Modern Instrumental Methods of Analysis

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Module no. #07

Lecture no. # 40

Chromatography -1 Introduction

Welcome to our class on chromatography I had explained in detail about the introductory part of the chromatography in the last class what I had told you was that the term chromatography embraces a family of closely related separation methods based on the observations of doctor Michael tweet and his experiments which were done somewhere around nineteen hundred and 3 to nineteen hundred and 6.

The importance of chromatography lies in the its use as an analytical separation tool rather than the preparative technique. So, it serves as a means of resolution of mixtures for the isolation and partial description of the components whose presence may be known or suspected; that means, you should know the components beforehand if you want to separate them.

In chromatography the components to be separated are distributed between a stationary phase and a mobile phase and the mobile phase is the a liquid usually a liquid or a gas and stationary phase is a solid the solid is loaded onto into a column in which it is fixed.

And the mobile phase is allowed to percolate through the stationary phase. So, the mass transfer between the mobile phase and the stationary phase occurs either because the molecules of the mixtures are adsorbed on the particle surface or absorbed onto the into or absorbed into the particle pores or they partition into pools of liquid held on the surfaces or within the pores this process is known as sorption.

So, the sorption is the main technique which we use in chromatography separation of the components in a sample is based on the fact that rate of the travel of individual

components is basically ruled by the mass transfer or partition coefficient of the molecules between the mobile phase and the stationery phase.

The partition coefficient of each component determines the rate at which the sample travels. So, each component would be travelling at its own rate depending upon the its partition coefficient

. So, after some time in travel what happens is every part component would be moving at different speeds and they would be distributed in space over the stationary phase and subsequently emerged out of the column as a single component if the stationary phase is long enough to permit the total separation of the components. but you can have a variety of stationery phases as well as mobile phases.

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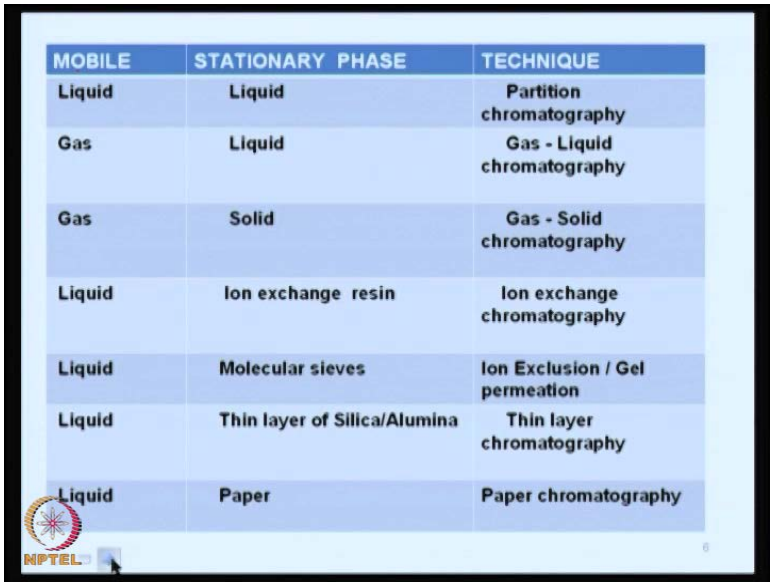

So, mobile phase you can have liquid and the stationary phase also can be a liquid and the technique corresponding technique is known as partition chromatography you can choose mobile phase to be a gas; that means, you can pass a gas through a stationary phase and the stationary phase can be a liquid and such a technique is known as gas liquid chromatography or g L c.

You can have gas and solid and the technique would be known as gas solid chromatography you can change the mobile phase to liquid and stationary phase to an ion exchange resin then we can have ion exchange chromatography then another choice

is you can attempt to separate the components according to their sizes and this can be achieved by using the molecular sieves. So, if you use molecular sieves as the stationary phase and mobile phase as the liquid then it is known as ion exclusion chromatography or gel permeation chromatography.

If your mobile phase is liquid and your stationary phase is a thin layer of silica or alumina then it is the technique is known as thin layer chromatography you can choose to do the separations on paper like cellulose and then your mobile phase is liquid you can have the technique is known as paper chromatography.

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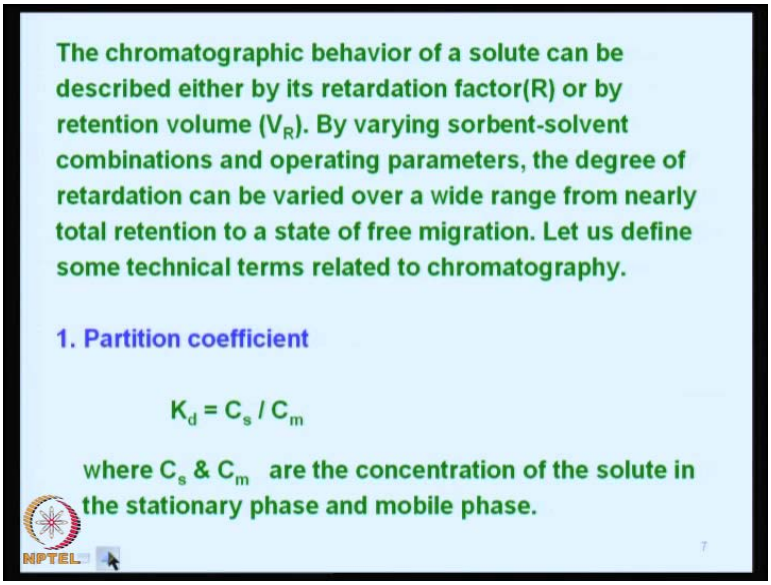

So, all these techniques are ah possible and they have all been developed over a period of time and many of their techniques which I have discussed earlier gas liquid chromatography gas solute chromatography ion exclusion chromatography and this gel permeation all these things have reached a very high level of ah very high level of sophistication and each technique can describe a chapter by itself. So, there are lots of things to learn, but it is important for us to know the important terminology of the gas chromatographic techniques and the chromatographic behavior of a solute can be described either by its retardation factor or by its retention volume that is V R.

By varying the sorbent solvent combinations we can and operating the operating parameters the degree of retardation can be varied over a wide range from nearly total retention to a state of free migration you can either have total retention or in a state of

free migration all you got to do is you should be ready to change the mobile phase using different mixtures of solvents.

So, let us define some technical terms related to chromatography here I am going to define. Firstly, partition coefficient. So, partition coefficient is defined as K d and K d is nothing, but the ratio of the concentration of the substance which you are trying to separate into the stationary phase that is that is denoted as C s and concentration of the same in the mobile phase that is C s by C m.

So, in most of the chromatographic papers research papers etcetera you would like to know what is the partition coefficient of this particular substance and in a given system that is for a given mobile phase and this stationary phase and for a given compound the partition coefficient is defined it would be its intrinsic property.

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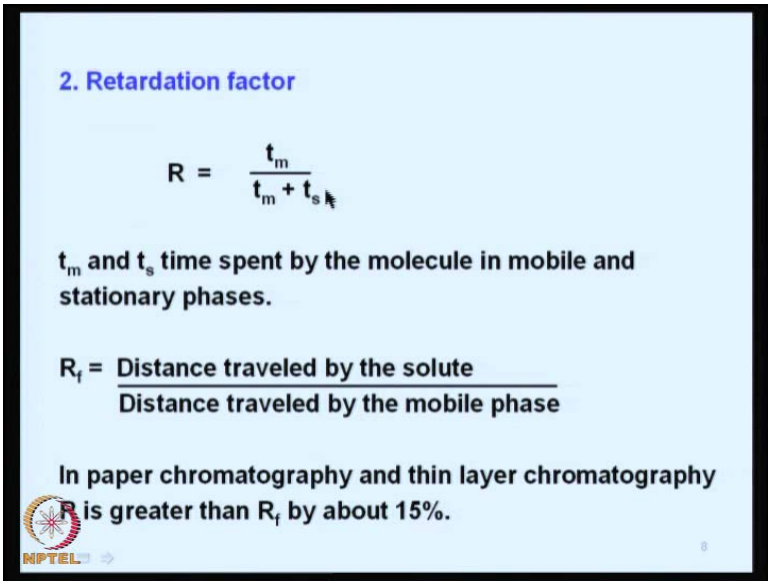

Then secondly, we would like to define another term known as retardation factor retardation factor is basically defined as the distance travelled by the solute that is the sample what you want to separate divided by the distance travelled by the mobile phase. So, it is also defined in terms of the times spent by the molecule in the mobile phase and stationary phase. So, R is defined as t m divided by t m plus t s.

That is time spent by the component in the stationary phase time spent by the component in the mobile phase and the numerator is time spent in the mobile phase in paper

chromatography and thin layer chromatography R is greater than R F that is retardation factor is greater than R F by about 15 percent

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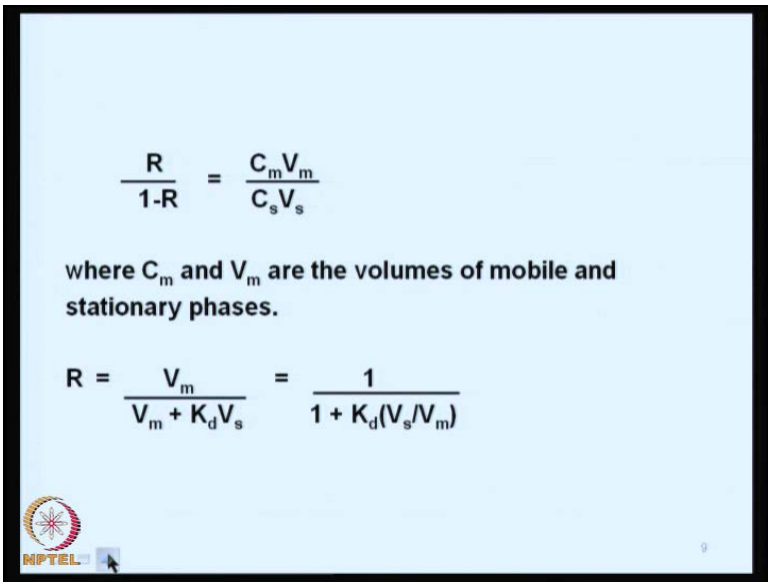

That means, the solvent or the mobile phase would be ahead of the mobile phase would be ahead of the stationary of the solute phase by about 15 percent. now, you can also define the retardation factor in terms of concentration C m into V m that is concentration of the mobile phase multiplied by the total volume of the mobile phase divided by 1 minus R that is concentration of the substance in the solute in multiplied by its volume.

So, where C m and V m are the volumes of the mobile phase and stationery phase. So, R you can also defined in terms of V m solely V m and V s that is given by V m divided by V m plus K d into V s because K d we are going to introduce here if you divide the top expression by C m you would end up with an expression something like this 1 over 1 plus K d into V s by V m.

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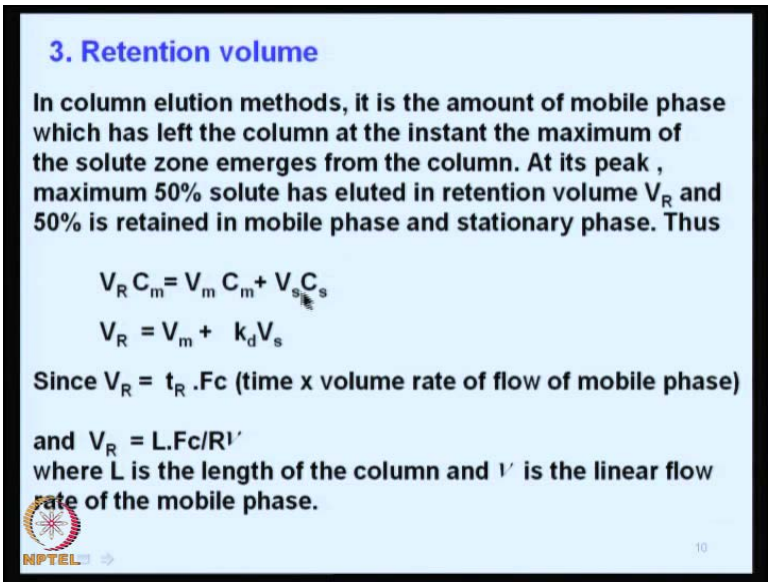

So, these are all different ways of expressing the retardation factor. So, another terminology that you come across very regularly is retention volume. So, in column elution methods it is the amount of the mobile phase which has left the column at the instant the maximum of the solute emerges from the column

That means when the substance leads the maximum substance has emerged from the column the amount of the mobile phase which has left the column corresponding to that is known as retention volume.

At its peak the maximum 50 percent of the solute has eluded in retention volume V R and 50 percent is retained in the mobile phase and stationary phase. So, we can write V R into C m is equal to V m into C m plus V s into C s that is volume in the mobile phase multiplied by its concentration plus volume in the stationary phase plus concentration in the its concentration of the solute. So, you can simplify this again V R is equal to V m plus K d into V s.

Since V R V R is also equal into the time that is retardation factor time multiplied by volume rate of the flow of the mobile phase we can write V R is equal to t R into F c and V R is equal to L into F c by R mu where L is the length of the column and mu is the linear flow rate of the mobile phase why I am introducing all these terminology is because you would be coming across many of these terminology in your whenever you're dealing with research papers.

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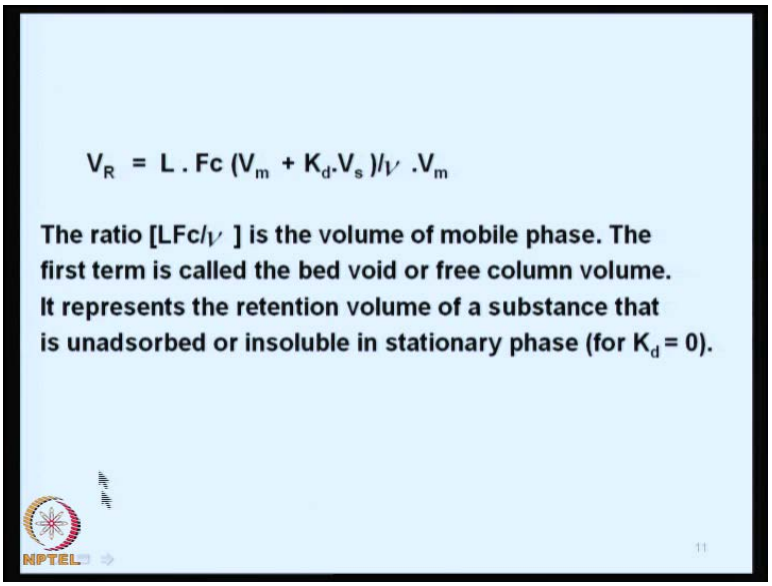

So, you can write V R is equal to L into F c multiplied by V m into K d V m plus K d into V s divided by mu into V m. the ratio of L into F c by mu is the volume of the mobile phase the first term is called the first term means L F c into V m is called the bed void or free column volume it represents the retention volume of a substance that is unabsorbed or insoluble in the stationary phase that is for K d is equal to 0.

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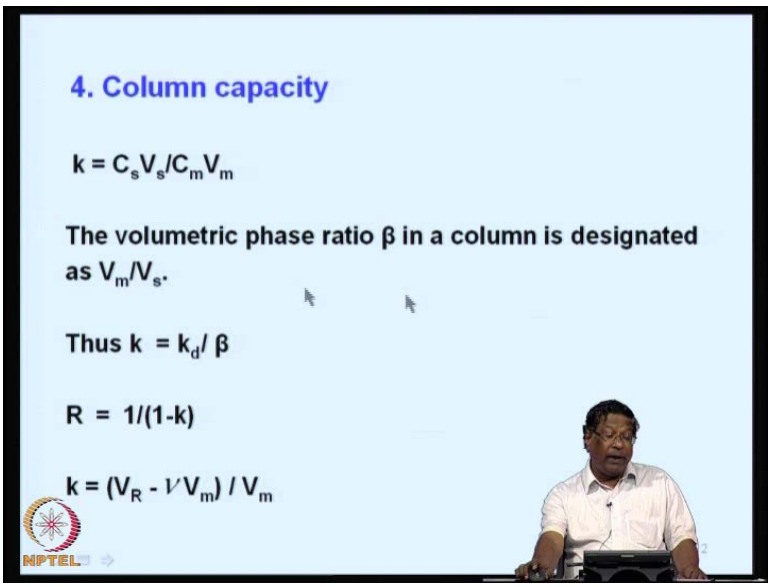

Then we can define couple of other terms that is column capacity what is column capacity it is the ratio of the concentration of the solute multiplied by volume divided by

C m into V m that is the concentration of the solute in the mobile phase multiplied by its volume.

The volumetric phase ratio beta in a column is designated as V m by V s

. So, we can write K is equal to K d divided by beta. So, R is equal to that is retardation factor again we can write in terms of the K values 1 upon 1 minus K or K is you can solve this for K in terms of V R and V m that gives you an expression of K is equal to V R minus mu into V m divided by V m

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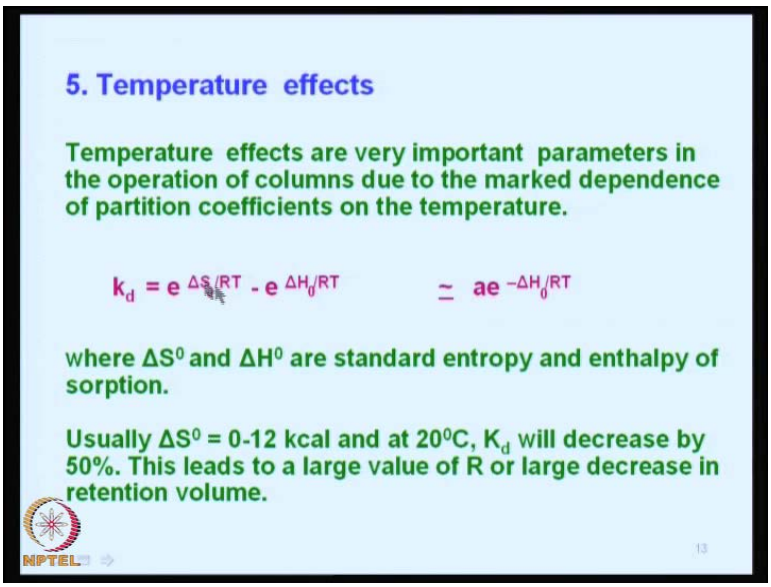

So, the other factor that is important in chromatographic separations is the temperature effect usually temperature effects are very important parameters in the operation of the columns due to the market dependence of the partition coefficients on the temperature for example, you can take a look at this expression K d is equal to e to the power of delta s naught upon R t minus e to the power of delta H naught divided by R t that is approximately equal to a to the power of a e to the power of minus delta H naught upon R t.

Here you should understand that delta s naught and delta H naught are the standard entropy and enthalpy of sorption. So, usually delta s naught is in the range of about 0 to twelve kilocalories and that is at 20 degree centigrade K d will decrease by about 50 percent this leads a large value of R or large decrease in the retention volume.

So, it is important for us to recognize the effect of entropy as well as the temperature I hope you'll appreciate the other terms in this equation that is K d partition coefficient R is the gas constant and t is the absolute temperature.

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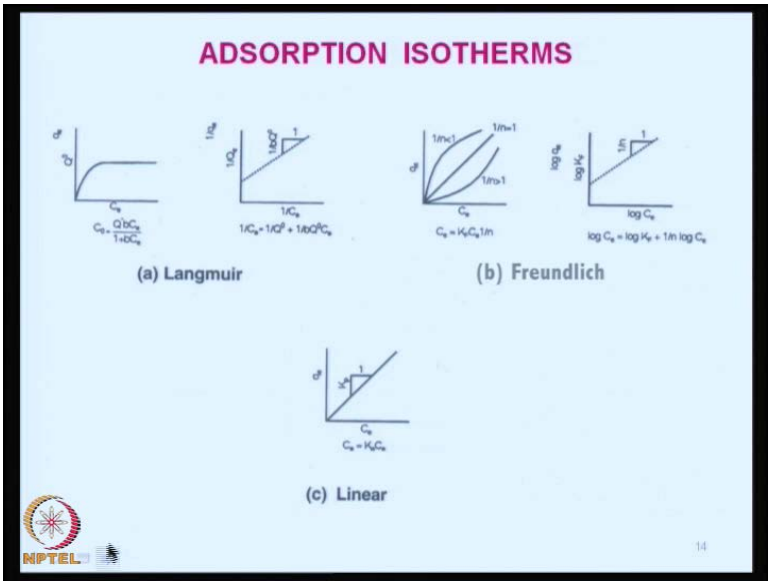

Now, you can look at these figures these are known as adsorption isotherms; that means, you can have different when the molecule component is absorbed onto this stationary phase its concentration keeps on increasing and the relation of the change in the concentration respect to concentration is given by the adsorption isotherms where there are 3 isotherms one is Langmuir isotherms and freundlich isotherms and other is linear isotherm.

In Langmuir isotherm we have the relation C s versus C d that is it will increase linearly and then it reaches a constant value. So, if you take a derivative this thing you would know that the slope is you can determine the slope and the from the slope you can determine the adsorption capacity.

Similarly, there will be changes with respect to other absorbance which need not be linear, but it can be linear also as shown here. So, different adsorption isotherms need to be plotted in chromatographic techniques usually and these adsorption isotherms have a critical role to play in the development of the chromatogram.

Now, what is a chromatogram in a in the development of a chromatogram the sample is generally introduced at the top of a column and I introduce the sample into the column on the top. So, the liquid stationary phase is held in the column and mobile phase will be passing through and I am introducing the sample through the column and when the mobile phase is allowed to percolate through a stationary phase the sample components developed into separate zones depending upon their retardation factor.

And these zones we can call them bends or peaks the this is the development of different compound peaks and zones with respect to the time is known as chromatographic development.

So, the development of the peaks are known as chromatograms the development of chromatograms may be conducted either by frontal analysis or elusion analysis or by displacement development how do you really generate the chromatogram; that means, you have to plot the concentration versus time essentially. So, this can be done either by frontal analysis or elusion analysis or by displacement development we have 3 techniques to develop their chromatogram.

So, what is frontal analysis in frontal analysis you'll appreciate that we pass the sample solution continuously through the stationary phase or the absorbent the active center of the absorbents are occupied by the more strongly absorbed component of the sample what you are passing through; that means, you have a column here and you are going to pass the mobile phase your mobile phase is the one which contains the components to be separated.

So, mobile phase is continuously passing through the stationary phase and the active centers will hold the components which you want to separate. So, the more strongly absorbed components are held in the on the near the edge before the in the beginning and less strongly absorbed components will be absorbed later and least strongly absorbed components should be coming out at the bottom

So, they would; that means, they would be coming along with the travelling the solvent comes out first followed by least strongly absorbed component followed by additional solutes which are more strongly absorbed; that means, your chromatogram would be containing the peaks which are least strongly components first chromatogram the second

peak would correspond to next strongly absorbed component and third would be the more strongly 3 more strongly adsorbed component.

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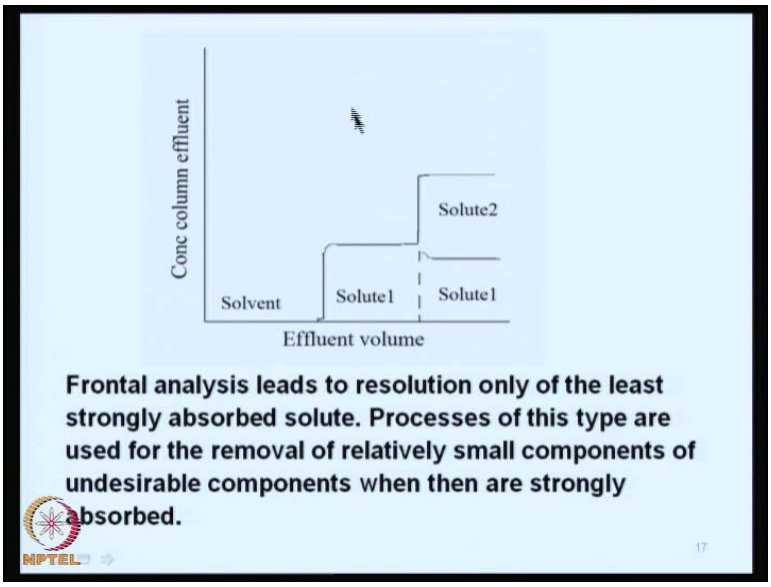

So, this is known as the frontal development. So, the you can look at this figure how we are going to end up with a chromatogram this is the concentration of the column effluent first comes out solvent and then the least strongly absorbed component that is solute 1 and solute 2 then the slightly more absorbed component and then you'll if there is 1 more component then you would be seeing solute 1 solute 2 solute 3 some more along this region; that means, the first peak will always correspond to only the pure solute.

So, this technique is usually employed for the separation of the least strongly absorbed solute. So, processes of this type are used for the removal of relatively small components of undesirable components when they are strongly absorbed.

Suppose you have a solution of a substance in which some impurities are there you want to separate the impurities all you have to do is go for frontal development and pass the sample solution containing the impurity through a column and the stationary phase would absorbed the impurities and lets lets out the other molecules which are least strongly absorbed. So, the application of this would be always in the preparation of small components and the mobile phase itself serves as the eluent

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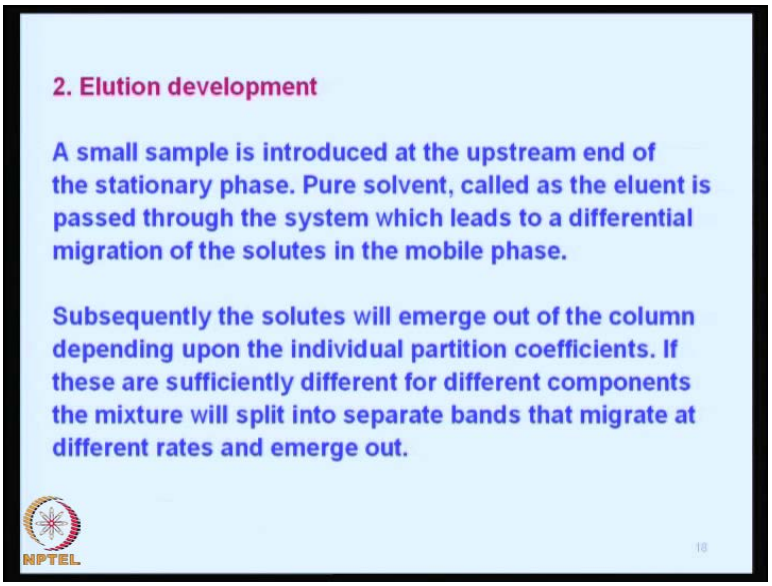

So, in elution development that mean now we are looking at the other aspects another method to separate is elution development. So, here what we do again we take a small again we take a stationary phase like this and then we pass the solvents or solutes which are a mixture of substances to be separated, but here the solute mobile phase is not the same as the substance which we want to separate is eluent is you are going to inject the substances which you want to separate and then you are going to pass some other solution which will start pushing the components out of the column or through the stationary phase.

So, here a small sample is introduced at the upstream end of the stationary phase. So, pure solvent though it is called as eluent and it is pass through the system which will which leads to a differential migration of the solutes in the mobile phase.

Subsequently the solutes will emerge out of the column depending upon the individual partition coefficients if the partition coefficient is very low then it comes out first if the partition coefficient is very high then it will be setting up several equilibrium throughout the stationary phase and mobile phase and it will come out later.

So, if these are sufficiently different for different components the mixture will automatically split into separate bands or chromatograms that migrate at different rates and emerge out.

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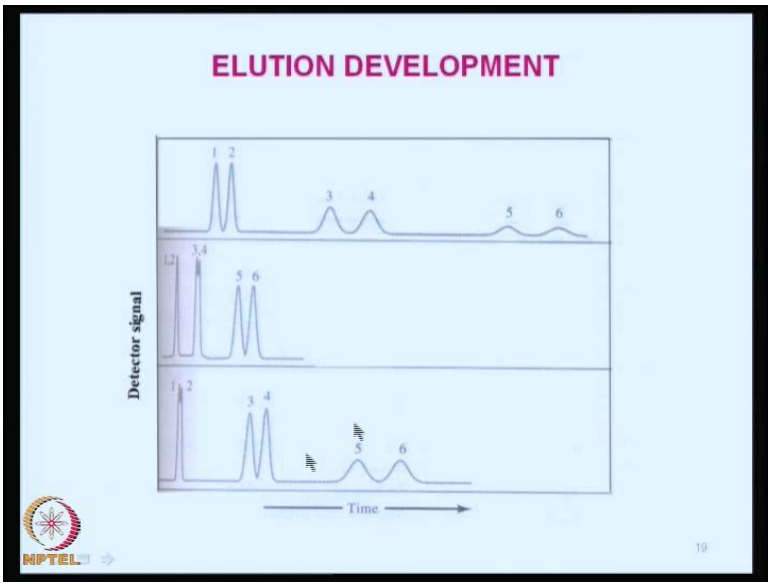

So, this is known as elution development you can see a figure like this that near the edge I have a substance mixture of substances which I have injected and then eluent is passing through like this and over a period of time the eluents would be coming out 1 2 3 4 5 6 like that and then the detector also would give you a signal like 1 2 3 4 and 5 6. So, this the chromatograms are always detected by a using a detector.

It can be it can be electronic detector or a specific detector which you which is chemically specific to the substance which you are separating, but what you can see here is the eluted substances are coming out 1 by 1.

And then after the first eluted compound the eluent reaches the base line then the second one will start then the third 1 4th 1 etcetera, but the time in which they appear are also distributed in the same scale.

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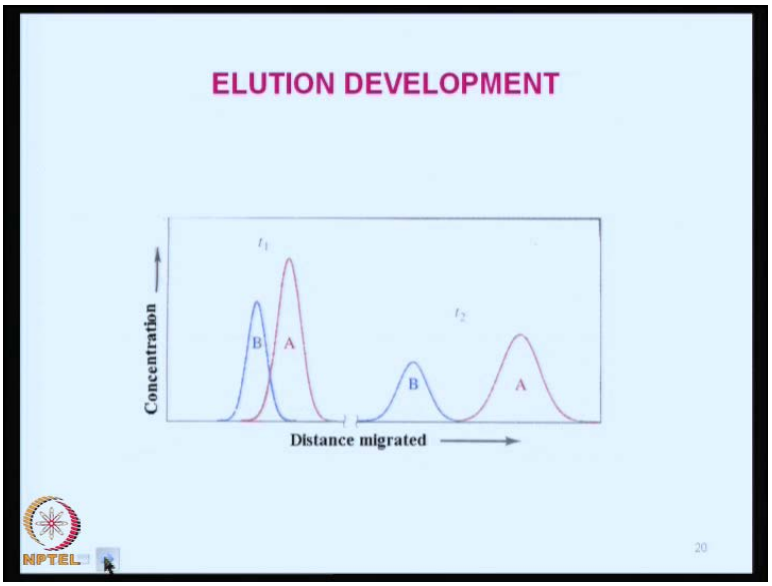

So, elution development initially is like this basically it is a mixture of substances here will be after sometime they separate out like this a little bit and then if you permit enough time that is denoted with a gap here they would be separating from each other into different chromatograms and then emerge out of the column as single components along with the eluent of course,.

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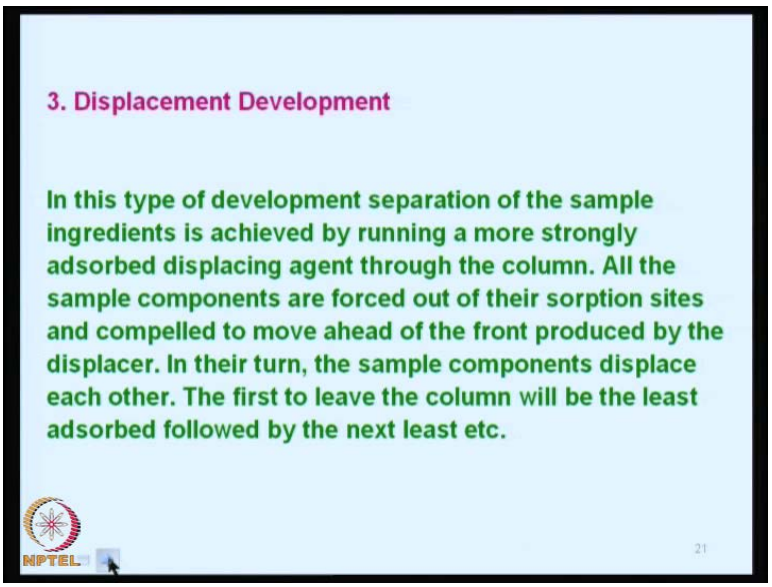

So, this brings us to this is another way of eluting here in the previous case what we are having is is the substance which is different from the substance you want to separate; that

means, the solutes and the mobile phase are different. So, you have a choice of increasing the partition coefficient by the choice of different mobile phases mixtures etcetera whereas, in the frontal development you have no choice it just contains 1 or 2 impurities which you can pass through and columns stationary phase and collect the purified substance.

Now, the third one is displacement development in this type of development separation of the sample ingredient is achieved by running a more strongly absorbed displacing agent through the columns; that means, you are going to add all the substances and they get held up in the column.

They do not come out whatever is the eluent what you have chosen for the system all of them will be held in the column now if you want to separate them out you have to push it through using a substance which is more strongly absorbed.

So, all this sample components have to be forced out of their sorption sides out of this options sides and compelled to move ahead of the front produce by the displacer in their turn the sample components is displaces each other.

The first component last component which is least strongly absorbed will come out first and then the second last will move to the last component and then it will come out like that. So, the first component which is most strongly absorbed will come out last So, you have a choice of choosing the mobile phase or varying the polarity of these molecular of the solvents for the separation of the components solutes

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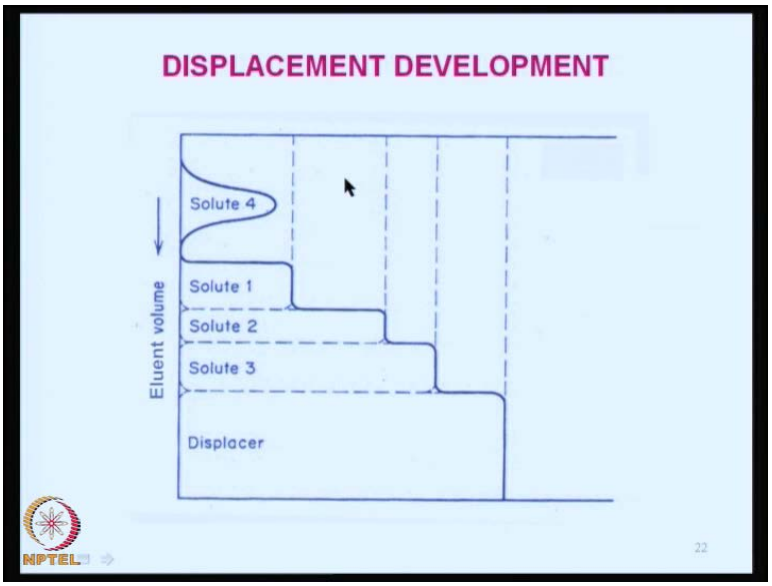

This is known as displacement development here you can see I have given a graphical presentation here all the solutes are there and then there is solute 1 solute 2 solute 3 and solute 4 a fluent volume is like this actually it should be eluent, but fluent is also fine. So, the it comes out like this and then once the displacer is there all the 3 of them will be coming out individually 1 after

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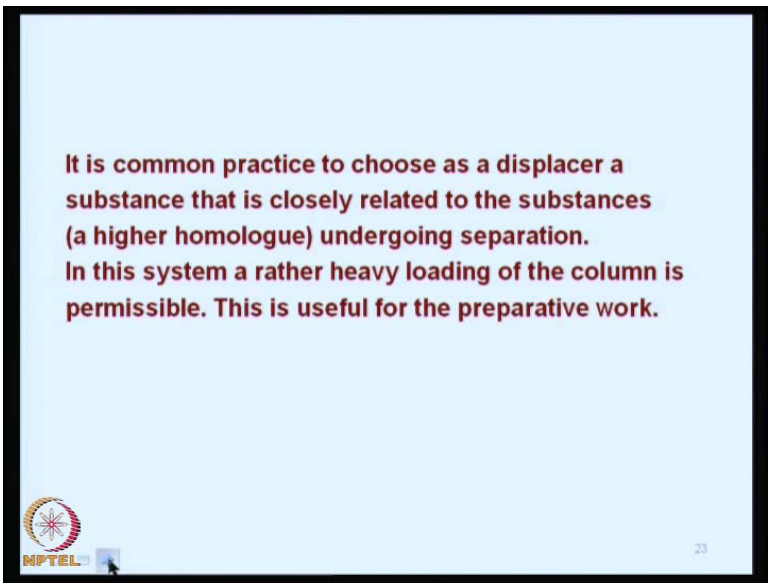

Another by occupying the sides occupied by the previous component which is absorbed so, it is the common practice to choose as displacer a substance that is closely related to

the substances which you want to separate probably a higher homologue would do this job.

So, if you want to separate a butane probably pentane will do the job if you want to separate pentane hexane will do the job something like a higher homologue in this system a rather heavy loading of the column is permissible; that means, you can take the substances can be in higher concentration. therefore this is useful for preparative work; that means, you can use it for industrial production also

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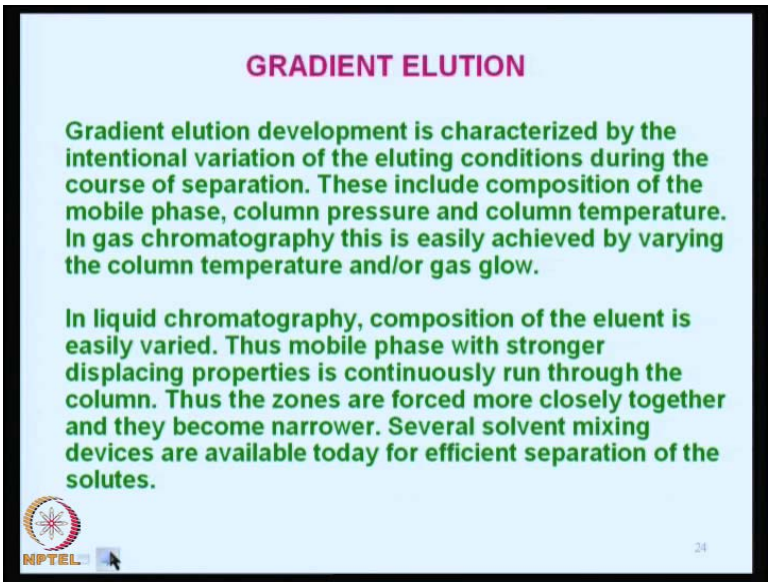

Only thing is you will have to scale it up to gram level or kilogram level depending upon what you would like to achieve the separations So, we can talk a little about the gradient elution. So, the gradient elution development is characterized by intentional variation of the eluting conditions during the course of separations; that means, they include composition of the mobile phase you can change it change the components

You can take two substances and keep on varying the composition of one another; that means, florets of 2 or 3 eluents and that is one aspect second aspect is you can change the column pressure if you are using gas as a mobile phase or you can change the column temperature during the separations etcetera in gas chromatography this is very easily achieved by varying the column temperature and or the gas block.

So, in liquid chromatography composition of the eluent is easily varied. So, there mobile phase with stronger displace in properties is continuously run through the column.

Thus the zones are forced more closely together whenever you are changing the mobile phase. So, the if the mobile phase is rather more polar the chromatograms will be forced nearer each other because they will be coming out much faster than the eluent.

So, the zones are forced more closely together and they become narrower several solvent mixing devices are available today for efficient separation of the solutes you can choose solvents which can vary the through several through several container and you can vary the composition of one solvent with respect to another on a continuous basis also.

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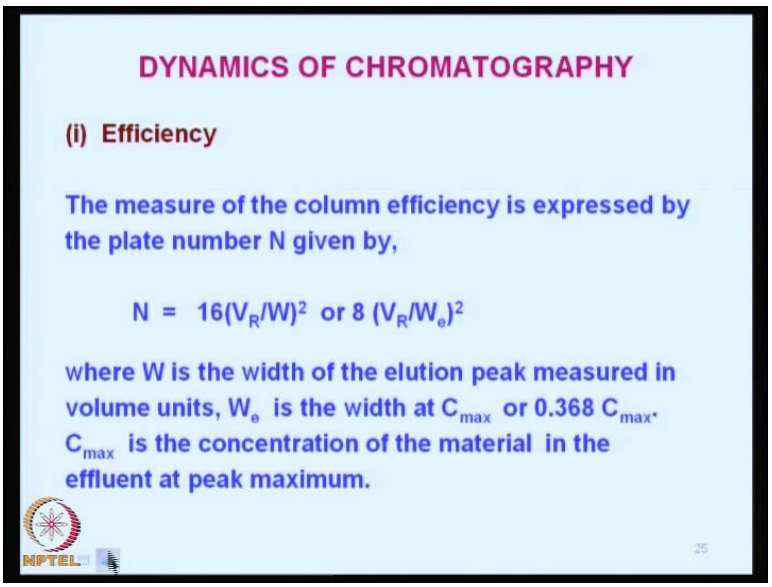

So, such efficient separation can be obtained if you have the capability or interest in separating the substances which are held very strongly. So, the dynamics of chromatography let us discuss a little bit about the dynamics that is let us talk about efficiency the major of the column efficiency is expressed by what is known as plate number the notation is n and it is given by 16 multiplied by V R divided by W whole square or 8 times V R by W square whole square.

Where W is the width of the elution peak measured in the volume units that is how many times the width is from the volumes employed and W e is the width at C max called maximum concentration or 0.3 6 8 times C max concentration max C max is the defined

concentration of the material in the effluent at its peak maximum So, if you know the C max you can determine the plate height that is you the column diameter you know the V R and you know the W e.

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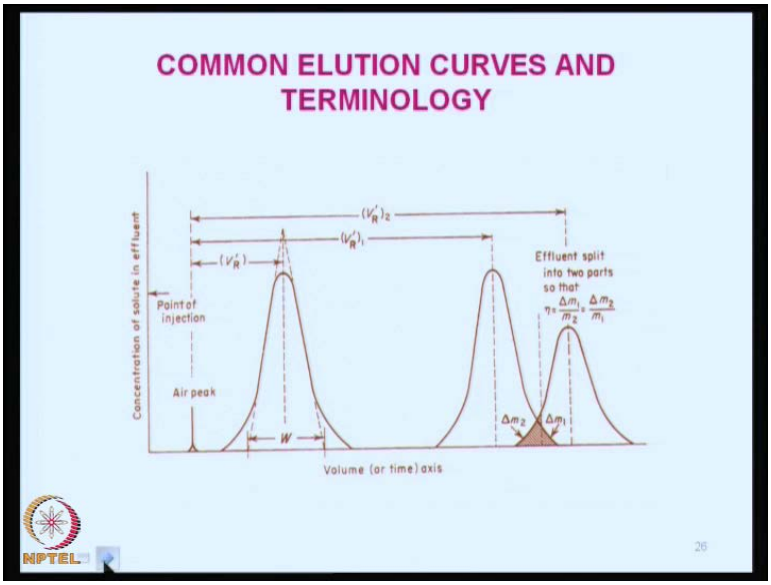

So, column elution curves and terminologies can be defined like this in terms of this curve. here I have plotted concentration of the solute in the effluent on the vertical axis and this is the point of injection that is here for standardizing the chromatogram I am introducing a small gap and that is known as air peak; that means, nothing will come out, but there will be a small peak which will tell me that the chromatogram has started and then I have 1 peak and second peak third peak etcetera and the distance from the air peak to the middle of the first chromatogram peak is known as V R dash.

Similarly, V R 1 dash for the second substance etcetera and this would be V R 2 dash that is for the third substance. So, V R dash V R 1 and V R 2 etcetera we can define and the axis is volume or time axis.

So, you can see in this case the width of the column the width of the chromatogram is very well defined whereas, in this case the it is there is some amount of overlapping of m 1 and m 2 in this case. So, this tells us that there is some more work raise required to separate the mixtures completely.

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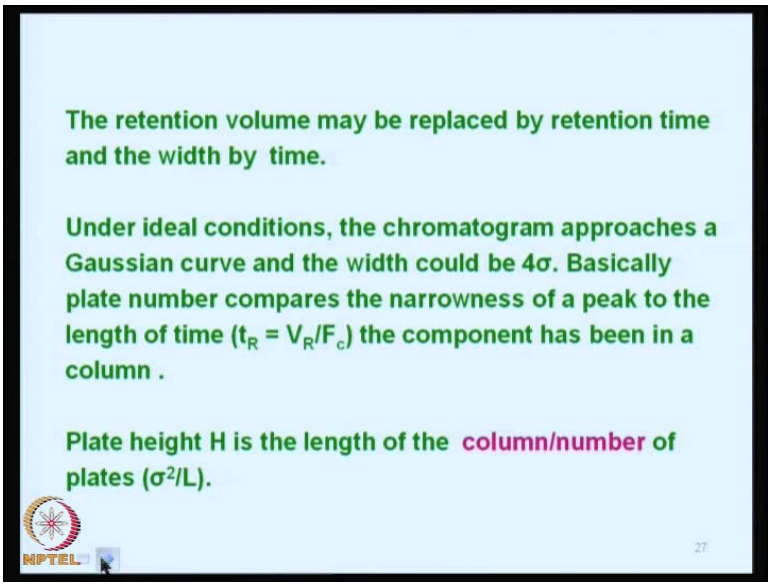

So, the retention volume may be replaced by retention time and width also by time under ideal condition the chromatogram approaches a gaussian curve if you remember the previous figure this is basically a gaussian figure all these figures are gaussian figures; that means, with a simple lambda max and then separating each other well after the median.

So, the chromatogram approaches the gaussian curve only under ideal conditions and the width will be 4 sigma that is the standard deviation basically plate number compares the narrowness of a peak to the length of the time that is t R is equal to V R by F c and the components that is component has been in a column.

So, plate height H is the length of this column divided by the number of plates that is sigma square by L

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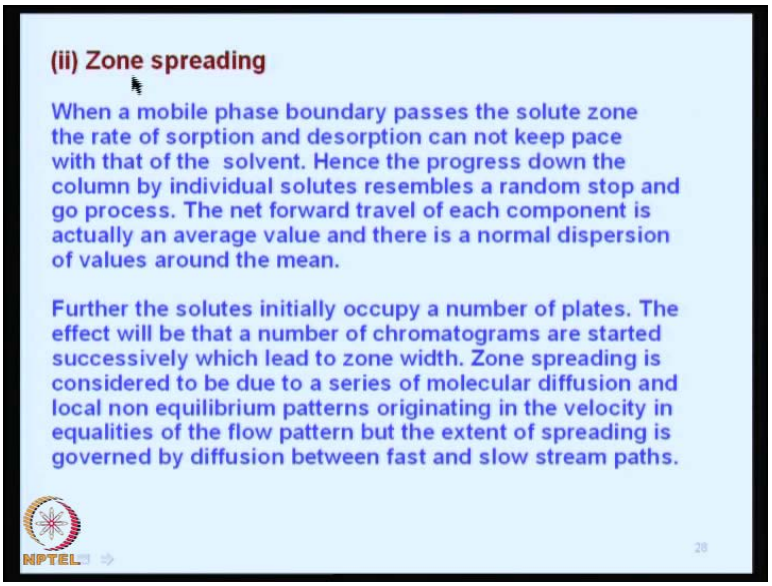

Now we can talk a little bit about zone spreading when a mobile phase boundary passes the solute zone the rate of sorption and desorption cannot keep pace with that of the solvent because the solvent would be running away faster that I have already told you that it runs about 15 percent 0.1 5 percent more than the solute front.

So, the progress down the column by individual solutes resembles a random stop and it can it is basically a stop and go stop and go stop and go process the net forward travel of each component is actually an average value and there is a normal dispersion of values around the mean; that means, when the curve is like this the means value there is always a normal distribution further the solute initially occupy a number of plates the effect will be that.

A number of chromatogram are started successively which lead to zone width they separation occurs, but they are not very well separated, but the number of separations always leads to zone banning. So, zone spreading is consider to be due to a series of molecular diffusion processes and local non equilibrium process also will take part originating in the velocity in equalities velocity will be different they. So, they would be all these processes or a molecular diffusion and non equilibrium patterns result in the inequality of the flow pattern for the different components, but the extend of spreading is govern by diffusion between the fast and slow stream paths.

What we are trying to say is zone spreading is considered to be a molecular diffusion and non equilibrium processes which leads to velocity in equalities the extend of the what use the effect what you see in the chromatogram is governed by the diffusion between the fast and slow processes the result is different and the cause is different the result is seen, but cause is molecular diffusion and non equilibrium patterns.

So, the resolution again the degree of separation is always a problem common to all chromatographic methods in truth complete separation of a substance can never be achieved there is a always a small catch when people say that I have separated totally hundred percent it is never possible.

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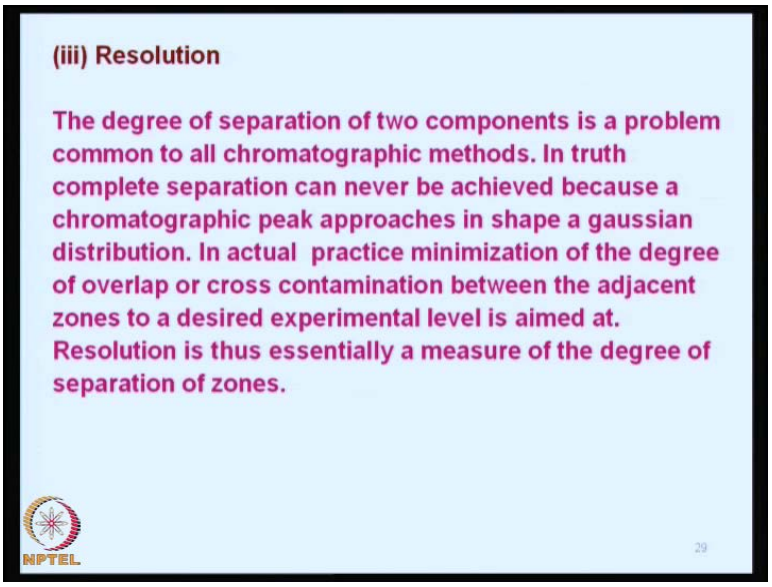

So, in truth or in theory complete separation can never be achieved because a chromatographic peak approaches in shape a gaussian distribution it is never reaches the base value in actual practice minimization of the degree of overlap or cross contamination minimization of cross contamination also between the adjacent zones to a desired experimental level that is aimed at if you are able to see well separated chromatograms one would say that the experiment is fairly successful, but no amount of separation can achieve hundred percent result in chromatography or for that matter any other process where separations are aimed at because it is always a process of the separation of the substance in the it is a partition process in the stationary phase as well as in the mobile phase mobile phase as well as in the stationary phase.

So, the resolution is therefore, essentially a measure of the degree of the separation processes its now and never an absolute value

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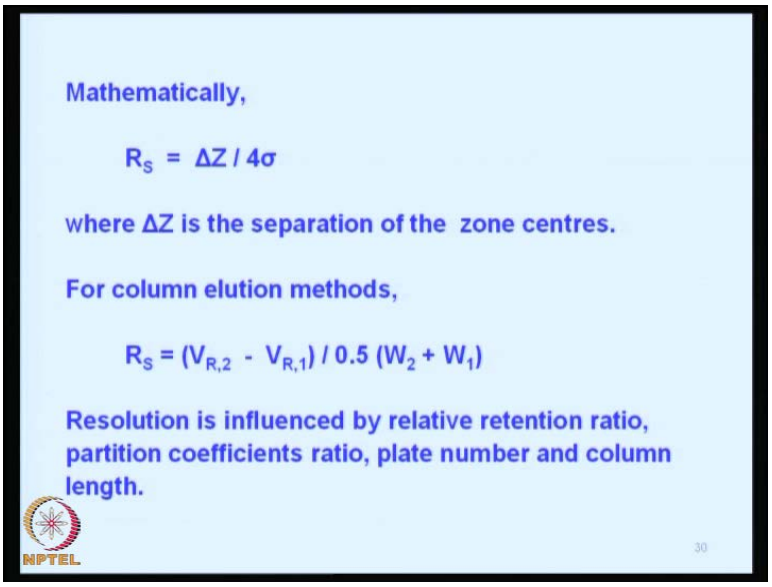

Mathematically we can write something like this R s is given by delta Z divided by 4 sigma that is 4 times the standard deviation. So, where delta Z is the separation of the zone centers and for column elution methods we can write something like this we are two that is the second component V R 1 that is first component divide by 0.5 times width of the second peak plus width of the first. So, the resolution is influence by the relative retention ratio and it is influence by partition coefficients ratio it is influence by plate number it is influence by column length.

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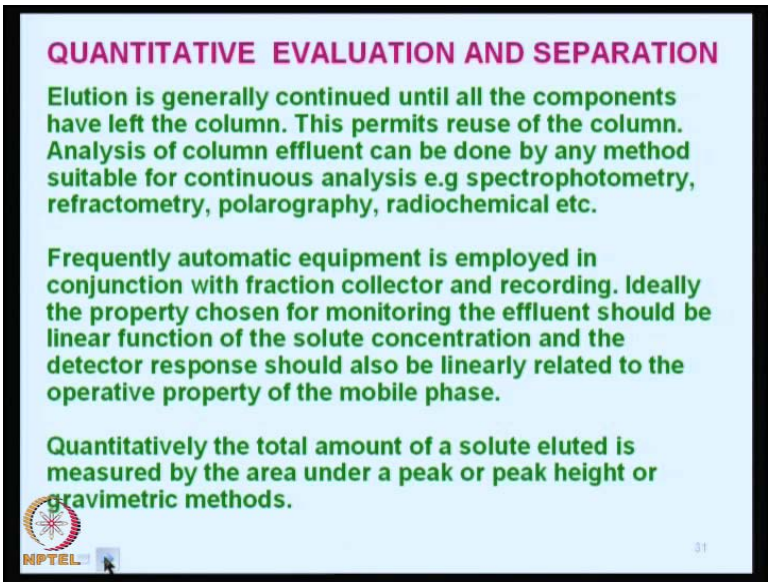

So, resolution is never a one way traffic it depend upon. So, many factors which are basically at variance with each other so, elution is generally continued until all the compounds have left the column this permits the reusing of the column because once you are able to once you are able to separate the component once you are able to separate the component you should be able to reuse the column. So, the analysis of column effluent can be done by any method suitable for continuous analysis; that means, the column effluent needs to be monitored and you can monitor the column effluent whether it contains the components or it is pure mobile phase if the substance contains the components if the eluent contains the components then the components can be analyzed by spectrophotometers refractometry poltragraphy any other radio chemical method infrared.

So, many other ways, but the moment you see the substance is not responding to any of the analysis technique you can be fairly sure that the eluent is has become pure and the component have left the column that is the signal for you to restart another experiment till that time you have not sure whether the column can be reused or not whether the separation is being achieved or not.

Frequently automatically recruitment is employed in conjunction with fraction collectors also suppose the components are very valuable then you would like to collect the component and you would also like record the substance the chromatograms therefore,

ideally the property chosen for monitoring the eluent should be lenient function of the solute concentration.

A very simple and straight forward assumption that is the substance what you want to determine should be a linear function of the solute in the solvent that is mobile phase and the detector response should also be linear with respect to the component that is being separated that is the ideal condition.

So, quantitatively the total amount of a solute eluted is measure by the area under the chromatogram under a peak or you can use the area a peak or peak height or you can even recorded on a piece of paper cut the graph paper and weigh the paper that is one of the very well known quantitative techniques used very regularly about 30 40 years before. So, that is known as quantitative chromatography analysis if the separation is achieved properly and the chromatograms are well developed well separated from each other a peak can be weighed directly.

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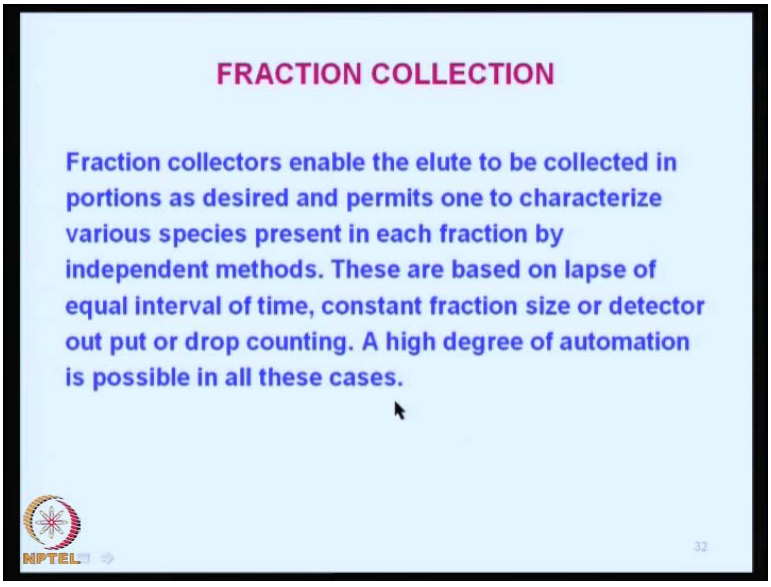

Now, I am talking about fraction collection that is a fraction collectors what are their jobs their job is to enable the elute to be collected in portions as desired and permits one to characterized various species present in each fraction by independent methods.

These are basically based on the laps of equal interval of time; that means, you can collect the sample at equal interval of time and then analyze them or you can go for

constant fraction size you can say I will collect only ten m L of the sample every time and then I will analyze the samples or you can say the detector output or drop counting I will collect after every hundred drops whatever comes I will have number of fraction collectors. So, a high degree of automation is possible in all this cases that is the advantage of fraction collection and several several other components can be analyzed in this way

So, far I have given you a glimpse of the chromatography techniques and now I would like to move on to gas chromatograph which I would like to deal in more detail other chromatographic methods are also there and they can be very easily were given the technical input that is terminology and the plate height and another chromatographic terminology.

You can go through them and then adopt suitable technique for your presentation for your work also, but what I would like to discuss with you is about the gas chromatography why I have chosen gas chromatography is because it encompasses almost all the principle that we have discussed in chromatography in 1 single technique.

Therefore I would like to go slightly in more detail with respect to gas chromatography and also introduce you to the tremendous advances that have occurred in the gas chromatographic instrumentation gas chromatographic development and then with respect to methods development. So, it triple since 1 of the most important development in chromatography which has been over taken only by the high pressure liquid chromatography that to only since last 20 years

So, gas chromatographic represents almost all the parameters that one would like to learn at this stage now let us see what is gas chromatographic in detail. So, the gas chromatography differs from other forms of chromatography in that the mobile phases is a gas; that means, you have to take the pass the gas through a column continuously from a cylinder preferably and the components are separated as vapours.

They are not separated as liquids the separation is accomplished by partitioning the sample between the gas and a thin layer of non volatile liquid thin layer of non volatile liquid which is coated on a solid phase; that means, the stationary phase contents 2 components 1 is a stable support phase on which a liquid is coated as a thin film.

So, it is known as gas liquid chromatography a sample containing the solutes is injected on to a heated block why heated block because the sample has to be vaporized also in the form of respective vapours and that should be mixed with the gas which we are using as a mobile phase and as a plug of vapour by the gas and the gas we call it as carrier gas the. So, we have 3 components in gas chromatography.

One is the carrier gas which serves as the mobile phase and your sample which should be converted into a vapour which will be swept away into the column by the incoming carrier gas and the it will be swept away as a plug of vapour and the solutes are separated by the stationary phase and then desorbed by the fresh carrier gas the moment the plus of vapour passes through the stationary phase it get separated according to the partition coefficient and the mobile carrier gas is forcing the sample that is vapour is getting sopped and desorbed because there is a continuous pressure of the gas coming through the stationary phase So, the fresh carrier gas serves to desorbed the components of the gas

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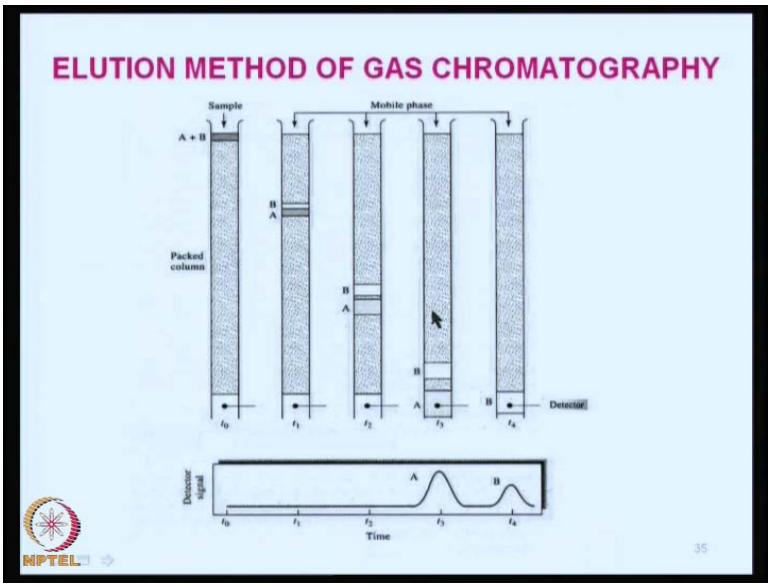

Now elution method of gas chromatography is something like this that is I have A column here and the sample is here A plus B I have a packed column and in this column you can see this small small particles on which liquid is coated, but that is not visible and the sample is introduced from the top and then I have the packet column gases passing through and then this plug will start moving at its own speed and depending upon the

partition coefficient and then it passes on and on here the separation is almost complete here they are well separated and here one is eluted sample A is eluted and here sample B is being eluted

So, the detector signal will always show very few place a detector somewhere here at the end of the column it will show you a basic straight line that is from the mobile phase and then component A is coming out and then followed by component B is coming is out

This is how the separation of gas chromatography separation of the components occurs in the gas chromatography now the process of separation in each is repeated in each plate sas the sample is move towards the outlet because we know that the a gas cross stationary phase can be represented as number of theoretical plates in which the separation will be taking place.

So, as the in each plate the sample get absorbed and then gets desorbed moves on to the next plate again its get absorbed and desorbed and like that it will be continuing throughout the column. So, each solute will travel at its own rate through the column and the bands will separate into distinct to zones depending upon the partition coefficients and the band spreading.

The solutes are eluted one after the as I have told you and we will continue our discussion in the next class how we can detect a series of signal resulting from the concentration changes and rates of elution on the recorder as the plot of time verses the composition of the carrier gas stream .