Modern Instrumental Methods of Analysis

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Lecture No. # 43

Gas chromatography-3 Applications

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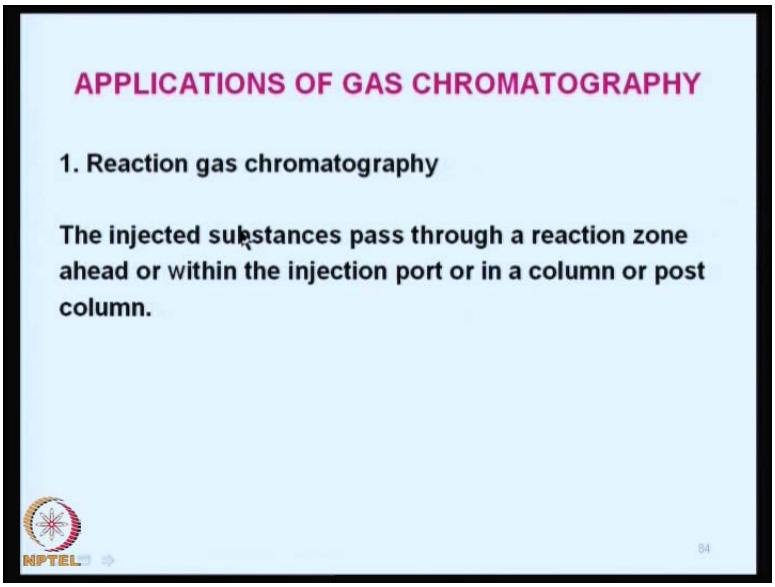

We were discussing the applications of gas chromatography. In that first of all we had taken reaction gas chromatography, and the idea is to inject the substances that passed through a reaction zone ahead of the injection port or within the injection port or in a column or a post column. So, the reaction before the column would facilitate formation of different compounds which will be easily separated as components, but post column reactions would be normally meant for identification of the substances, that are already separated something like derivatives derivatization etcetera.

So, we had discussed about the straight chain alkanes, and cyclic alkanes and branched alkanes mixtures, if we have. We had discussed that 5 angstrom unit molecular siev molecular siev can be used for the separation, and we had discussed that around 200 and 68 degree centigrade hexane is not absorbed from a mixture of hexane, heptane, and octane. So, if hexane is not absorbed; the hexane will be coming out. So, other

substances would be absorbed. So, this is some an example of a reaction chromatography.

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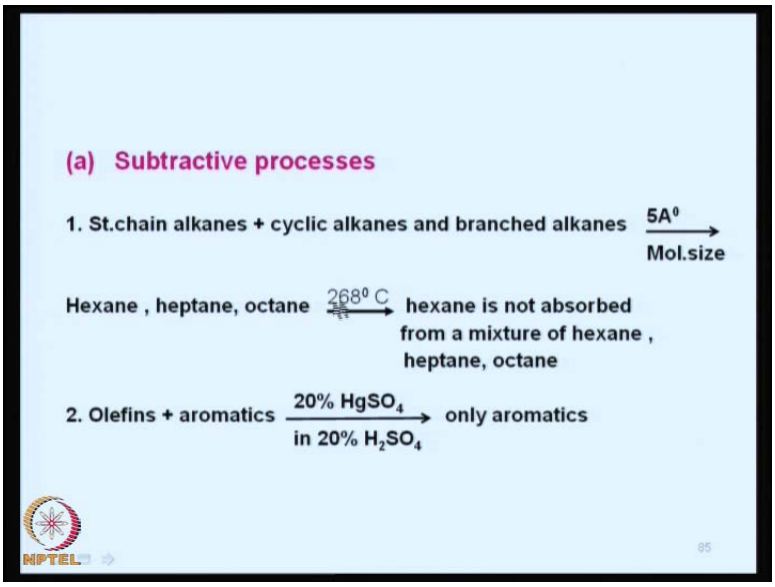

Another reaction chromatographic technique I had discussed is about the olefins, and aromatic mixtures. So, there we have discussed that 20 percent mercuric sulphate, if we use in solution of 20 percent sulphuric acid; only aromatics will be coming out of the column, and the olefins would be absorbed in the solutions. Remember that this is not a separation process on massive scale, it is only in the micro liters scale. So, a small quantity of the reaction zone, small quantity of the reactants as well as small portion of the column can be converted into reaction gas chromatography.

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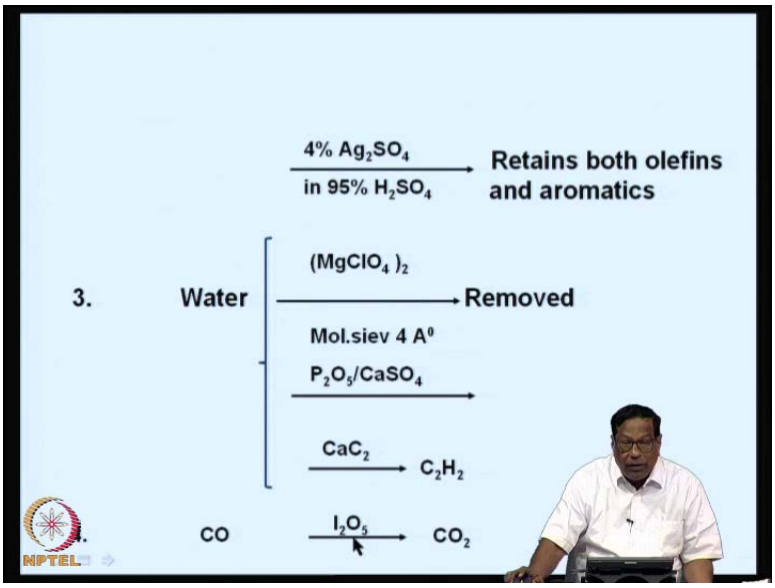

So, if suppose we have both we use 4 percent silver sulphate, in 95 percent sulphuric acid would be retaining both the olefins and aromatics; that means, all the unsaturated compounds would be absorbed by using this mixture.

Now, suppose we have water; you want to remove the water, because water could be sometimes an impurity, it may not be necessary to determine the water contact. So, you would like to remove it from the column. So, for that we can use magnesium perchlorate solution or a molecular siev or we can use P 2 O 5, that is phosphorous pentoxide, and calcium sulphate, and then calcium carbide. In all these things water will be removed except in calcium carbide, if the water content would there would be a reaction generating the acetylene. This is a very standard reaction any organic chemistry text book would would be dealing with this reaction, you can look up the details of the reaction in these things.

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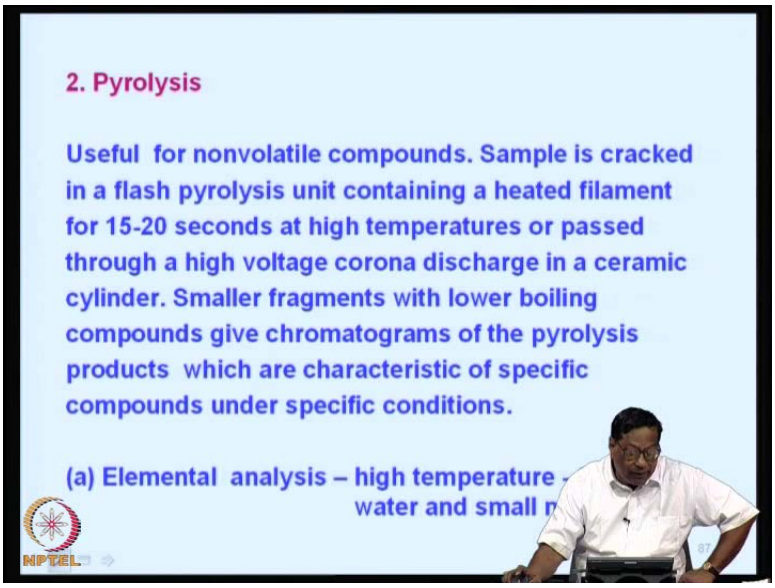

Then similarly if you have cobalt carbon monoxide, we can use iodine pentoxide which will oxidize it to CO 2 which can be easily separated by gas chromatography. And then second type of substances reaction is very useful, because if you have the nonvolatile components many of them would not elute. Therefore, what we do is we subject it a flash pyrolysis; that means, pass the components through a hot wire, something like heated filament probably you can use a platinum or tungsten for about 15 to 20 seconds at high temperature or you can pass through even a high voltage corona discharge in a ceramic cylinder.

So, smaller fragments are are generated with lower boiling points, and such compounds will give chromatographs of the pyrolysis products which are characteristics of the specific compounds that is parent compound, and are the specific conditions what we have employed for the gas chromatography. Elemental analysis basically happens during pyrolysis at high temperature, and very simple products like C-carbon, CO 2 water and small smaller molecules are usually generated.

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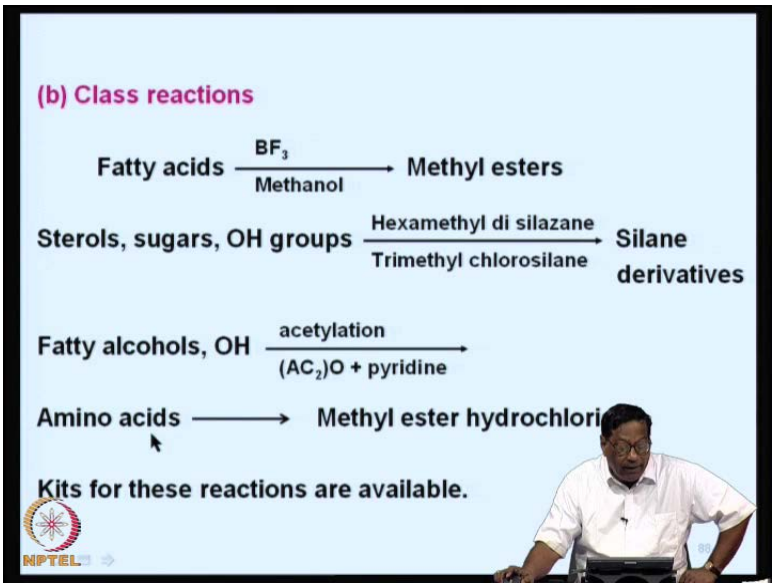

Now, there could be you can conduct reactions as class reactions; for example, if you take fatty acids make them react with boron trifluoride in methanol medium, you would be generating methyl esters, and this methyl esters having lower boiling points would be very easy be to separate instead of fatty acids, which are having higher boiling points and they could be solids many of them.

So, other reactions which I want to detail here are sterol, sugars, and OH groups, and there is things we can react with hexamethyl di silazane, and trimethyl chlorosilane to get silane derivatives which can be easily chromatographed. Similarly, fatty alcohols and or OH groups; here OH groups can be converted into a acetylation, but the acetylation reactants are acetic anhydride, and pyridine - in pyridine medium. And again you would be generating the acetylation products which can be easily separated.

Amino acids is also similarly you can convert them into methyl ester hydrochlorides, and a an interesting aspect of such reactions are the that several reaction kits are available for conducting the reaction gas chromatograph. So, you can depending upon your applications, you can buy different kits, and then incorporate either in the pre column or injection phase, the injection port or post column, etcetera. And then they can be easily separated.

Now, you can I want to discuss with you something about program temperature gas chromatography. Here, what is happening is suppose we have compounds having a wide

range of boiling points. And you would know you intuitively, but in chromatography gas chromatography compounds with lower boiling points would be coming out first, and then higher boiling points would be coming out later. And all the separation depends upon the partition coefficient.

Now, compounds with higher boiling points would be have would be low having low volatility, and in general they will not come out. Suppose, we have a mixture of compounds, which are having low boiling as well as high boiling mixtures compounds in a given mixture. Then, you will it will take enormously long time to separate the compounds for which the analyst may not have the time or the patience. So, as the what we normally do the trick is while the column gas chromatography is being operated, we plan to raise the temperature of the column. And in a standard manner of about 4 degrees per minute or 10 degrees per minute 5 degrees per minute like that.

So, that as the lower volatile compounds compounds with lower boiling points would be coming out, but at the same time slowly we are increasing the temperature of the column. So, that non volatile components would also gets sufficiently volatilized, and start moving down the column; subsequently they will emerge out.

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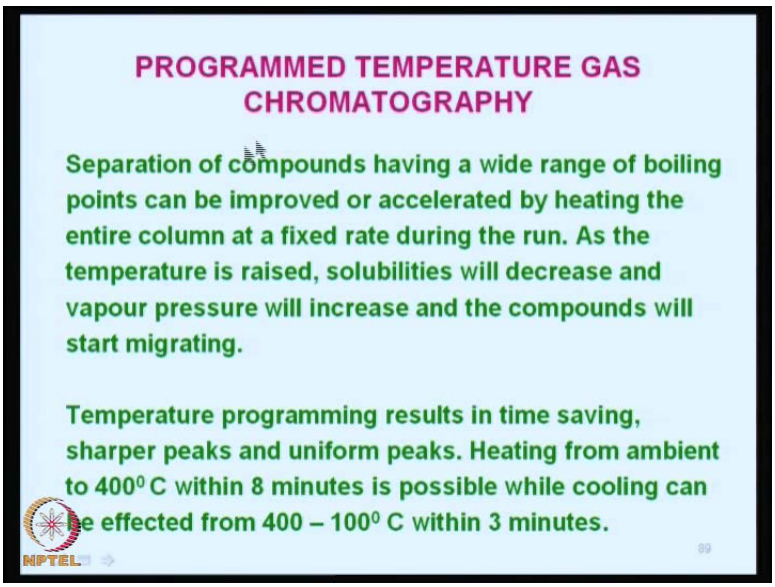

So, here we can see in this slide that what I have written is that the wide range of boiling points can be the separation can be improved or accelerated by heating the entire column

at a fixed rate during the run. As the temperature is reached raised, solubilities will decrease and vapour pressure will increase, and the compounds will start migrating.

So, temperature programming basically results in time saving, sharper peaks, and uniform peaks. So, heating from ambient to 400 degree centigrade within 8 minutes is possible, while cooling can be effected from 400 to 100 degree centigrade within 2 to 3 minutes. So, that is the advantage of gas reaction gas, that is temperature programming.

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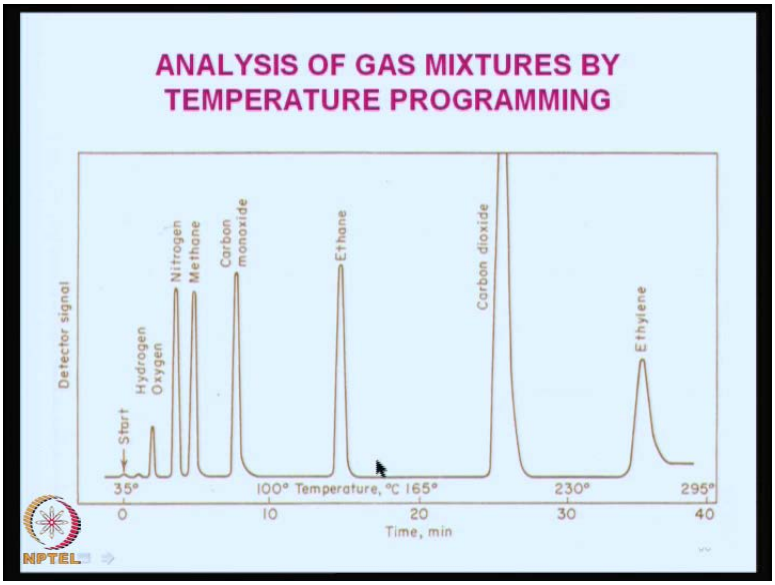

So, program temperature gas chromatography is a very useful technique for example, you can see in this slide that we we have hydrogen, oxygen, nitrogen, methane, carbon monoxide, ethane, carbon dioxide, etcetera, and then ethylene, etcetera. You can see that these around the the starting temperature is 35, and around 100 degree centigrade you would get mostly hydrogen, oxygen, nitrogen, methane, carbon monoxide. In this range all of them will come out, but the ethane will not come out, until the temperature is reached around 140 to 150 degree centigrade. And then even the elusion time is approximately 15 minutes.

So, if you do not heat, then the graph would you would not be able to see the graph in a single screen, and you may it may extend beyond the screen, and you will have to compress, but if you are heating the column subsequently the whole graph can be obtained same thing is to with respect to carbon dioxide. And here in this case, we can imagine that a pyrolysis has already been carried out, because most of the products are

very simple products like hydrogen, oxygen, nitrogen, methane, ethane, etcetera, and carbon dioxide also would be one of the products, but that comes out around 200 degree centigrade.

And then the time is also about 28 minutes elution time, and ethane ethylene would be still longer and it would be coming out around 295. This example very clearly illustrates, that the temperature has got a very performed effect on the separation of the compounds.

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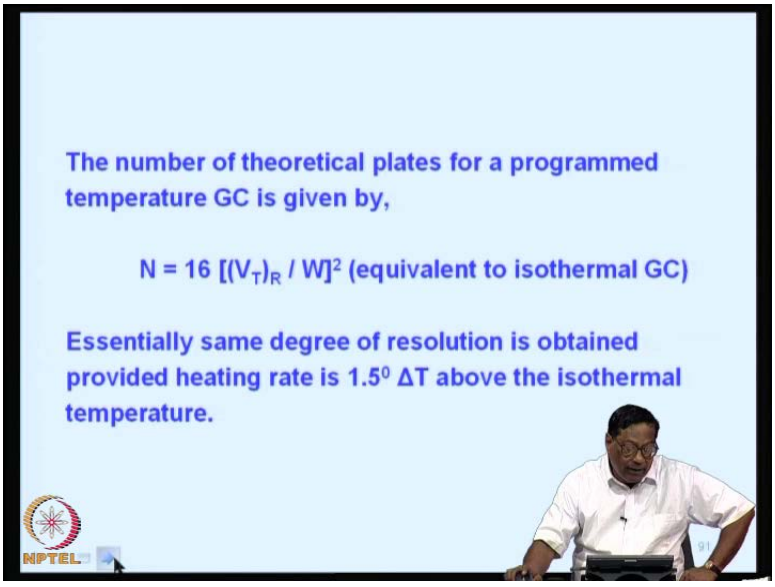

So, the number of theoretical plates for a programmed temperature gas chromatography is usually given by this expression, that is n is equal to 16 V T R divided by W whole square that is equivalent to isothermal GC. Essentially the same degree of resolution is obtained provided heating rate is around 1.5 degree degree on 1.5 delta T above the isothermal temperature, this is only a guideline; it is not it does not happen all the time.

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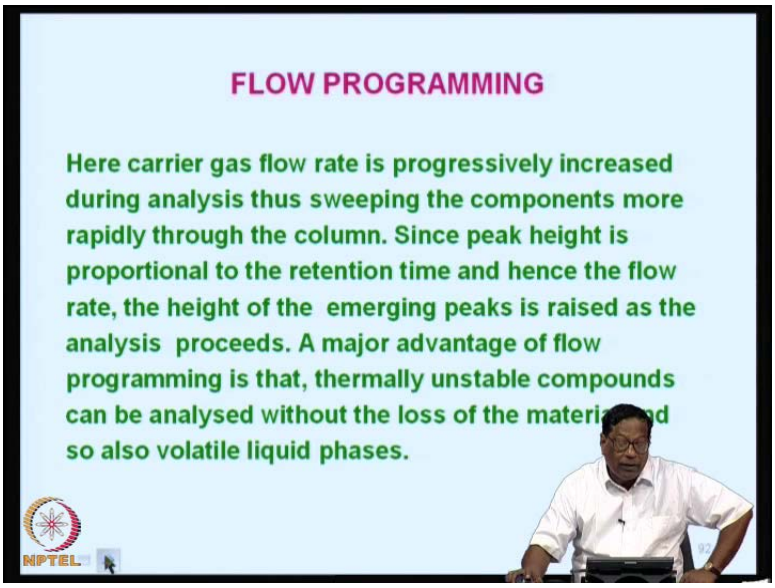

Now, I would like to discuss with you about flow programming. Now, we have discussed about temperature programming, where it was not possible to separate low volatile components as well as very high volatile components, in a single run. Now, we have raised the temperature, and separated the substances, there there is an inherent assumption that the temperature raising of the temperature does not decompose the substance, it does not destroy the compound. Now, suppose we are dealing with enzymes, and proteins, and other things as you keep on raising the temperature, there is a danger of substances getting decomposed at higher temperature. So, instead of getting the products as such, that is the chromatograms of the substances which you want to separate, you would be separating the compounds which have underground decomposition.

Sometimes it is not acceptable quite often; therefore, we use another trick that is known as flow program. What we do here, is here the carrier gas fluoride we keep on increasing, and then during the analysis when we increase the carrier gas fluoride, sweeping the we are almost forcing or sweeping the components from one theoretical plate to another theoretical plate.

So, but we will be doing it more readily through the column, since the peak high it is proportional to the retention time, and hence the fluoride, the height of the emerging peaks is raised as the analysis proceeds, because the compound keeps on coming out

much faster than it would have if the fluoride was had been kept constant. So, major advantage of flow programming is that thermally unstable components can be analyzed without the loss of the material, and so also the volatile liquid phases can be separated very easily. In this slide, that I have put the basic requirement.

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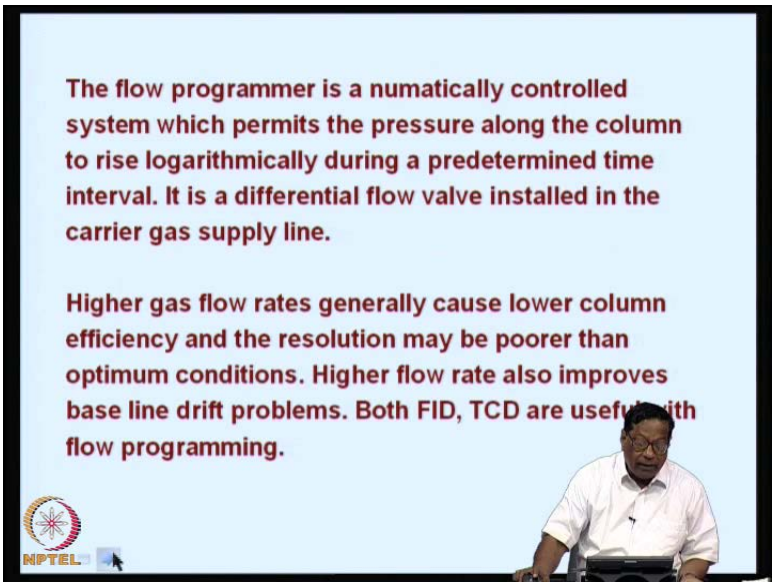

And in the next slide, the flow programmer is numatically a controlled system incorporated in the gas chromatographic instrument. You can do it, even with a computer by giving a MS dos command to increase the fluoride. So, that permits the pressure along the column to raise logarithmically during a predetermined time interval. It is a differential flow wall installed in the carrier gas supply line basically.

So, higher gas flow rates generally cause lower efficiency, but the resolution may be poorer, and then the optimum conditions, this is the sort of restriction quality reduction or except, but a higher fluoride also improves the base line drift problem, the base line drift will remain steady during much of the time, because small variations in the fluoride does not matter, when you are have increasing the fluoride. So, both FID and TCD should be useful with flow programming.

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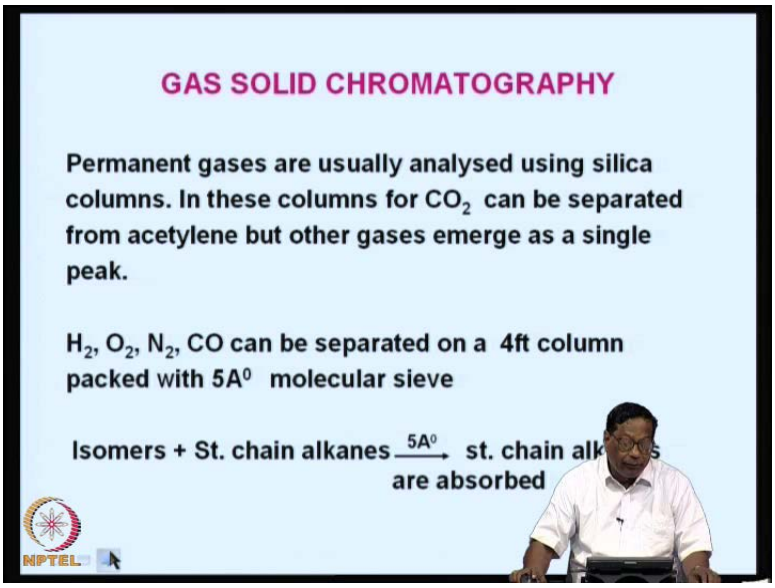

So, there are other aspects like, gas solid chromatography. So far, we have been discussing only the gas liquid chromatography. Now, what I would like to do gas chromatography can also be used to separate the substances, which are in general substance a column materials which are solids by themselves. Earlier, we had used the columns, and column support material was coated with a high boiling liquid material which could be polar, non polar or intermediate polar, etcetera etcetera.

Now, what we are trying to do is we are using the solid support itself, as a column material. So, that is known as gas solid chromatography, it is not very very well developed, but useful all the way, because it is not a separate section or separate part of gas chromatography, you can buy a column without using the liquid support on the support material. So, here what is the importance? The importance is permanent gases are usually analyzed using silica columns; in these columns carbon mono dioxide can be separated from acetylene, but other gases emerge as a single peak.

So, if you look at the slide, we can simply use a 5 angstrom molecular siev to separate isomers, and straight chain alkanes, then what happens is straight chain alkanes are absorbed, and other isomers are not absorbed. So, you can find out how much of the isomers are present.

For example, here hydrogen, oxygen, nitrogen, carbon monoxide, etcetera. They can be separated on a 4 feet column packed with 5 angstrom molecular siev, remember there is

no liquid there is no liquid material, that is there is no liquid support coated on the column material. it is just a column material which is (( )) and filled with 5 angstrom molecular siev.

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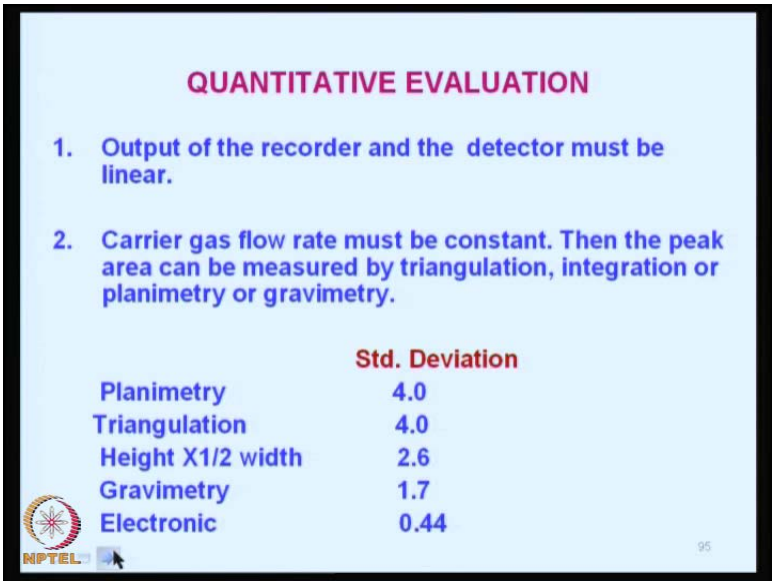

So, such reactions or such separations are known as gas solid separations. And they are useful anyway those who need to separate small gases etcetera, small quantities of gases water etcetera, small straight chain and isomers substances. They can be separated very easily using these things.

Now, quantitative evaluation I want to discuss a little bit, and generally what I would like to say at this point is quantitative evaluation is all about this course. The all during the course our emphasis has been on the quantitative estimations. So, if we use gas chromatography, as a quantitative tool to separate the substances, and measure the their separations. The output of the recorder, and the detector must be linear, that is one of the pre conditions that we assume that they must be linear.

So, the carrier gas flow rate must be always constant, then the peak area can be measured by triangulation or integration or planimetry or (( )) or gravimetry. What you can do is you can take the chromatograms on a graph paper, and count the number of squares, and for each component under the that, and peak of each component. And then, you can quantitatively evaluate, so many peaks correspond to so much of the quantity that we

have the injected, and then you can quantitatively estimate relatively, that is the separation efficiency.

So, triangulation also involves some sort of a an approximation, in assuming that each peak is something like a triangle. So, you have to draw a base line, imagine a triangle in which the whole peak is fitted, and then determine the quantity. Then, if you sometimes what people do is, because the triangle cannot be fitted very nicely, they go for height multiplied by half of the width, that is also possible. To determine the extent of separation, extent of each peak.

And then you can cut the chromatographic peaks, and weigh them; this is also a very simple process, and it can be done and then electronically of course, it is possible to integrate each peak as we have seen in NMR, and other areas. And you can see that there is some amount of uncertainty involved in all these cases; that means, in planimetry and triangulation there is a standard deviation of the reproducibility is around 4 percent. Whereas, if you measure the each peak by its height multiplied by its half of the width, then the standard deviation comes down to 2.6. And if you use gravimetry it is about 1.7 whereas, in electron - in the case of electronic integration it will be 0.44, now it is 90 percent of the gas chromatographic separations are evaluated electronically.

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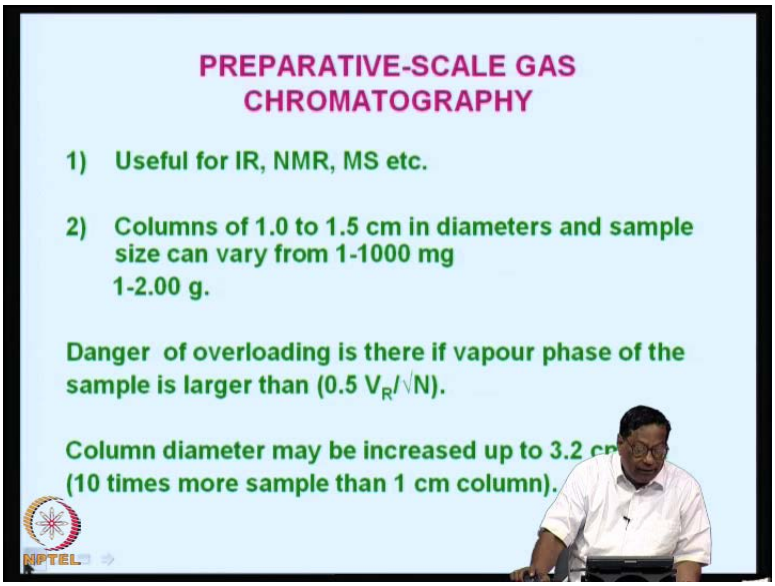

So, I am very sure that other methods are almost outdated, but for the sake of gravity I have introduced all these things, why because quite often we are faced with a situation

that electronic integration is not always possible. And we should be in a position to evaluate, and take care of the of the quantitative aspects even when we do not have the electronics, and computer at our disposal quite often it happens, that we are forced to deal with such things.

Now, so if we I will spend a few minutes on the preparative scale gas chromatography, the logic is very simple. If we are in a position to separate the components in micro scale or may a micro liter scale, why not do it for the scale it up at higher level for the quantitative separation. Theoretically it should be possible, and it has been made possible, and such separations if we are able to achieve from the impurities, and substances associated isomers etcetera. Then they could be directly connected with with MG gas chromatic with infrared NMR mass spectrometer, etcetera for the direct identification of the separated substances or they can be used for separation.

So, what we go in the case of preparative scale gas gas chromatography is that, they are useful for identification by in IR, NMR, and mass spectrometer, etcetera. In the requirement in each of these cases is that the columns diameters should be of above should be about 1 to 1.5 centimeter, and sample size also can vary from 1 to 1000 milligrams, that is you can even go up to 2 grams. So, a from few milligrams to 2 grams substances you can separate.

Now, there is a danger of overloading, if vapor phase of the sample is greater than 0.5 yard divided by root N, that is a standard indicator equation for us. So, that we we should be able to decide, whether a particular gas chromatographic separation can be successful or not, that is preparative gas chromatography. So, the column diameter also you can increase up to 3.2 centimeters diamp, that is the again the guideline here is the column diameter should be 10 times more than the 1 centimeter column, that is in preparative gas chromatography usual separations, if you want to expand from from gas chromatography to preparative gas chromatography column diameter should be increase by about 10 times.

So, another approach is repetitive injections instead of using the same column for bigger diameter, we can go for repetitive injections, and continue the separations; that is automatic injections. So, of small samples on narrow diameter columns something like, 3 by 8 or 3 4 th inch of diameter; the in such cases sample size can vary from 5 to 30

milliliters, and a high percentage of liquid phase is also essential. And the sometimes, we we use short fact columns that is known as high volume preparative work, that is very essential for high volume preparative work.

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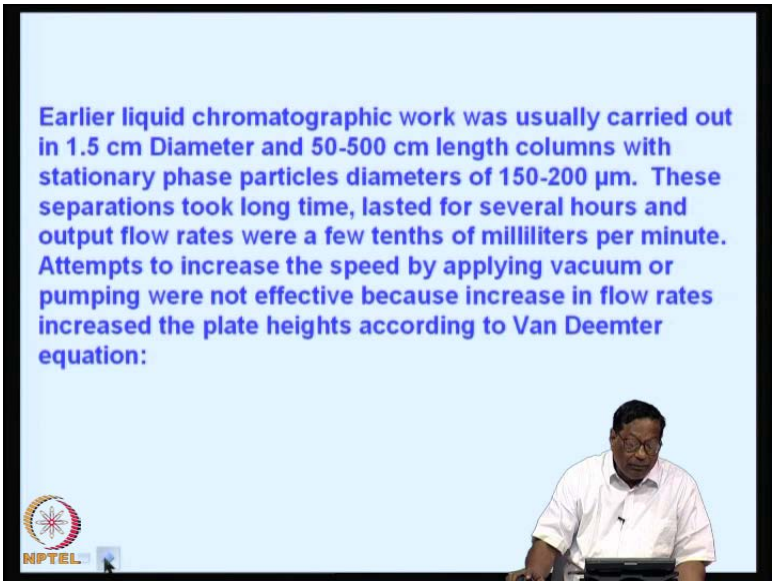

So, I would like to say that I have introduced you two different kinds of aspects of the gas chromatography, during my last discussion. And now I would like to spend a few minutes on the application of high performance liquid chromatography. What is important? In the case of high performance liquid chromatography is that separations from gas chromatography to high pressure liquid chromatography, it initially started as a curiosity, we have been doing separations of substances in columns using aqueous and non aqueous solutions.

So, substances which are volatile, we can go for gas chromatography, but suppose we want to separate inorganic ions, and then organic ions, proteins, and then plasma, blood plasma, and several other systems like that. It is possible to separate them using a simple liquid chromatography. Now, earlier liquid chromatography where cause usually carried out in 1.5 centimeter diameter, and 50 to 500 centimeter length columns; that is something like a view write that is 50 centimeter, and 1.5 centimeter dia is (( )). So, you can increase it by about maximum up to 500 centimeter length columns with stationary phase particles of 150 to 200 micrometer - diameter.

These separations usually took a long time, and they lasted for several hours and output flow rates of the few eluent, where a few tenths of millimeters milliliters per minute. So, it used to take very long time, and then you go to start the experiment go for coffee come back, go for lunch and come back by (( )) again still the compounds would not be separated. So, the atoms are made to increase the speed by applying vacuum or pumping, but these things were not very effective, because increase in flow rates increase the speed plate heights according to van deemeter equation.

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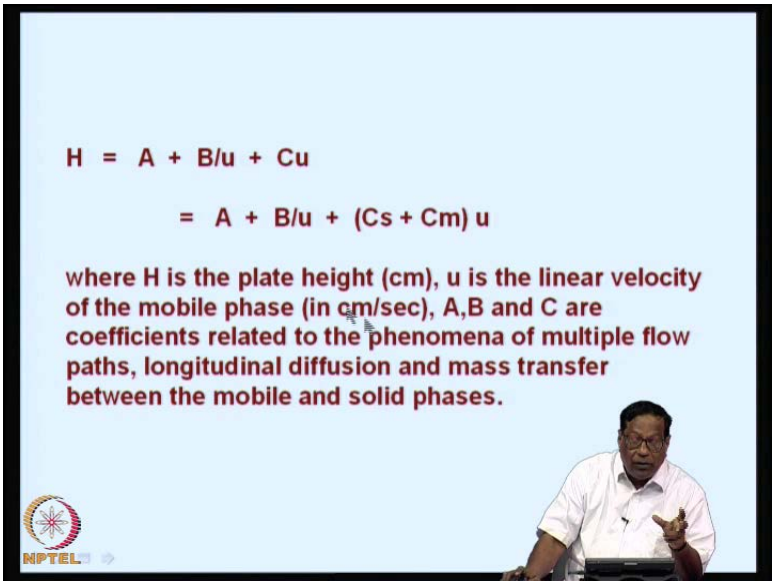

It is a very simple equation defined as H is equal to A plus B by u plus Cu, where A is equal A, H is the plate height, u is the linear velocity of mobile phase that is in the centimeters per second, A, B, and C are the coefficients related to the phenomena of multiple flow paths. And longitudinal diffusion, and mass transfer, etcetera between the mobile and solid phase. So, its not a very simple straight forward equation.

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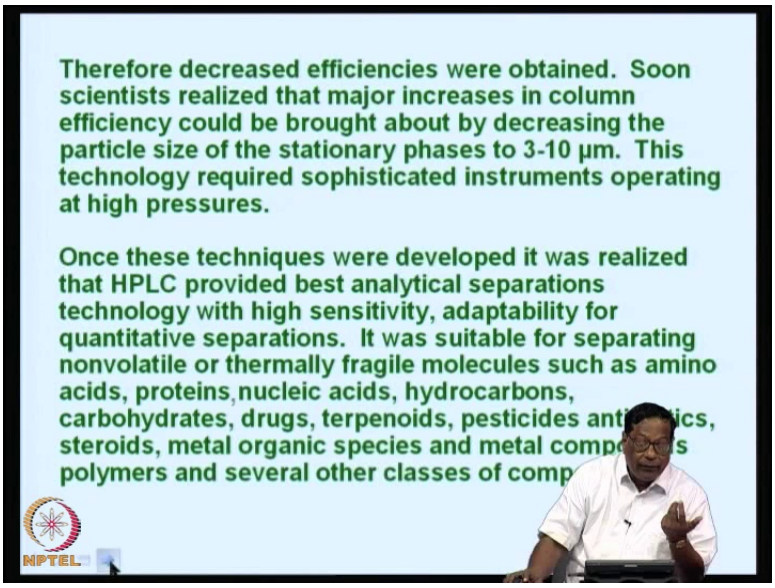

So, its not always very easy, it is not always easy to look for separations using high flow rates, and vacuums in liquid chromatography. We are not talking about gas chromatography now, we are talking about liquid chromatography.

Therefore because of all these restrains, what I have described here in longitudinal diffusion, multiple flow paths, and mass transfer etcetera. The efficiencies were always low. So, soon scientists realized that major increases in column efficiency can be brought about by decreasing the particle sides of these stationary phase to a few microns that is 3 to 10 micrometers.

This technology provided sophisticated instruments operating at high pressure these permits, high pressure operations. So, once these techniques were developed it was realized, that high pressure liquid chromatography provides best analytical separations taking separation technology with high sensibility, adaptability for quantitative separations.

It was suitable for separating the non volatile or thermally fragile molecules like what I have discussed earlier, that is amino acid, proteins, nucleic acids, hydrocarbons, carbohydrates, drags, terpenoids, pesticides, insecticides, and several other antibiotics, steroids, metal organic species, and metal compounds, polymers, and several other classes of compounds could be separated using liquid chromatography, and only if you are able to separate the the use 3 to 10 micrometer particles.

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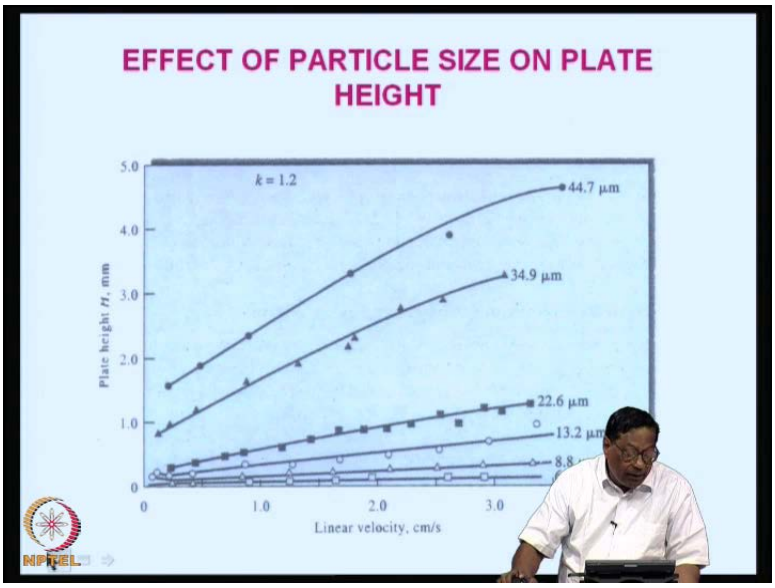

And from to the liquids through the the column, because the smaller the particle the liquid flow rate would be lower. So, to maintain the liquid flow rates at higher rates, we have to increase this vertical pressure. So, that is why it was known as high pressure liquid chromatography, and this chrome high pressure liquid chromatography was initially about about to 20, 30 years before, it was a laboratory curiosity. And most of the substances being separated, where there there simple inorganic solutions mixtures of flames or things like that, but subsequently once the once the separations were achieved. The instrumentation were developed, you will be surprised to know that between 1984 and 86 within 2 years, there were about 1500 papers, 300 national and international conferences, and more than 100 books, and review articles, etcetera published with in a span of 2 years, so that is real development.

And today the high pressure liquid chromatography is an is an essential component of any laboratory worth its name, which are dealing with the substances like amino acids and other things what I had told you, and the the substances are all these because we are able to increase the efficiency by increasing the particle size, as well as the flow rates because of under, because of the high pressure pumps. Now, we can see here in this slide, that the linear velocity and plate height I have drawn drawn here, we can see that if the particle size is 6.1 micrometer, linear velocity plate height is in millimeter, and we can see that the effect of particle size on plate height could be fantastic as shown in this figure.

For example, here it is 6.1, this is 8.8 micrometer, and the plate height would be steel wire, and you increase it up to 22.6 your your normal plate height is of the order of what 0.8. And if you increase it to 34 or for 35, then the effective plate height also will increase depending upon the linear velocity of the effluent of the eluent, and 44.7 is something phenomenon.

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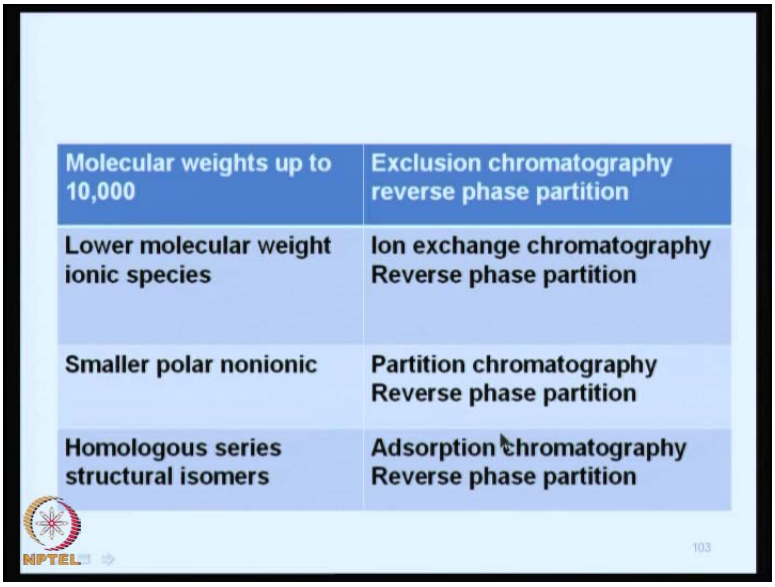

So, the molecular weight, the applications and the substances, the classification what we can do is we can have substances with molecular weights up to 10000 if we have, we can go for exclusion chromatography, and reverse phase partition. And then still our molecular weight ionic species can be separated using ion exchange chromatography, and reverse phase partition.

Smaller polar non ionic molecules can be separated again using liquid liquid partition chromatography with reverse phase partitioning, and homologous series structural isomers, etcetera can be separated using adsorption chromatography, and reverse phase partition.

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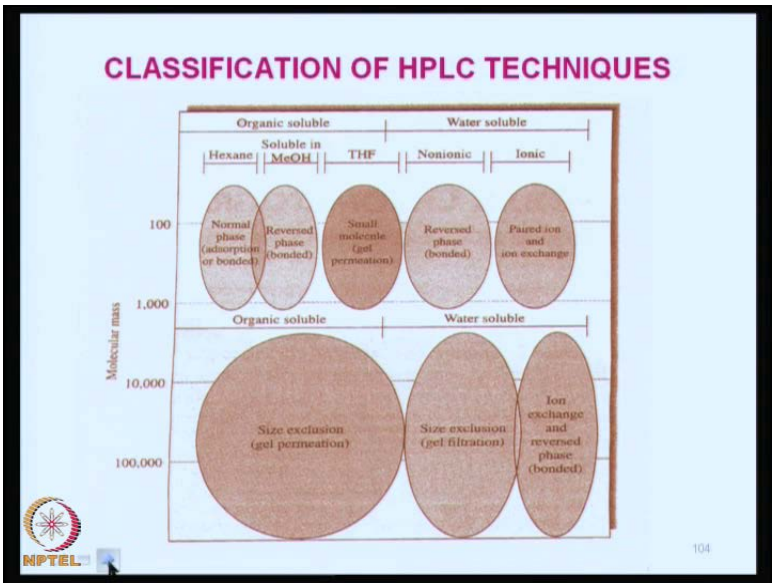

So, here is a least track of classification of the HPLC techniques, here in the top I have put organic substances, and here I have put the water soluble substances among the organics we can classify something like hexane soluble methane, this is soluble methane, this is THF, this is non ionic, this is ionic.

So, at one end we have almost non polar substance, and at the other end we have auto soluble and most polar substance. So, here I have drawn molecular mass and the techniques - possible techniques I have put here, that is size exclusion, that is gel permeation gel filtration, and ion exchange and reversed phase bonded technology for water soluble substances, and size exclusion for organic substances. So, size exclusion would work exclusively in for substances having 1000, 100, 1000 molecular weights, and from 10000 up to let us say about 1500 or may be around 2000 to 100000, and above the size exclusion is the technique.

Here, in if it is non polar substance we can go for normal phase normal phase or reverse phase also to some extent, if it have polar; that is adsorption or bonded columns. And then small molecules will be separated by gel permeation, and for non ionic and ionic substances we can go for reverse phase to substances bonded and ion exchange columns.

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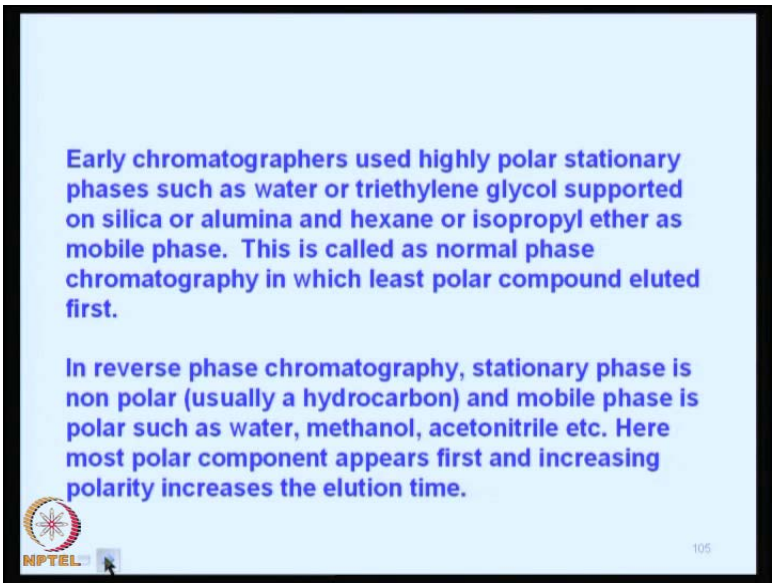

So, early chromatographic chromatographers used highly polar stationary substances phases, such as water or triethylene glycol; these substances were used on supported they used to on suppose used to cot the silica or alumina, and hexane or isopropyl ether etcetera as the mobile phase. This is called a normal phase chromatography, in which the least polar substance eluted first.

It is a it is stands to reason that if the stationary phase is polar, and silica or alumina are there, then the it is a separation of least polar substance coming at first and non polar substance gets absorbed, and comes out later. This is known as normal chromatography. In reverse phase chromatography, what we do is take the stationary phase as a non polar substance, usually a hydrocarbon; and mobile phase we are changing it to polar phase; that is what our methanol, (( )) etcetera. Here the most polar component appears first, the on increasing the polarity, increases the illusion time.

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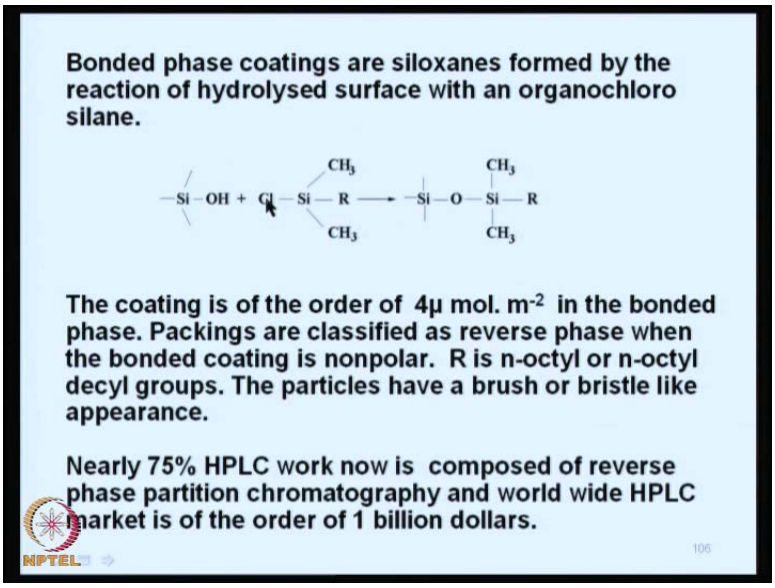

So, the bonded phase coatings are usually siloxanes formed by the reaction of hydrolyzed surface with an organochloro silane. The you can see in this slide that Si OH group are coated with ethyl groups, and then what you have is silicon bridge followed by methyl groups. The coating is of the order of about 4 micro moles per square meter in the bonded phase. So, packings are classified as reversed phase when the bonded coating is non polar, this you will have to understand, because reverse phase separation is the most followed technique in HPLC.

So, here I have put R – R is a n octyl or n octyl decyl groups, the particles have a brush or bristle like appearance, and nearly 75 percent of HPLC work is composed of RPPC, that is reverse phase partition chromatography. And worldwide HPLC market is of the order of about one billion dollars, already more than several lacks of HPLC instruments are there all over world in or spread over, all over the world in almost every laboratory worth its name.

So, pumping pressures of several 1000 pounds per square inch are required. So, equipments tends to be more elaborate and expensive, because we have to subjects the liquids to high pumping pressure. So, reservoirs you would need of 200 to 1000 m l that is the liquid phase eluent equip with spares, vacuum pumping, distillation system, heating stirring, dust filters, milli per filters, and their vacuum constitute other HPLC components.

So, sometimes we either we use a single solvent as a mobile phase, in that case we call it isocratic dilution, illusion. A mixture of 2 or 3 solvents also we can new program it to increase the polarity in the series of steps or continuously and that is known as gradient illusion illusion.

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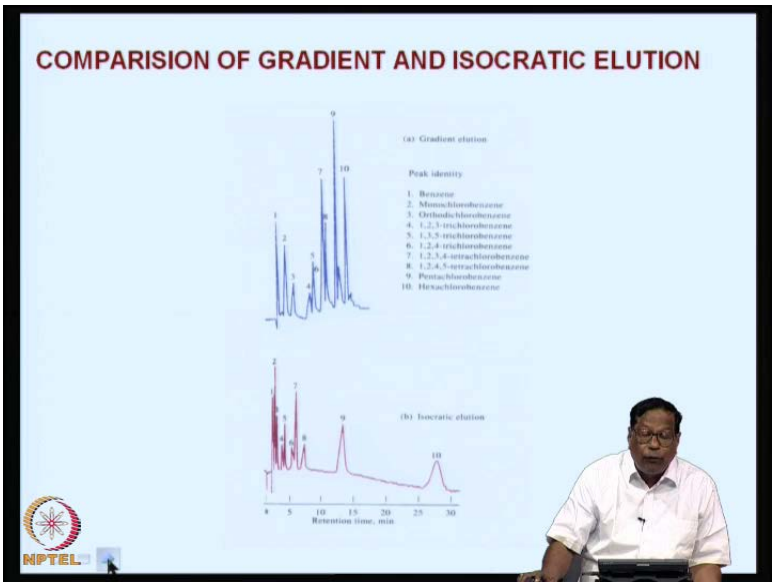

So, comparison of gradient and isocratic illusion I am showing here in this case. So, the top figure is a gradient illusion figure in which the substances like, benzene, mono chlorobenzene or ortho dichlorobenzene, 1-2-3 trichlorobenzene, etcetera. They are isomers have been separated in such a beautiful fashion, if I use a gradient illusion. If I use a single eluent, we can see that the same substances are separated in this ray, and it will it takes lot of time, and it increases the the working expenses. So, it is better to go for gradient illusion in these things.

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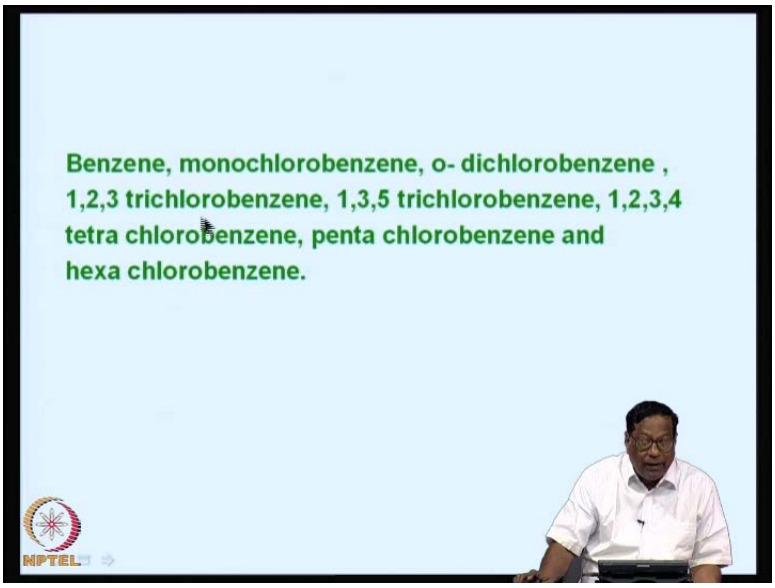

So, this these are the substances which have been separated in the previous slide, that is benzene, monochlorobenzene, ortho dichlorobezene, etcetera. And this I am not going into details of the of these instruments especially, because we are we are in a position to understand that the complexity, and the capability of HPLC is always dictated by the substance about the column material as well as the operator efficiency.

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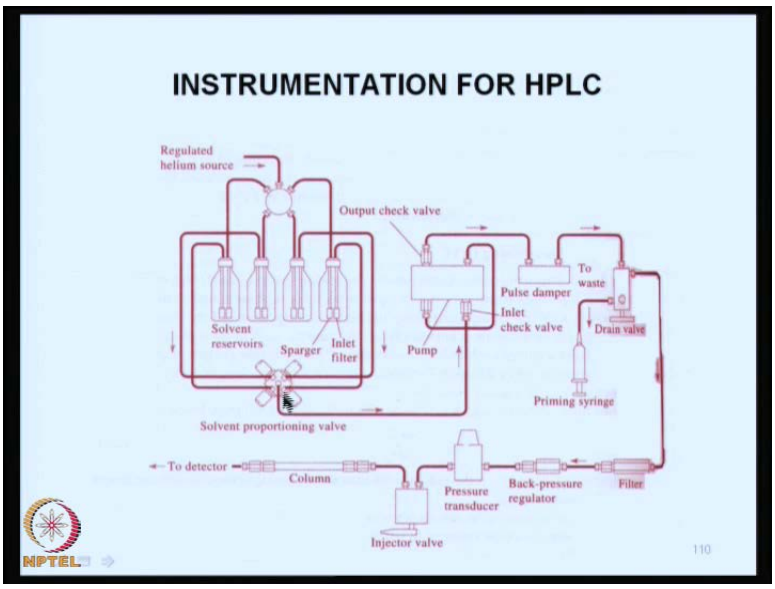

So, here is a small list of the basic equipments schematic diagram of course, and the here this is the regulated helium source, and then we have solvent reservoirs 1, 2, 3, 4 here,

and then I have from all these things, there is a sparger, then the mixture then for gradient illusion, etcetera. And solvent proportioning valve is there, and then it is taken to a pump and output check wall is there, then we a have a pulse then that is also the liquid should be delivered in a uniform manner. We got much this things, and then we have a small drain wall followed by a priming pumping priming syringe, and then the substance enters a pre filter, a back pressure regulator, pressure transducer, and then in injector valve followed by the column, and the end of the column we do have some sort of a detector to separate the component to identify the separated component.

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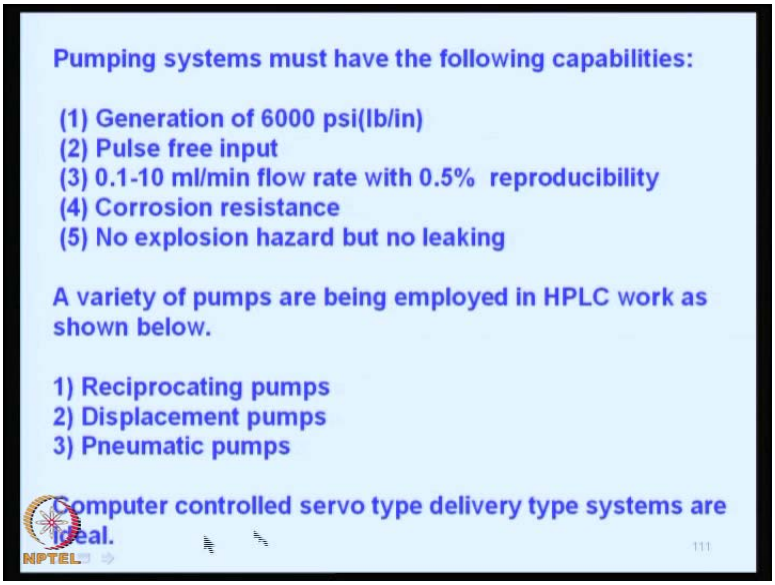

So, this is essentially a schematic diagram of the substance of the equipment of HPLC, and there are lot of developments, and I am not going into the details basically, because this is almost like a last lecture. And I would like to leave something for you to learn on your own, but I want to give you the basic requirement of the gas chromatography.

For example, we can see here that pumping systems must have the following capabilities. One is generation of 6000 psi or lb's per inch, and what we need is pulse free input, and flow rate should be between 0.1, and 10 milliliter per minute with 0.5 percent reproducibility that (( )) places great demand. And then the substances should be corrosion resistance, because here using polar as well as non polar substances etcetera.

And especially when you are working with pressures there is an explosion hazard. So, the explosion hazard must be taken care of there should not be any explosion, and there should not be any leakage - the moment leakage comes HPLC fails immediately.

A variety of pumps are being employed in HPLC work as shown below that is reciprocating pumps, displacement pumps, pneumatic pumps, etcetera, and computer controlled servo type delivery types system are usually ideal. Now, the sample injection I would like to just spend a few minutes, I will introduce you to the concept now, and then request you to look up the aspects what I am going to enumerate on your own. So, that we can you can have a the it is something like an assignment which I have taught you for so long, that you would like to I would like to leave it to you as an assignment.

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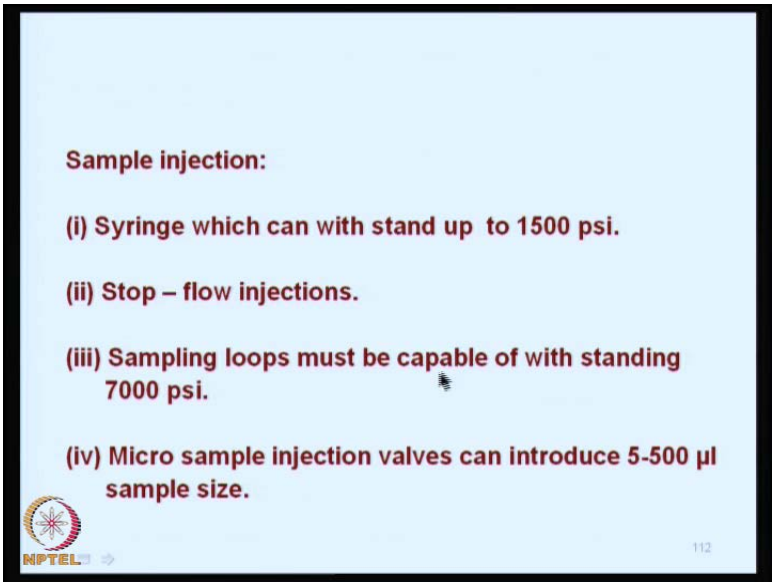

So, the sample injection is a syringe which what you through a syringe which can stand up to 1500 psi which is very very important, and then you need to have stop flow injections. Sampling loops we can incorporate, but they should be capable of withstanding up to 7000 psi.

Micro sample injection valves also can introduce samples of 5 to 500 micro liters. Now, columns again I cannot over the importance of columns cannot be over emphasized, you have to understand that columns are the heart of the any chromatographic separation. So, the stainless steel are heavily world glass tubes of 10 to 30 centimeter, you know in a compare to gas chromatography, HPLC columns are slightly smaller and they should,

because maximum length is about 30 centimeter about they should be capable of withstanding 6000 psi 4 to 10 millimeter inside diameter, and 0.5 micrometer particles to provide 40 to 6000 plates per minute per meter.

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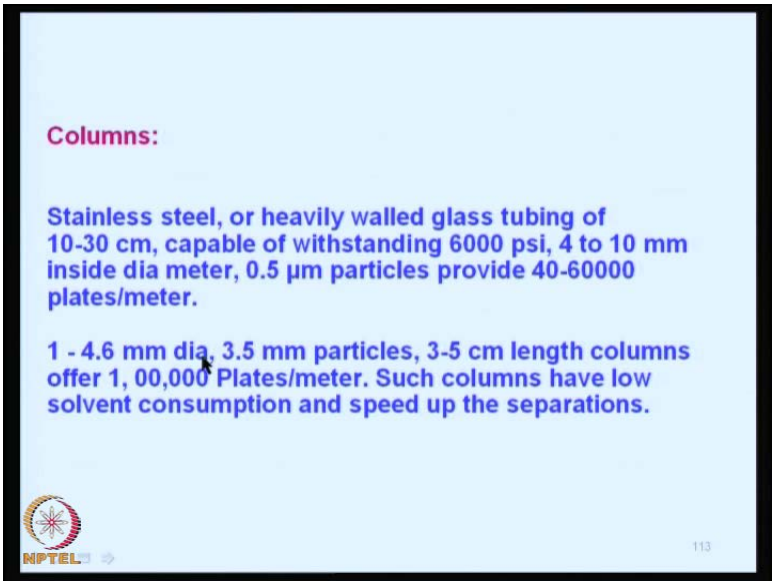

So, the particle size if you have, if you employ column of 1 to 4.6 diameter, 3.5 micrometer particles, and then 3 to 5 centimeter length, this columns are for approximately 100000 plates per meter. Actually there is a small mistake here, it should be micrometer, and it will be corrected subsequently such columns have low solvent consumption, and they can speed up the separations.

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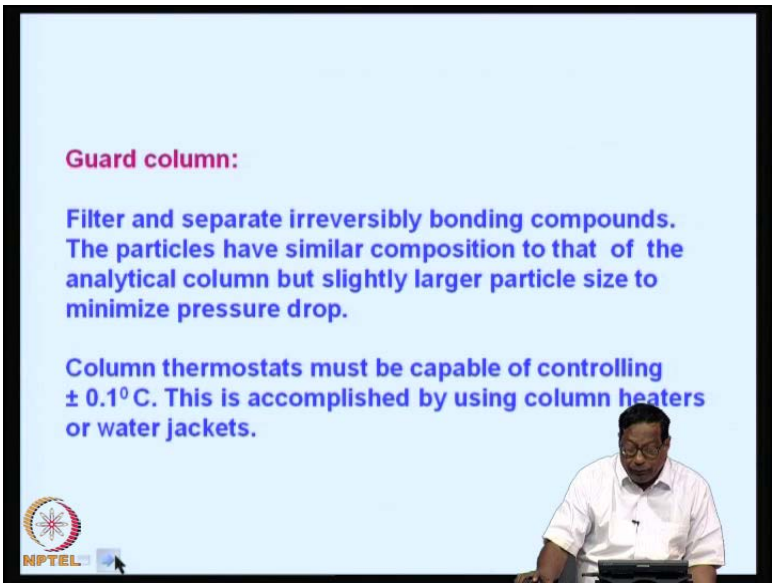

Now, guard column is the another important concept in HPLC, that is they should feebly able to filter, and separate irreversibly bonding components, (( )) particles should have similar compositions to that of the main analytical column, but slightly larger particles size to minimize the pressure drop. We do not want the pressure drop to happen in the guard column, because guard column is only a precautionary column.

So, the column thermostat must be capable of controlling temperatures of about plus or minus 0.1 degree centigrade, this we usually accomplish by using the column heater or placing the column in a heated water jacket, and the temperature can be maintained very successfully.

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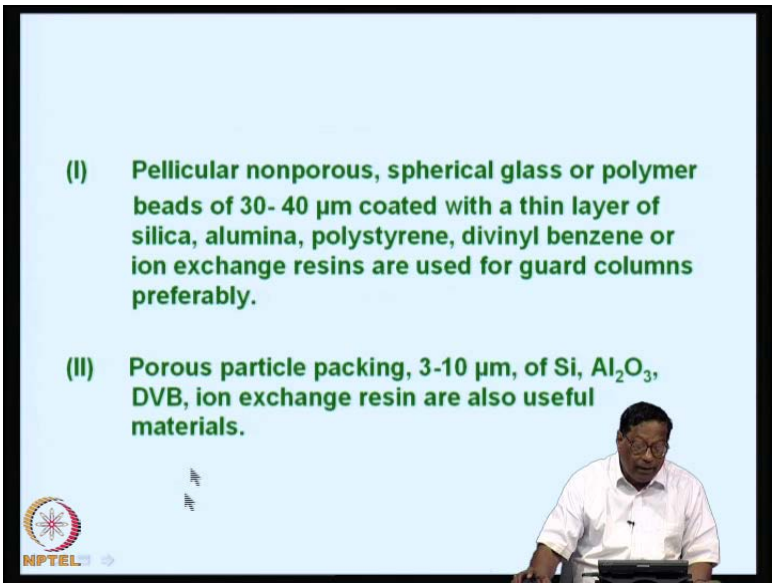

So, there are pellicular nonporous particles, spherical glass or polymer beads of about 30 to 40 micrometers coated with a thin layer of silica, alumina, polystyrene,s divinyl benzene, etcetera. These are the usual some particles compose support column material, and or you can even use ion exchange resins for guard columns. And porous particle packings of 3 to 10 micrometers of silica, and the silica alumina DVB ion exchange resins are also useful materials.

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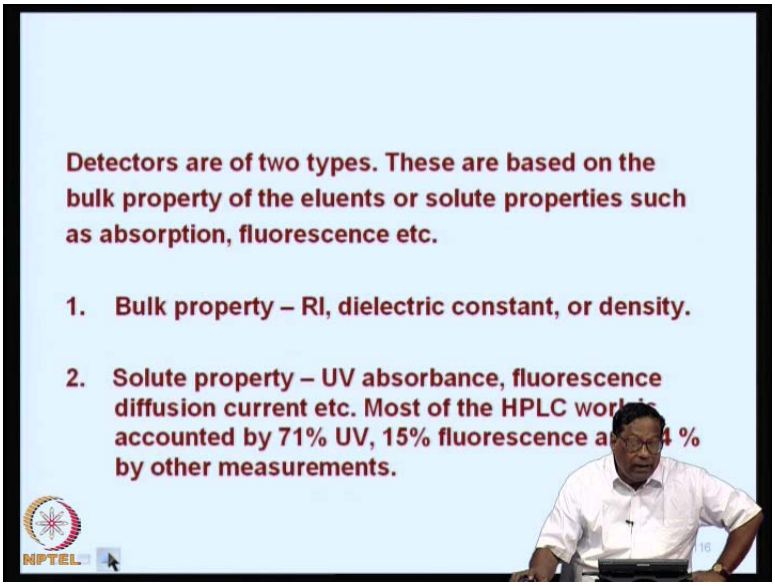

So, the that brings us to the detectors, generally detectors are again based on the bulk property of the eluents or the solid properties, such as absorption, adsorption, fluorescence, etcetera. The bulk property detectors are are there with the reflective index dielectric constant or density. So, the bulk property means, we can determine any substance irrespective of the chemical nature, and solute properties our detectors are based on the materials which are being separated, and their characteristics.

For example UV absorbance, florescence, diffusion, current, etcetera. Most of the HPLC work; however, is accomplished by 71 percent ultra UV detectors, and 15 percent of florescence, and 14 percent by other measurements.

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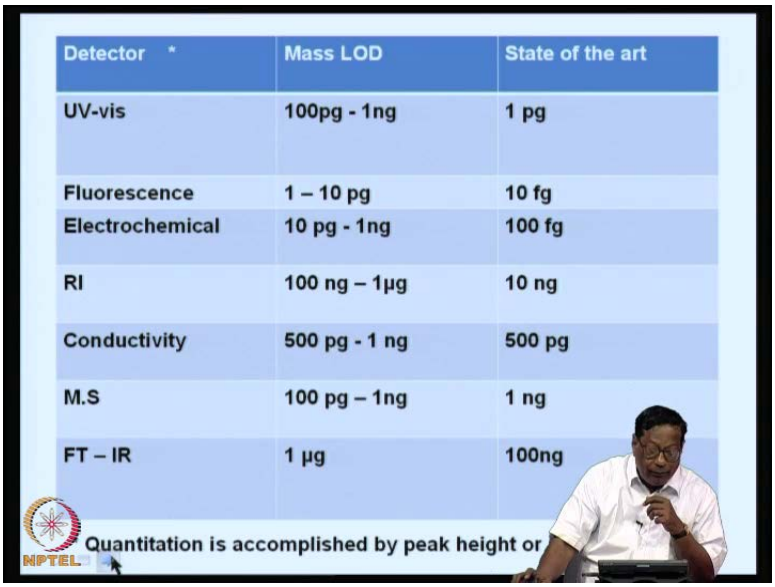

So, you can if you want to buy a chromate HPLC, you can go for RI, and UV that should suffice for most of the routine work; here I have tried to put the detectors, and mass LOD; that is limit of detection. And then state of the art; for example, in UV visible detector you can have loading of this match, but the detection limit is of the order of one pictogram; that is how how much the the technology as advanced for the determining the detection of the substances.

Fluorescence is (( )) 10 femtograms, electrochemical is 100 fimtograms that is about 10 raise to minus 13, RI is 10 nanograms, conductivity is the 500 picograms, and then mass spectrometry if you couple with HPLC it will be about 1 nanogram, and the FT-IR if you use you can determine up to 100 nanograms. So, the one can have an idea about the

importance of the specific detection limit, and the power of high pressure liquid chromatography.

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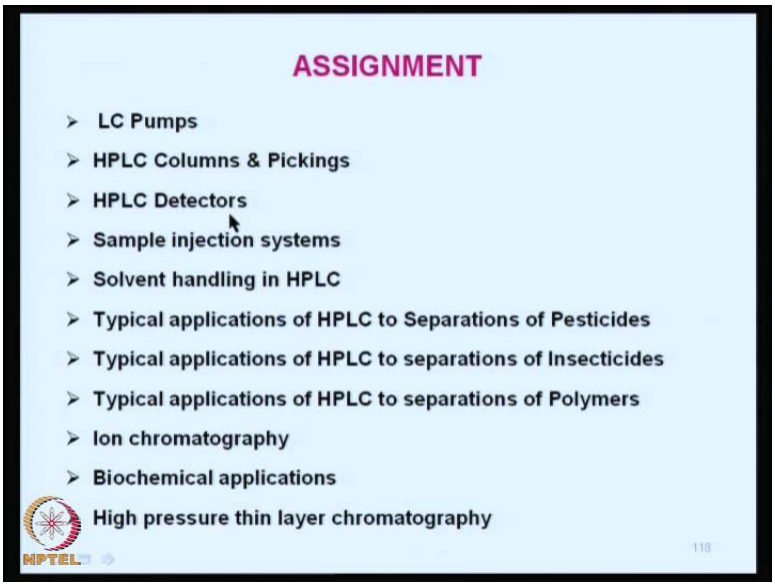

Here, I have a list of small assignments, and if you do this if you gather information in on the style, what I have taught you so far on LC pumps, HPLC columns, and HPLC detectors, sample injection system, sample handling, typical applications to pesticides insecticides the polymers, ion chromatography, biochemical applications, and high pressure liquid chromatography, I am sure you will be able to do and justice to high pressure liquid chromatography, and you will be an expert in this thing.

And what I would like to this brings us to the end of our course on NPTEL, I would like to thank you for being with me all these lectures, and I have tried to give you an insight into the different kinds of chemical analysis, and to be performed, and that are relevant to modern science, and with our worried.

For example in the initial courses, we have discussed that we had we had started right from the the atomic structure, and then we have tried to understand some of the optics basic instrumentation followed by the by schematic diagrams of how to combine them together to make an instrument with the course what you have undergoing, in this course I am sure you will be able to assemble a detector depending upon the substance, you want to separate our determine or identify.

So, we have had taught you about the optics, I have taught you about the chemical properties, and I have taught you about the schematic diagrams, and principals of how the detectors and other thing works. And based on these things we have measured, we have moved on to different kinds of separations, and then I different kinds of identification initially we did conduct from of the as our studies on spectrophotometry, fluorescence, x ray techniques, and then there are other substances other techniques.

Atomic absorption we have spent quite a lot of time, and then surface analysis, and then electro thermal electro thermal analysis, hydride generation, cold weeper mercury followed by infrared, etcetera. And several other techniques followed by electrochemical techniques. Among them we have studied in detail about the paleography, and which is a very very important technique with respect to electro with respect to electrochemical techniques, and I would have taught you if the time at permitted on sensor send other related techniques which are the in things now.

That is you just carry a sensor, and then determine the ambient as well as the non ambient circumstances, the chemicals which you would like to determine, subsequently we have move on to the chromatography, where we have spent lot of time; in gas chromatography understanding the principals and theoretical aspects, etcetera followed by HPLC.

I have not taught you HPLC as it should have been taught, because I want to leave an impression on you that, now you are capable of studying things on your own. That is what I want to impart to you along these things. So, with this with this comments, I will leave you now with all the best wishes, and I would like to thank you for being with me during all these lectures, and I wish you all the best, suppose you need something more please contact me any time. Thank you very much.