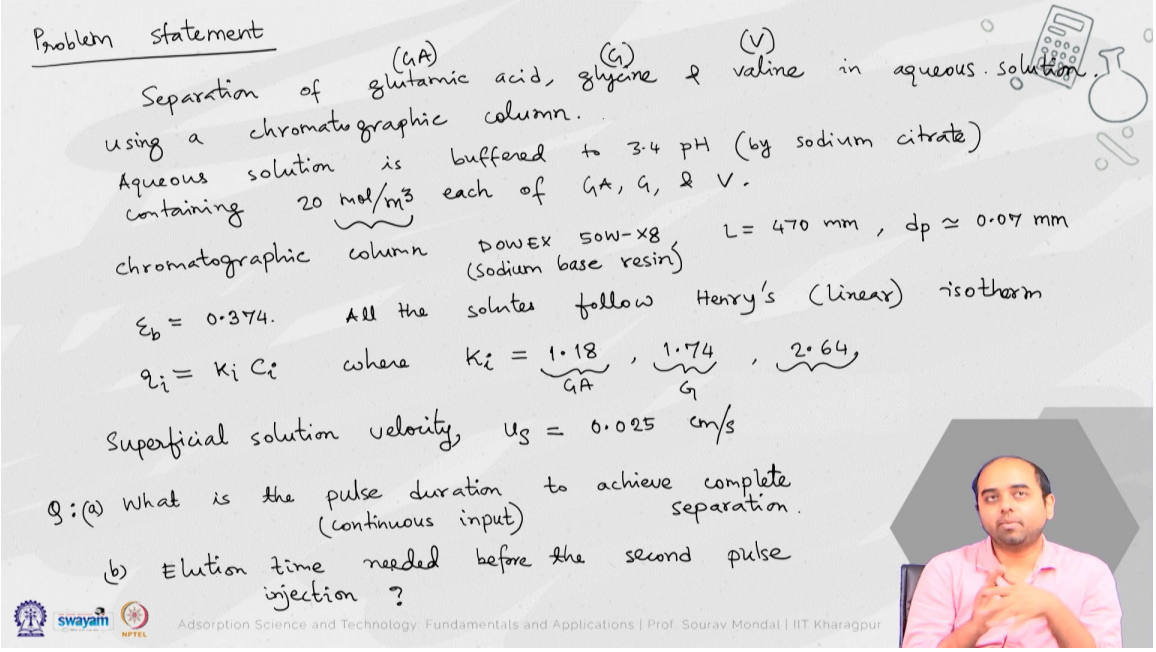
Lecture 35 | Chromatography: Illustrative Problems 2

Hello everyone, welcome to this last lecture on this week on chromatographic separations. So, in this class we are going to talk about an interesting problem related to the separation of three compounds in aqueous solution and particularly you know what should be the pulse duration for each of these components to be completely separated before starting the next pulse. So, this is a particular way of how you do you know column chromatography for actually production of these high purity streams of these components. And essentially this comes under this category of you know displacement based or the frontal based techniques that we have talked about in one of the previous classes in this week. So, this is a particular you know problem because this technique of frontal displacement is blessed understood with with the help of a problem. So, let us look into the problem straight away.

So, the idea is to separate the problem statement is separation of glutamic acid glycine and valine. So these are all amino acids in aqueous solution in aqueous solution, using a chromatographic column. Now, some of the properties is that the aqueous solution is buffered to three point to a specific pH because these are all very sensitive to pH and the solute solvent or the solute mobile phase interaction and the solute in the mobile phase and the solute in the solid phase or stationary phase interaction is highly dependent on the pH and ionic strength. So, using sodium citrate this is how the buffer is treated now this contains, containing you know 20 moles per second.

meter cube each of. So, this is you know this concentration of these each of these components each of glutamic acid glycine and valine. So, I use this abbreviation glutamic acid glycine and this is valine. The chromatographic column that is used generally for amino acid separation is DOWEX 50W, these are like you know classifications of

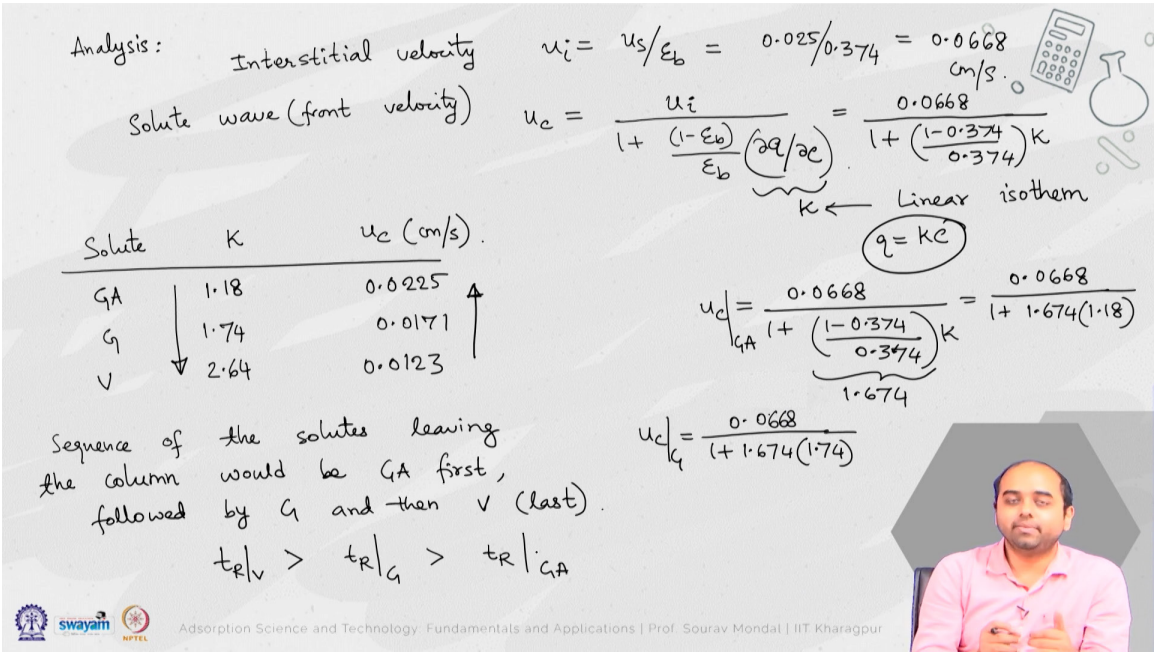
different columns and the length is 470 mm this is very long almost half a meter long and the particles so this is a actually a resin column sodium base resin so this is a sodium base resin. particle diameter is 0.07 mm and bed fraction bed porosity is 0.374. So, all these all the solutes follow Henry's law all this linear isotherm that is q i is equal to k i c i where k for k i is are for glutamic acid is 1.18. So, this is glutamic acid then for glycine it is 1.74 and it is 2.64 for valine. Superficial solution velocity u s is 0.025 cm/s actually very low. You can work out from the superficial velocity what is the flow rate of the mobile phase.



So, now the question is what is the pulse duration? So, the here the pulse is not a injection of a you know in similar to a delta direct delta function. So, pulse is like a continuous input. So, what is the duration of continuous input or this pulse duration. So, this is the continuous input that is needed to achieve complete separation and the second part is time duration or the illusion time I would before elution time needed I would say needed before the second pulse injection So, after all the three components have been successfully removed from the system then only you can start the second pulse injection right. So, what is that time of the extended elution that one needs to introduce into the system before the second pulse or second input is possible ok.

So, now this is the problem statement now let us look into the solution or the analysis. So the first thing that we need to do is to calculate the interstitial velocity interstitial velocity that is u i is you know this separation sorry superficial velocity divided by the porosity and in this case if you put the numbers. This comes to around 0.0668 centimeters per second. Now next is the solute wave solute wave or a solute front velocity which is we

have already know the formula, this is d q by d c i right or I can write let us not confuse this index i with the interstitial velocity this d q by d c . So, in this case this is nothing, but k we know for Henry's law for linear isotherm this correlation of k times q c, dq/dc turns out to be k constant. So, using the numbers here you can see that this is 0.0668 divided by 1 plus 1 minus 0.374 by 0.374 times the k of different components. So, for the different solutes if you try to test tabulate the k values and the you know this, solute wave front values in terms of centimeter per second so for the glycine the value is one point one eight and u c for glycine can be worked out as point zero zero six eight and this one is one plus whatever this term that we have one minus point three seven four by point three seven four this value is 1.674 time k. So, this is 0.0668 by 1 plus 1.674 and for glutamic acid this value is 1.18 k. So, turning out this value turns out to be 0.0225. So, of course, all the solute wave front velocity will be less than the you know this interstitial velocity.

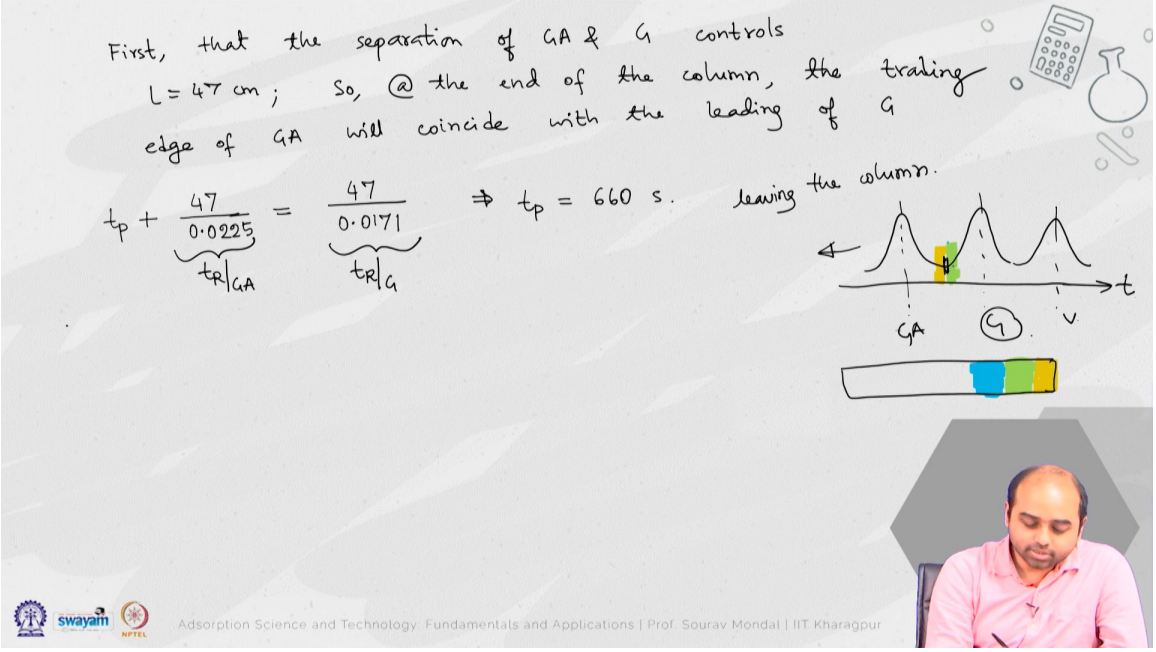


For the case of glycine similarly you can work out what would be the interstitial velocity. So, this would be for glycine. So, this is for glutamic acid for glycine this is 0.60668 by 1 plus 1.674 multiplied with 1.74 this is 0.0171 for valine so this is the value is 2.64 and this is quite less almost half of glutamic acid, right. So, it is understood that from the k values as you see the k value increases from glutamic acid to valine and as a result the solute wave front velocity decreases, you know, in the case of from starting from glutamic acid to valine. So, which suggest the sequence of the solutes leaving the column. So, sequence of the solutes leaving the column would be first would be GA to be first followed by glycine and then valine so valine would be the last or in other words the

mean retention time of valine would be the highest um followed by glycine and followed by glutamic acid so the mean retention time tR of valine would be larger than tR of valine glycine which is larger than tR of glutamic acid.

So, hence the valine would be the solute that will be leaving at the end or a final solute that will be leaving this column. These solutes or solute bands would be leaving in sequence. If you recall the introductory class of this week there I have talked about how the solute bands are actually propagating and moving in this column and since the solute wave front velocity of valine is the smallest that will move slowly compared to the other solutes. So glutamic acid will be leaving the first from this column. So in the first case we assume that the separation of this glutamic acid and glycine controls the overall you know situation and in this case the bed length is 47 centimeter just remembering.

So at the end, so at the end of the column, the trailing edge of glutamic acid, the trailing edge of glutamic acid will coincide or will be close with the leading edge. So, what I mean is this if there are three solutes which is leaving the column the first one would be this for glutamic acid and immediately after that would be the glycine and then valine. So, then the ideal scenario after the glutamic acid wave front has completely moved out of the column it is time for the next one to start and hence the peak of that next column will be like the leading edge so this is like how they are leaving the column right leaving the column. So, this is the time. So, first would be the glutamic acid.



So, as soon as the glutamic acid finished you know it is the leading edge of the this glycine that will start to come out. So, in the column this would be like bands of first the band of sorry first the band of you know this glutamic acid then we will have the band of glycine followed by the band of valine. So, essentially when the final amounts of glycine glutamic acid leaving the column that is like this stage. So, this is like the stage where we are having the interface of glutamic acid and glycine. So, in this case when glutamic acid leaves the system the trailing edge will coincide trailing edge of glutamic acid will coincide with the leading edge or the leading front of the you know this glycine and in the chromatogram this would look like the start when of the peak of you know this glycine.

So, if you try if we try to find out the time needed for the pulse to achieve this scenario which is tp would be 47 centimetres divided by the velocity of wave front velocity of you know this glutamic acid. I mean this time should be equal to the time needed for the or the mean residence time for the wave front of glycine to come in the picture. So, this is the mean residence time of glutamic acid, this is the mean residence time of glycine right. So, the pulse duration would be such that the the it it reaches till the point when the glutamic acid has completely flushed out of the system. So, thus the time it needs to reach the.

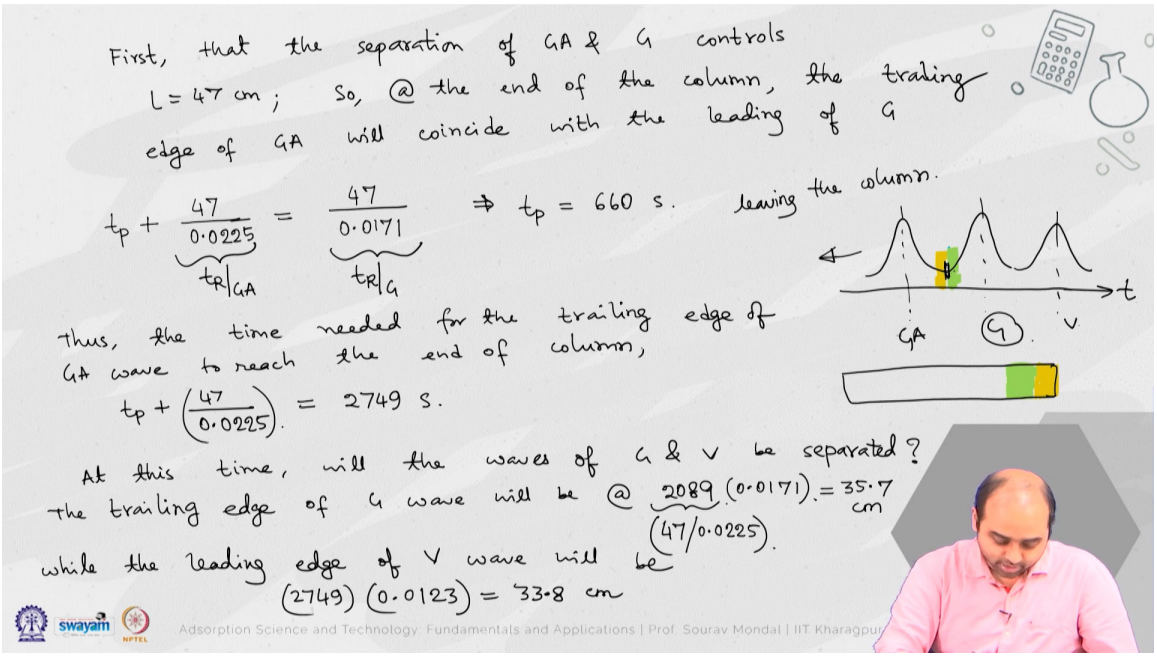
So, this is how we calculate that the time it needs. So, this is essentially the time needed

for the sorry this time of mean residence time of the glycine is also equivalent to the time needed for the trailing edge of the glutamic acid to reach the end of this column. right. So, if you see this if you put this work out this numbers this tp comes to around 660 second. So, thus the time needed for the trailing edge of glutamic acid wave or front to reach the end of column would be tp plus 47 by 0.0225 and that turns out to be 2749 second right. So, this is the time when completely the glutamic acid has moved out from the column. So, at that time the question is at this time will the waves of glycine and valine be separated. So, that is something we have to check. If that is not the case then that controls the separation and that has to be considered.

So, the trailing wave we have to find out the location. So, the trailing of this glycine sorry the trailing edge of glycine wave I should say the trailing edge of glycine wave will be at what this 2089 multiplied with 0171. So, 2089 is the mean residence time of the glycine 47 by 0.0225 right.

So, that multiplied with 0.0171 gives 35.7 centimeter right 35.7 centimeter and while the leading edge leading edge of valine wave will be at that point will be 2749 times the valine solute wave front velocity 0123 that is equal to 33.3. So, it clearly shows that the trailing edge in the column the trailing edge of valine sorry of glycine will be further ahead by almost you know 2 centimeters of difference from the leading edge of the you know valine wave.

So, if we maintain this you know time of 2, 7, 4, 9 seconds for the pulse input. That is when the trailing edge and the leading edge of glutamic acid and glycine will be just coinciding and in that case there will be a difference of almost 2 centimeters in the bed between the trailing edge of the glycine with the leading edge of valine. So, it means that here one will have. So, as as soon as it leaves towards the column. So, here we will be having a difference of almost 2 centimeter.



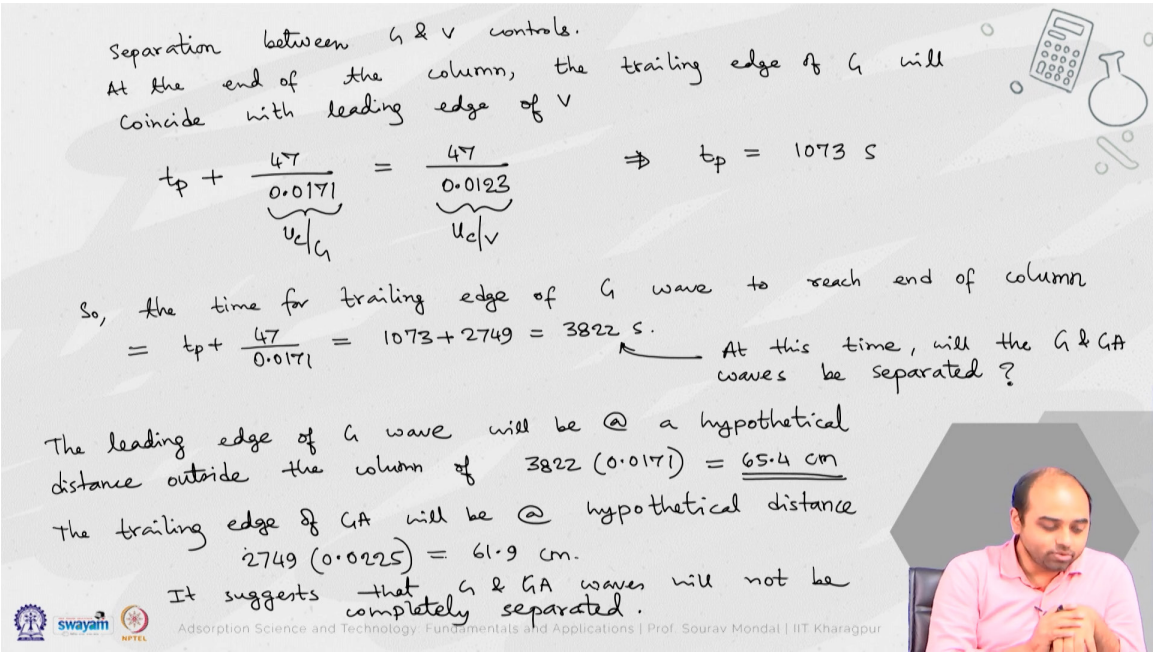
So, this will actually look this picture will actually look something like this where there is a finite difference between the the glycine and the valine wave front. So, this is like glutamic acid wave front this is glycine and this is valine. So, that is how these 3 peaks can be separated. Now, let us assume that the separation between. We want to see if the pulse input can be reduced.

Separation between lysine and valine controls the process. So, in that case at the end of the column we use this idea that the trailing edge of glycine will coincide with leading edge of valine. So, in that case the pulse input time would be something like this. So, this is the solute wave front velocity of valine and this is the solute wave front velocity of glycine and working out the numbers the pulse input duration is 1073 second. Now the important thing to check at this point is whether the trailing edge when what happens when the trail I mean the the separation between the glycine and the glutamic acid waves.

So, the time first thing to work out is the time for trailing edge of this glycine wave to reach reach end of column would be t p plus 47 by 0.0171 and if you put the numbers this turns out to be 1073 that is t p and this is 2749 this is 3822 second. So, now the question is at this time, at this time will the glycine and glutamic acid waves or wave front will those be separated? Of course, the leading edge of glycine wave will be at a hypothetical distance outside the column of this 3822 seconds times 0.0171 the glycine wave front

velocity and that is around 65.4 of course, this is outside the column, but we want to find out whether that will be separated or not and the trailing edge of.

So, this is like the glycine is in front of sorry glutamic acid is in front of glycine. So, the trailing edge of glutamic acid will be at again hypothetical distance distance somewhere . So, this is the time 2749 times the wave front velocity of glutamic acid and this is 61.9 centimeter. So, this value is lower than the value where the leading edge of glycine wave will be there.

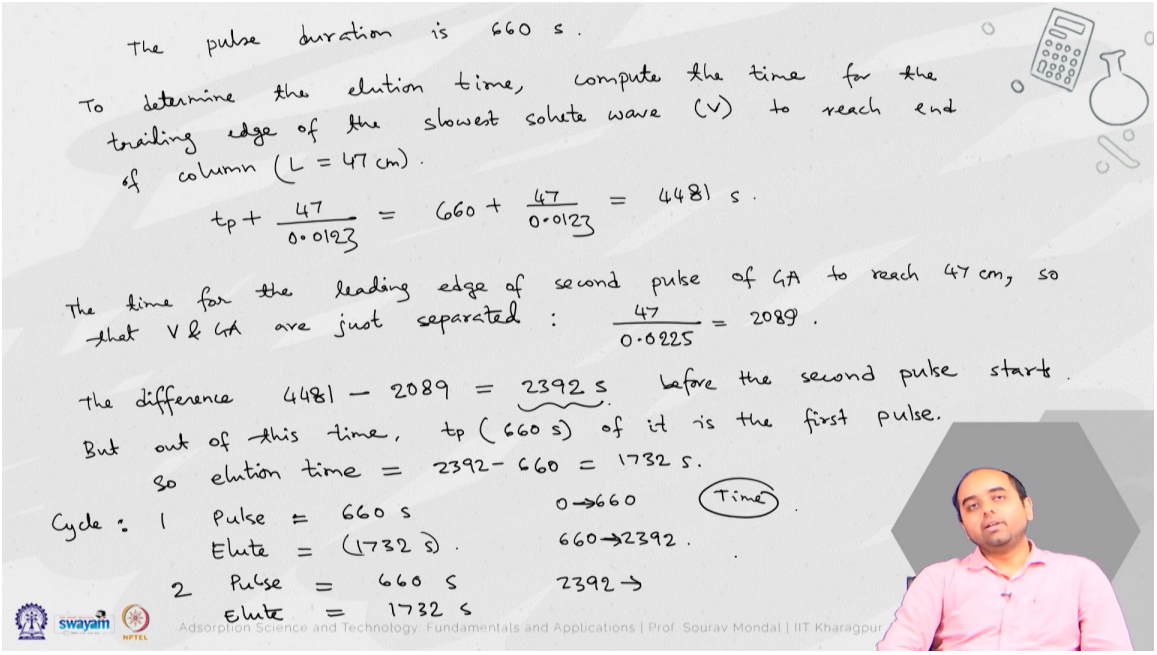


So, it means, that it suggests that glycine and glutamic acid waves will not be completely separated. So, in that case, we realize that the that the separation between glycine and valine cannot be considered as the you know this controlling factor instead we can consider the separation between glycine and glutamic acid can be the controlling factor. So, in that case the pulse duration, the pulse duration that is needed in this case is 660 seconds. Now to determine the so this is the first part next part is to determine the elution time deletion time we have to compute the time for the trailing edge of the slowest you know solute wave in this case this is valine to reach end of column that is 47 centimeter. and this time is what this time is pulse time plus 47 divided by 0.0123. So, using a value of 660 plus this number this gives us a value of 4481 seconds. Now the time for the leading edge of second pulse of glutamic acid to reach 47 centimeter at the end of the column so that VA and GA are just separated is also very important we do not want in the second pulse when the GA comes the VA of the previous pulse trailing edge also coincides. So, there has to be a separation just separate and this time would be the time

taken for the glutamic acid to reach the end of the column is 2089. So, the difference this 4481 that is the time taken for the lead trailing edge of valine to go out from the to go out from the system minus the time needed for the you know this wave front to reach the of the second pulse to reach the end of the column is 2089. This is the difference that so, this is around 2392 is the difference that is needed this much second before the second pulse can be started.

But out of this time out of this time out of this time E p or 660 seconds of it is the first pulse. So, illusion time is 2392 minus 660 this turns out to 1.72 seconds. So, the cycle looks something like this if you try to get a picture of the time. You send in a pulse of 660 seconds first from 0 to 660 seconds then you use a elution or elute the system for 1732 seconds then you so this is like fast pulse this is like then you inject the second pulse again for 660 seconds so like this again the second elution would be of another 1732 seconds So, this is like it is getting added up you know this is I am just putting this symbol.

So, the actual time would be added up in all of these steps. So, first is like 0 to 660, then in the second case you will be adding up. So, if I try to write the you know the time of the operation. So, first is like 0 to 660, then is from 660 to 2392 seconds. Then it is from you know I mean this period you will do this illusion that is covering this value of 1732 seconds.



So, from again 2392 another 660 seconds if you add this will be like the second pulse and so on it will be continuing like this. So, that is how the time you know clock or the time of operation would be proceeding for this calculation. So, this is a very practical example of frontal you know chromatographic separation system where you do this pulse and elution in synchronization to separate you know more than 2 or 3, 4 compounds and you get you know high purity compounds separated or you know captured from the exit or from the end of this column. So, longer is the length of the column the better is this separation efficiency. Thank you everyone I hope all of you like this week on chromatographic separation.

In the next week we are going to talk about ion and ion exchange and which is also a very nice and interesting application of adsorption science.