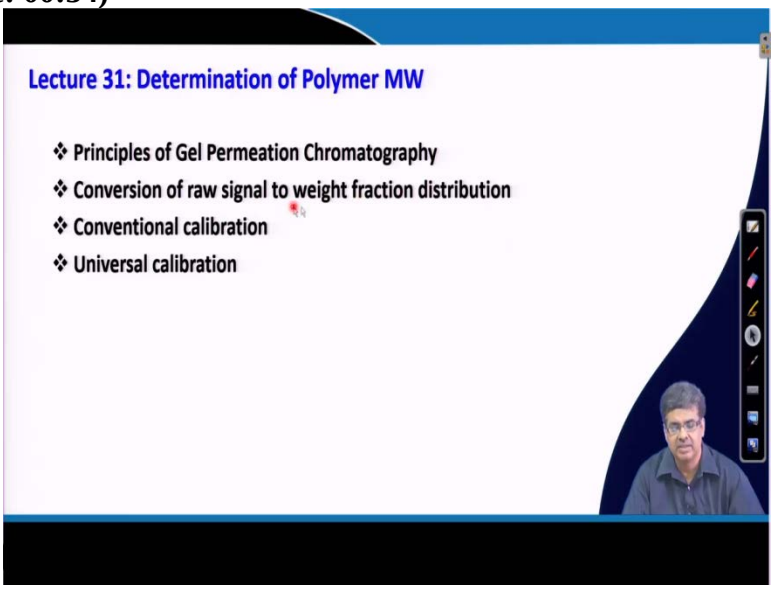
Introduction to Polymer Science
Prof. Dibakar Dhara
Department of Chemistry
Indian Institute of Technology – Kharagpur

Lecture – 31
Gel Permeation Chromatography

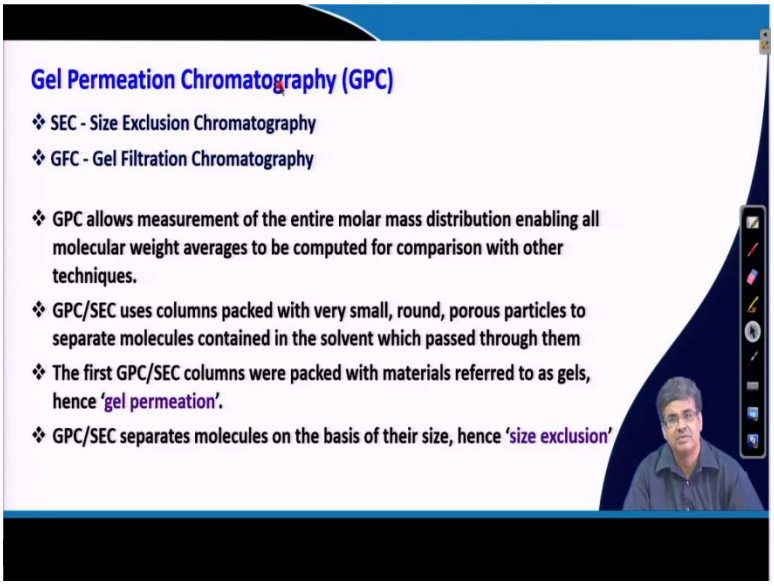
Welcome back, in today's lecture I will discuss about gel permeation chromatography which is another technique to characterize polymer molecular weight.

(Refer Slide Time: 00:34)



In this lecture I will talk about the principles of gel permeation chromatography, how to convert raw signal from a detector to weight fraction distribution, conventional calibration and universal calibration.

(Refer Slide Time: 00:50)

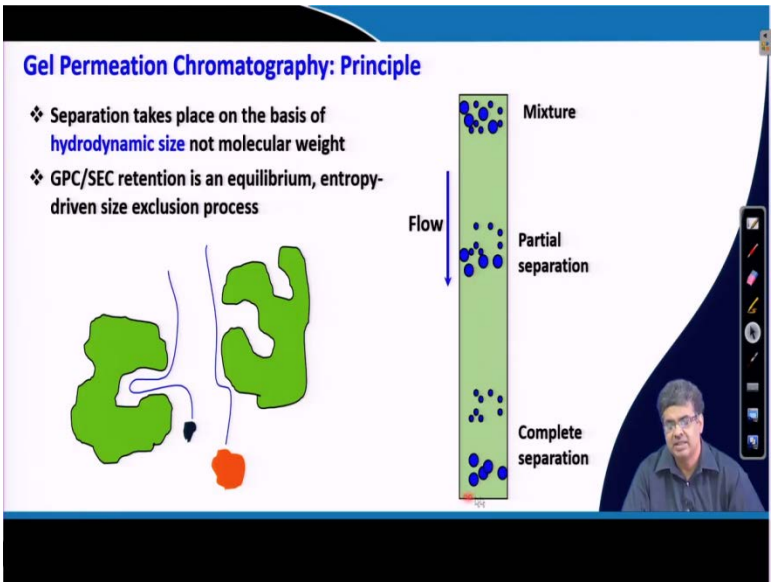


Gel permeation chromatography is also known as SEC, size exclusion chromatography, and GFC, gel filtration chromatography; this GFC term is very frequently used for protein characterization in biophysical or biochemistry laboratory. GPC allows measurement of the entire molar mass distribution. Remember, we discussed various techniques and in most cases, the techniques used give us either M_n or M_w or M_v.

GPC allows the measurements of entire molar mass distribution, which enables all molecular weight averages namely M_n, M_w, M_z, M_v to be computed for comparison with other techniques. GPC uses columns packed with very small round porous particles to separate molecules contained in the solvent, which pass through them. The first material, which was used to pack the column, was based on gels and that is the reason why this gel permeation term appears in this technique.

This technique separate molecules or polymer molecules, to be specific, based on their size; I will talk about this size in coming slides. And this term size exclusion chromatography, as you know, is a technique for separation.

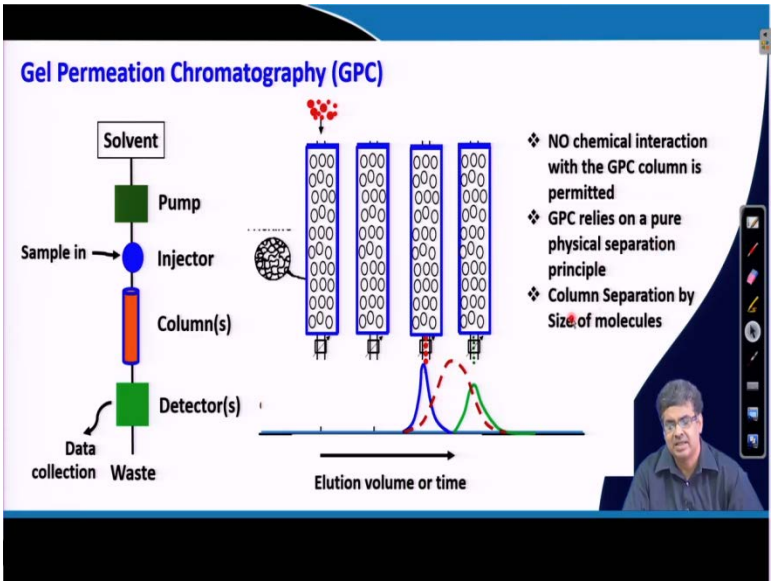
(Refer Slide Time: 02:54)



The principle of gel permeation chromatography or GPC is that in this case separation takes place based on hydrodynamic size not molecular weight, remember we are talking about hydrodynamic size. Now in this technique the separation or the retention of polymer molecules is an entropy driven size exclusion process. For example, if I schematically show that these are the pores in the column. And if we pass molecules of different sizes, then the small molecules will enter within the pores, hence will take longer time to come out of the column. The large molecules will not able to enter the pores in the same extent as the smaller ones. Hence, these large molecules will come out of the column faster. Now in this case it is not so rigid as it shown here, polymer molecules are flexible. So the entry to the pores or the restriction of entry to the pores for polymer molecules is driven by entropy of the polymer molecules inside the pore and outside the pore.

If one polymer molecule goes inside the pore, it actually loses some entropy. And this loss is more for larger sized polymer chains, hence the tendency of larger molecules to get into the pores will be much lower compared to the smaller ones. So once again, it is entropy driven size exclusion process. So if I just think this is a column and this is the flow of the solvent along with the polymer molecules, and at the beginning, I have a mixture of two size - one large and one small. With time the larger ones will move faster and eventually the large molecules will come out of the column before the small ones.

(Refer Slide Time: 05:39)



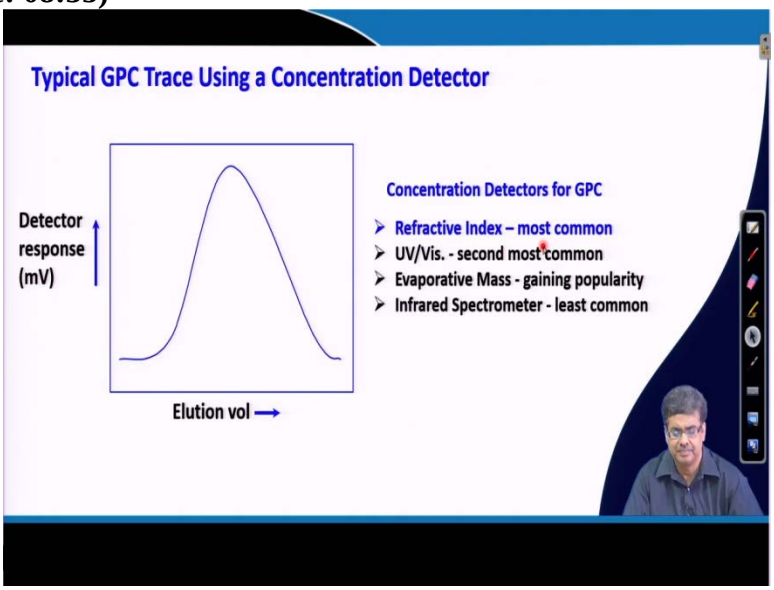
This is a typical schematic diagram for a GPC instrument. We have solvent at beginning and there is a pump, which pumps the solvent at a steady flow; there is an injector after that, it could be manually injector or an auto injector, which inject a polymer solution, dilute polymer solution in the system. The polymers go through the column, they are separated according to the size, and detector presence at the end of the column will detect the polymer molecules.

At the end, a computer will process the data. There could be one or more than one detectors depending upon the type of accuracy or type of measurement one trying to do. So again if this is the flow of the sample in the column and if these are the scenarios at different times, then at beginning one inject the sample and with time the larger ones actually come out, then medium ones come out and then small ones come out later. And this is a typical scheme of how the pores looks like.

Now in this example there are two distinct size of polymers, one large and one small. So we have two distinct peaks, but in case of real polymer sample the difference is not such distinct, there is a continuous variation in polymer size. So in case of a real polymer sample, we will get a single peak with the more broader distribution. The volume of the solvent or time that is required to take these molecules, polymer molecules, out of the column is called elution volume or elution time.

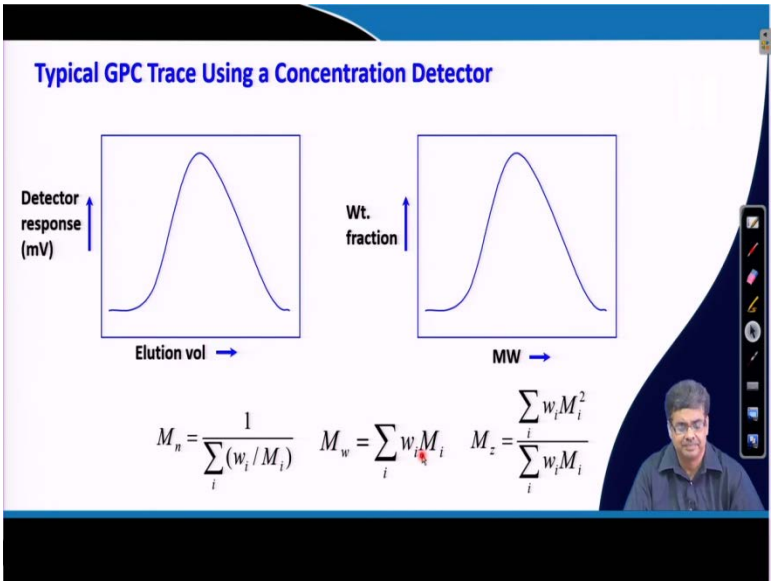
So in this case, once again, no interaction between the polymers with the GPC column is permitted. So the enthalpy of interaction is zero, GPC relies on a pure physical separation principle as I discussed and column separation is by size of the molecules, the hydrodynamic size of the molecules.

(Refer Slide Time: 08:35)



Now this is a typical chromatographic trace, in y-axis, we have the detector response and in x-axis, we have elution volume or sometimes we can write elution time as well. This is a typical signal we obtain from a concentration detector; the detector actually determines the concentration of the polymer sample in the fraction that is coming out of the column. There are different types of concentration detectors possible; the most popular is the refractive index detector. It could be a UV visible detector but this is of limited use because in this case the polymer has to be UV visible active. There are other detectors as well which are slowly becoming more regular in usage but the most frequently and most common usage for a concentration detector is the dRI (differential refractive index) detector.

(Refer Slide Time: 09:47)



Now we have this typical GPC trace. If you remember that to find out the molecular weight of any sample, we must express this distribution in terms of weight fraction or number fraction as a function of molecular weight. We will prefer weight fraction because as I discussed in the beginning lectures that with weight fraction distribution it is easy to calculate the different molecular weight averages.

For example, M_n can be obtained by

$$M_n = \frac{1}{\sum_i (w_i / M_i)}$$

M_w from

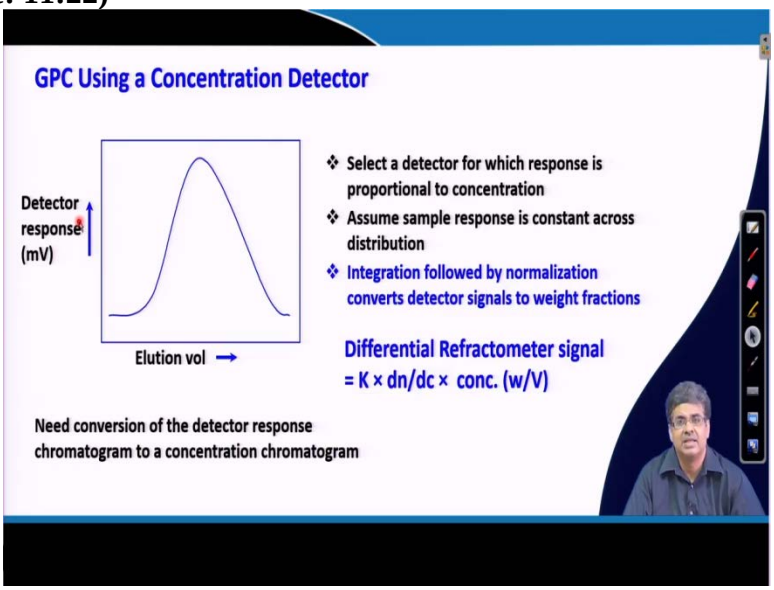
$$M_w = \sum_i w_i M_i$$

M_z from

$$M_z = \frac{\sum_i w_i M_i^2}{\sum_i w_i M_i}$$

which we have discussed earlier. Now these expressions are deduced assuming that there are discrete samples, w_i is the weight fraction of i-th polymer chain in the mixture, but in practice the chain length of the polymers are continuous. So in practice, in software, the summation is replaced by integration. So that the values can be obtained by integration of these quantities.

(Refer Slide Time: 11:22)



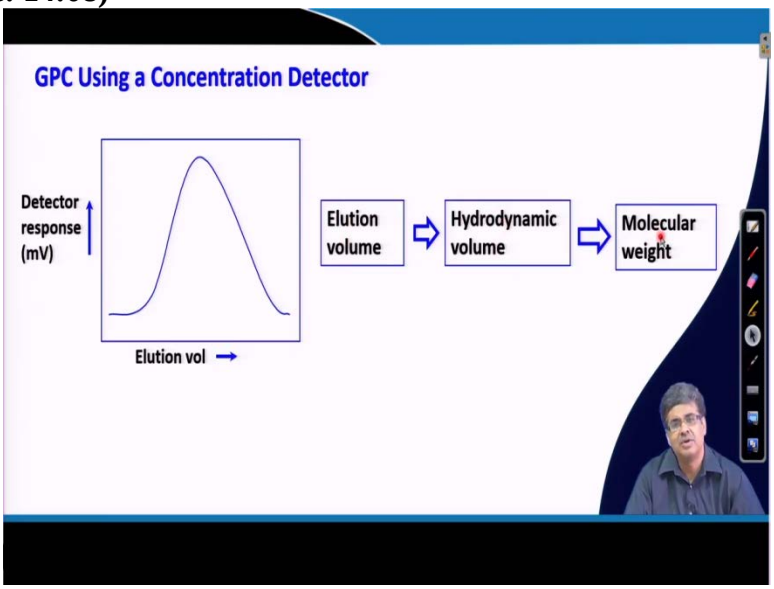
Therefore, we need to convert the detector response to weight fraction response and elution volume to the molecular weight as I discussed in the last slide. First, we need the conversion of a detector response chromatogram with concentration chromatogram. For that do we need to select a detector for which response is proportional to the concentration of sample and this proportionality factor does not depend on the molecular weight of the sample. Which means that we can assume that a sample response is constant across molecular weight distribution. If these two conditions are met, then weight fraction can be obtained by finding out the area of each slices and then dividing each slice by the entire area under the curve. So by integration followed by normalization, we can convert the detector signal to weight fraction. We can take a slice and we can find out the area and then divide that particular area with the entire area under the curve to get the weight fraction of the polymer chain having molecular weight in that particular slice.

One such detector is differential refractometer detector for which the signal is proportional to a constant $\frac{dn}{dc}$ and concentration, again concentration is mass/volume concentration not the mole/volume concentration.

Differential refractometer signal = $K \times (dn/dc) \times conc.(w/V)$

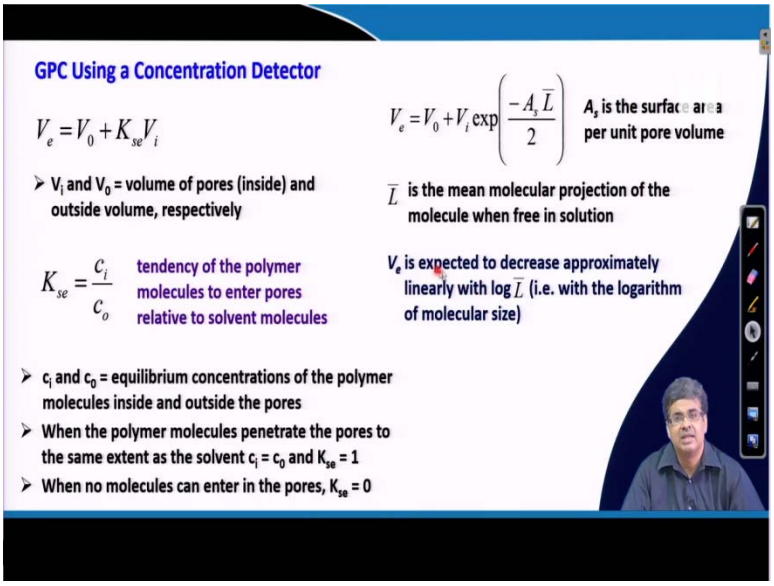
We have discussed $\frac{dn}{dc}$ term earlier; it is the refractive index increment with concentration. Therefore, in this case we assume that, this K and $\frac{dn}{dc}$ values do not depend on the molecular weight of the sample. The detector signal only depends on the concentration of the sample present in this solution. In that case, we can do an integration followed by normalization to convert the y-axis response to a weight fraction.

(Refer Slide Time: 14:08)



Now we need to convert the elution volume to molecular weight. We know that elusion volume is related to hydrodynamic volume, which is related to molecular weight. But they are related, not directly proportional.

(Refer Slide Time: 14:28)



We can express that elusion volume, Ve, in terms of V_0, which is the volume of the outside of the pores and V_i is the volume of the pores, meaning the inside volume of the pores.

$$V_e = V_0 + K_{se} V_i$$

V_0 is the outside volume and V_i is the inside volume and K_{se} is an equilibrium constant which is the ratio of concentration of the polymer inside the pores and outside the pores.

$$K_{se} = \frac{c_i}{c_0}$$

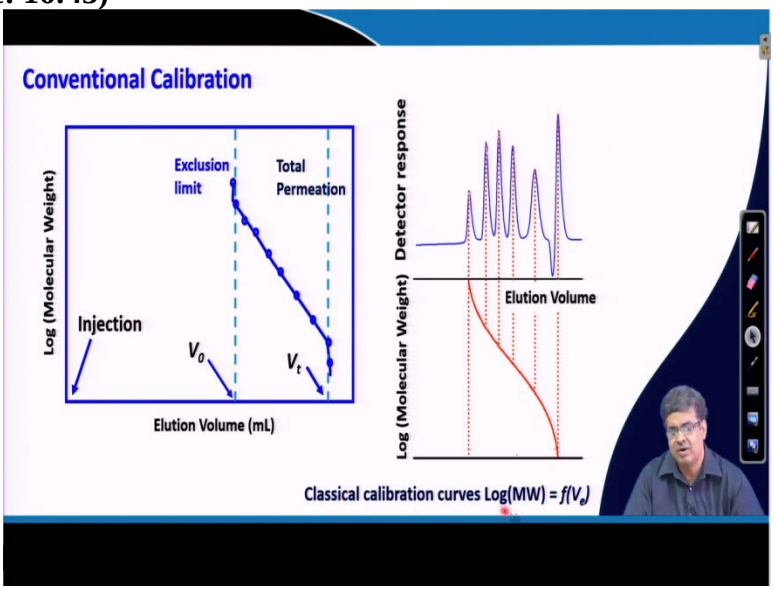
Therefore, it gives the tendency of the polymer molecules to enter pores relative to the solvent molecule.

If the polymer molecule penetrate the pores in the same extent as the solvent molecules then $c_i = c_0$ and this equilibrium constant is 1. And if the polymer size is so large that none of the molecules can enter the pores then K_{se} is 0. We can express V_e as

$$V_e = V_0 + V_i exp\left(\frac{-A_s \bar{L}}{2}\right)$$

by expressing K_{se} in this term where A_s is the surface area per unit pore volume. $\bar{L}$ is the mean molecular projection of the molecule when free in the solution, for a spherical molecule this is the diameter. Hence, we can expect that V_e to decrease approximately linearly with $\log\bar{L}$ which is related to the molecular weight of the polymer chains. That means we can also expect that V_e changes linearly with $-\log$(molecular weight).

(Refer Slide Time: 16:43)

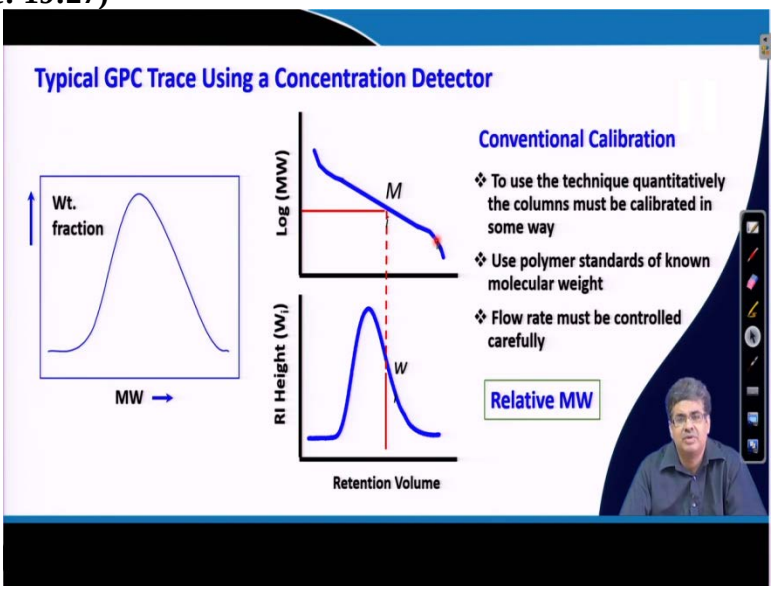


So we can plot log(molecular weight) with elusion volume and if the molecular weight is above this particular value then none of the polymer molecules enter the pores and all of them come together without entering the pores, so this corresponds to the V_0 which is the volume of the available volume outside pores. So if the size of the polymer molecule is large it will not enter, the elution volume will be equal to the volume available outside the pores.

Now if the polymer molecular weight very low, below this then all of the polymer chains will enter equally to the pores and again will come out together. We call that size as total permeation and the earlier size in terms of large molecular weight range we call this exclusion limit. And in between we will have a linear relationship between log(molecular weight) and elution volume as I discussed in the last slide.

So in practice, one will inject different polymer samples with very narrow polydispersity index or dispersity, which are called polymer standards, and measure the elution volume for each of these samples and then plot log(molecular weight) versus elusion volume. This is the calibration curve, which will give us the molecular weight of an unknown sample if we know the elution volume. This is a classical calibration curve where $\log(MW) = f(V_e)$

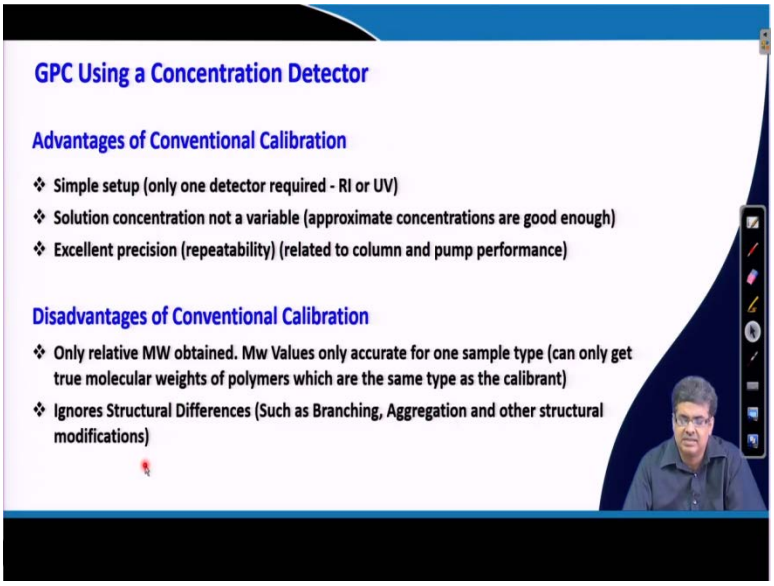
(Refer Slide Time: 19:27)



So in this case if we have this GPC trace then for each point or each slices we have corresponding molecular weight value from the calibration curve which was constructed as described in the last slide. Which means, for each of these slices or each of these points, we can have a molecular weight number from this calibration curve. We have already discussed how to convert this y-axis signal to weight fraction signal.

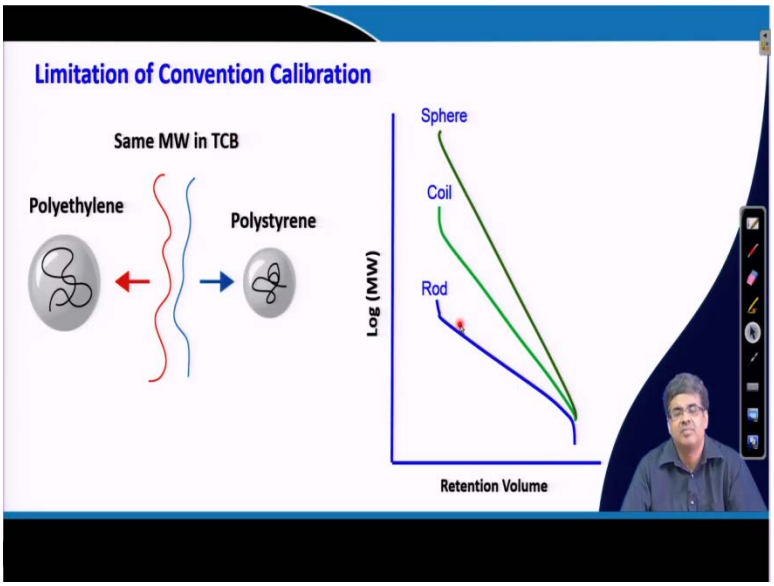
Hence, we can convert this raw signal to a weight fraction versus molecular weight trace form that we can get the molecular weight distribution, M_n, M_w and so on. This is called conventional calibration and for using this the columns must be calibrated and we use polymer standards of known molecular weights as I describe in the last slide for this purpose. Flow rate must be controlled carefully. There should not be any fluctuation in the flow rate. Now we know that from the raw data we can actually convert the raw signal to be a weight fraction versus molecular weight signal and then using the software we can get the values for M_n, M_w and M_z and the other values using the formulas I have shown in the first slide. Now in this case this molecular weight values are obtained from a calibration curve, which was constructed using a set of polymers having known molecular weight values. So these molecule weight values are relative to the samples which were used for calibration. That is the reason we call this molecular weight data as a relative molecular weight data, not absolute data.

(Refer Slide Time: 22:31)



Now advantage of this conventional calibration is that it is simple, we need only one detector either RI or UV and the solution concentration need not be known very quantitatively or very accurately. Approximate concentrations are good enough and very accurate data are obtained as long as we maintain the column and pump performance well. But the disadvantage of this conventional calibration is that this is only relative molecular weight, molecular weight values only accurate for one type of sample. For example if we are using polystyrene standards for the calibration in a particular solvent, say in THF, then if we do a measurement for an unknown polystyrene sample in THF, the values which will be produced by this method would be accurate. But if you use some other polymer sample then the obtained molecular weight number will not be very accurate. This method ignores the structural differences like branching, and other structural modifications.

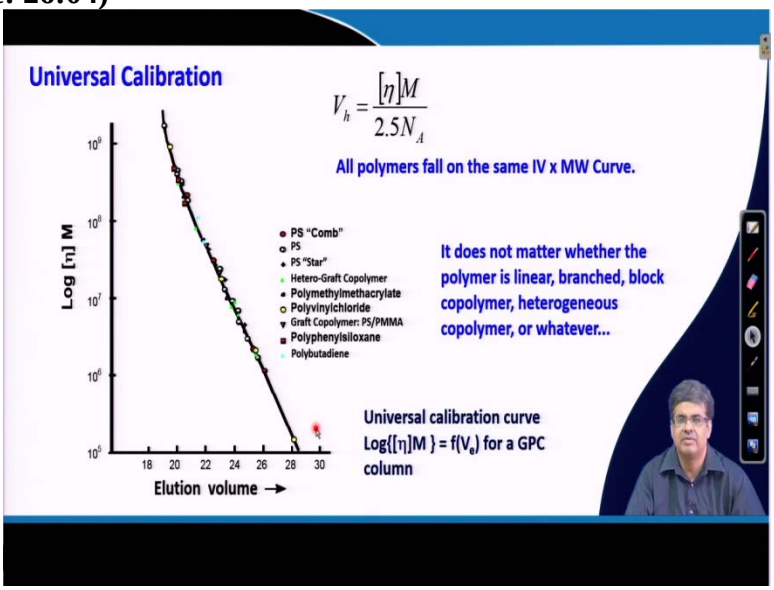
(Refer Slide Time: 23:56)



I will give you an example. Consider one polyethylene sample and one polystyrene sample of same molecular weight. In trichlorobenzene solvent, if we look at these sizes, polyethylene has larger size in this solvent compared to polystyrene. Hence, the elusion volume for polystyrene will be higher than polyethylene and this relative measurement technique will show lower molecular weight of polystyrene and higher molecular weight of polyethylene, because the separation is based on size not based on molecular weight. However, in actual term, they are of same molecular weight,

Also, if the shape of the polymers are different, even for same type of polymer molecule, because of branching and hyper branching, we will have different molecular weight values. The spherical shape polymer having higher molecular weight will come at the same elusion volume of a sample, which is of rod shape or is elongated and have a much lower molecular weight. Hence, if we use this relative technique the molecular weight numbers will be different or it will be more erratic if the shapes of the polymer molecules are not same.

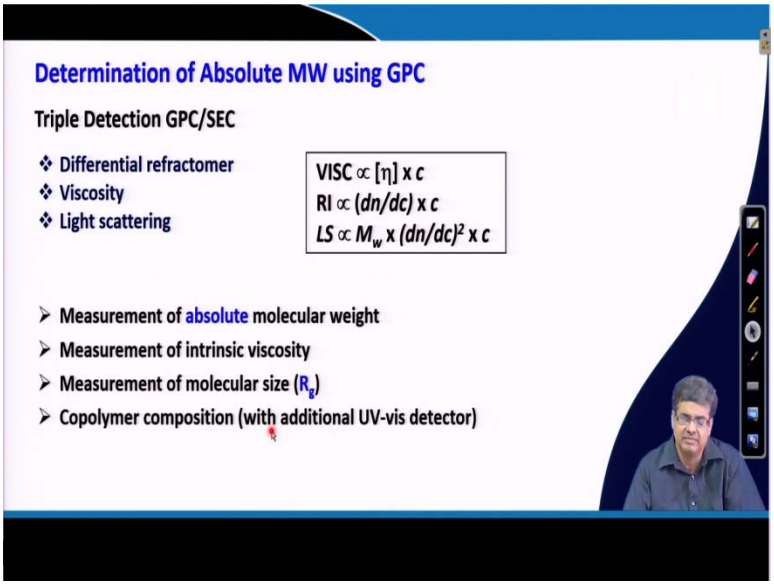
(Refer Slide Time: 26:04)



There are different ways to use universal calibration to remove this relative measurement, for example, we have we know this equation where this hydrodynamic volume depends on the multiplication of intrinsic viscosity and molecular weight. If we plot V_h with $[\eta]M$ then no matter what is the polymer, what is the shape, these all samples will fall on the same intrinsic viscosity multiplied by molecular weight curve, as we know elution volume depends only on the hydrodynamic volume, $V_h = \frac{[\eta]M}{2.5N_A}$.

So if in this case, instead of plotting log(M), $\log[\eta]M$ is plotted against elution volume and in that case you can see that different polymers, even polymers have different shapes, fall in one straight line. Therefore, we can use this universal calibration to find out more accurate molecular weight. But, in that case we need to have intrinsic viscosity number for each slices for that we need an online viscometer which will determine the intrinsic viscosity for all the samples. So for universal calibration curve, $\log\{[\eta]M\} = f(V_e)$, for a particular GPC column. It does not matter whether the polymer is linear, branched, block copolymer heterogeneous. If we know the intrinsic viscosity and if we know the molecular weight of the sample then we can construct the calibration curve from which we can determine the value of molecular weight for unknown sample using this calibration curve, this is called universal calibration.

(Refer Slide Time: 28:45)



We can also use other techniques to determine the absolute molecular weight and for that we can use triple detector GPC or SEC. The three detectors generally used are differential refractometer, viscosity and light scattering detector, the response of these three detectors are shown here. For example, the response in viscosity detector is proportional to intrinsic viscosity multiplied by concentration.

$$VISC \propto [\eta] \times c$$

The response in differential refractive index detector, as we discussed earlier also, is proportional to $\left(\frac{dn}{dc}\right) \times c$

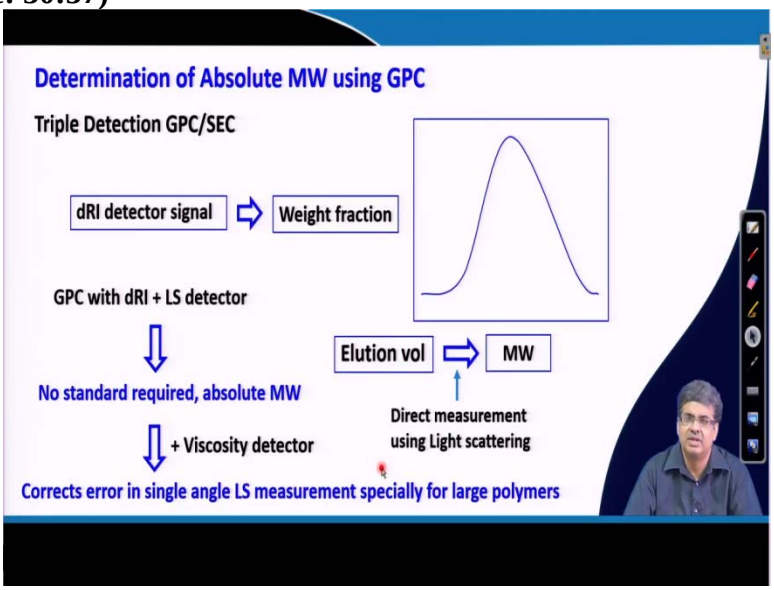
$$RI \propto (dn/dc) \times c$$

and response of the light scattering detector is proportional to $M_w \times \left(\frac{dn}{dc}\right)^2 \times c$.

$$LS \propto M_w \times (dn/dc)^2 \times c$$

Again, this concentration is mass/volume. So using this technique we can actually can measure the absolute molecular weight, we can also measure the intrinsic viscosity, we can also measure the molecular size, radius of gyration. And if we add another detector like UV visible detector, then we can determine the copolymer composition provided at least one of the copolymer segments or copolymer units is UV visible active. In this case we use four detectors, we call tetra detector GPC not triple detector GPC.

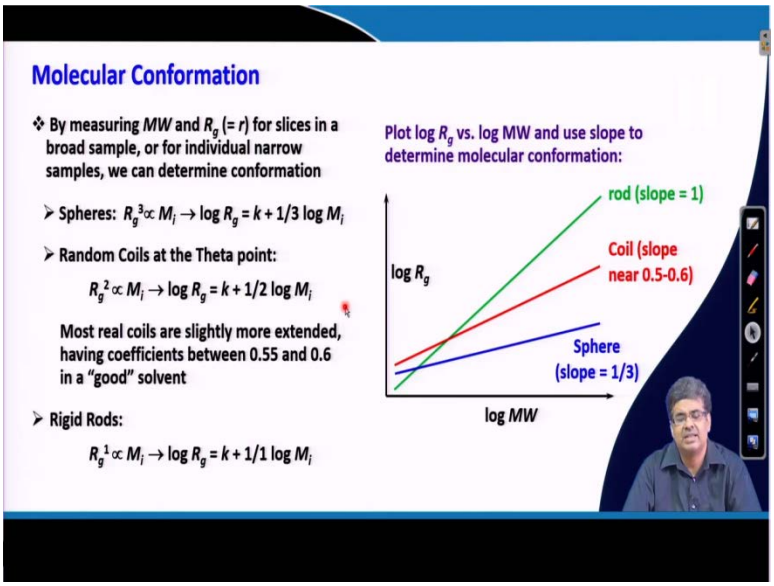
(Refer Slide Time: 30:37)



So how to do this absolute molecular weight measurement? We have a differential refractive index detector signal from which we can convert to weight fraction as we discussed earlier. From each elusion volume, we can use light scattering techniques to measure absolute molecular weight for each of these slices and then we now know the value of molecular weight of each slices and weight fraction. So from that we can calculate the absolute value without using any calibration curve. Now we have learned in last lecture that to know accurately the molecular weight using light scattering measurements we need to do Zimm plot where we need samples with multiple concentrations and we need the scattering intensity values at different angle. Now for this case, GPC, the concentration of each slices are extremely low. Hence, we can consider this concentration is almost close to zero and if we know the amount of sample that we have injected then from the total area under the curve we know the amount of sample that has come out is. Again assuming that whatever was injected has come out, we can get the values for concentration. Using one or two angles, we can measure the value of or we can extrapolate the scattering value at $\theta = 0$.

We have also seen that if the polymer size is small, less than 10 nm, then we do not require this angle dependent scattering data. So GPC with differential reflective index and LS detector will give you the absolute molecular data. I am not going in details about the process of using these three detectors for measuring absolute molecular weight, but I just briefly mentioned the principle of using triple detector in GPC to find out the absolute molecular weight.

(Refer Slide Time: 34:19)



We can also find out the molecular conformation. For that, we need to find out the molecular weight and the radius of gyration from light scattering detector. If we can find the radius of gyration accurately, and we can find the molecular weight data also accurately then using these expressions, we can actually get the idea about the conformation of the large polymer chain.

Spheres:

$$R_g^3 \propto M_i \rightarrow \log R_g = K + 1/3 \log M_i$$

Random coils at the theta point:

$$R_g^2 \propto M_i \rightarrow \log R_g = K + 1/2 \log M_i$$

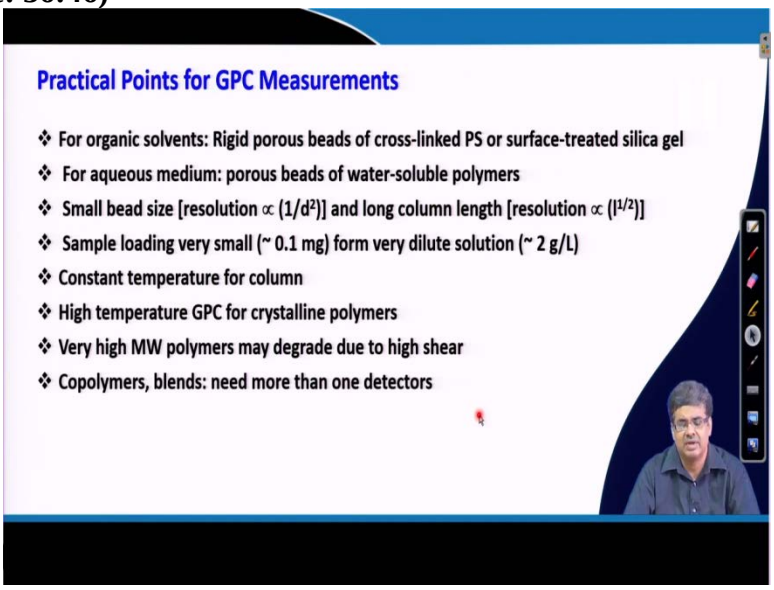
Rigid rods:

$$R_g^1 \propto M_i \rightarrow \log R_g = K + 1/1 \log M_i$$

Obviously, if the size of the polymer chains is low below 10 nm show then there will be no angular dependence or scattering angle, we will not be able to determine the radius of gyration by light scattering technique. In any way, if the molecular size is low then it will be flexible coil of roughly spherical type shape. Now we know that for sphere, R_g^3 is proportional to molecular weight for random coil, at theta point R_g^2 is proportional to molecular weight, and for rigid rod R_g is proportional to molecular weight. So we can plot $\log R_g$ versus $\log MW$ and use the slope to determine the molecular conformation or shape.

If its slope is one then it is close to rod shape. If it is one third, there is a perfect hard sphere type shape, and for most polymer molecule it will be value of 0.5 or 0.6 or 0.7 in that case the coil shape, and 0.5 the solvent is theta solvent. Therefore, if the polymer size is large, we can use light scattering detector to determine the R_g then we can get some idea about molecular conformation using this technique as well.

(Refer Slide Time: 36:46)



Quickly, let me say a few practical points about GPC measurement. For organic solvent, rigid porous beads of cross-linked polystyrene or surface treated silica gels are used; for aqueous medium, porous beads of water-soluble polymers like dextran are used. Small bead size and long column lengths are preferred for good resolution. Resolution is actually proportional to $1/d^2$, d is the diameter of the bead, and resolution is proportional to the length to the power half.

Sample loading generally is very small, about 0.1 mg, which is turned out to be very dilute solution, 2 g/L. If the difference in refractive index of the solvent and the polymer sample is low then we need higher concentration of the polymer in solution to get a more intense signal in RI. Temperature of the column must be maintained fixed, this is very important.

For crystalline polymers, high temperature GPC is required to solubilize the polymer samples. If the molecular weight is extremely high then while passing through the pores, the polymers might

generate high shear and that might degrade the polymers. So we should avoid injecting very high molecular weight polymers in the column. To characterize copolymer or blend we need more than one detector. With this, I come to the end of this lecture.