**Quantitative Methods in Chemistry**
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**Lecture-46**
**Detectors Employed During Chromatographic Separations**

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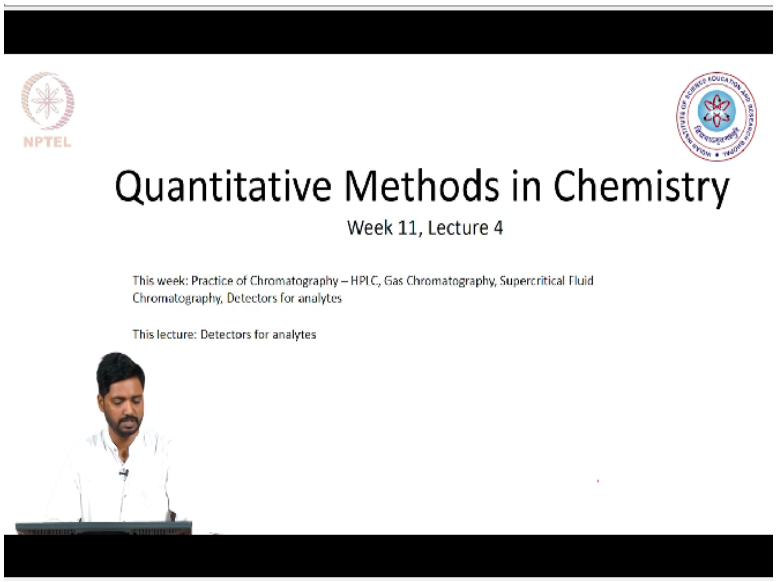


Hello and welcome back to week 11 lecture 4 for this course quantitative methods in chemistry. In this week our focus has been to understand how chromatography is practiced by researchers. And we have already seen that we can segregate the chromatographic separations based on the mobile phase that we use. For example, we can call it as a liquid chromatography if the mobile phase is fluid or liquid.

Similarly in gas chromatography, we saw that we use carrier gases, typically helium or nitrogen, to move our analyte through the column of the gas chromatography. And we also saw that there is something known as supercritical fluids. Specifically, we talked about the supercritical carbon dioxide, which is the most usual supercritical fluid used for chromatographic separations and even extraction processes.

So, we figured out that some of these chromatographic techniques are necessitated, because we want to isolate our analyte in highly pure form and some of this will be mandated also by the regulatory agencies. For example, when we are making a new drug entity, we are required to give the highest purity compound for testing. And moreover, we also need to identify all the impurities that may be present in our drug entity.

So, this lecture, our focus would be on understanding the principles of various detectors that are used for sensing the presence of the analyte in the eluent. So, let us move ahead and see what are the requirements that we would want to have with regards to the detectors that are used in chromatographic separations.

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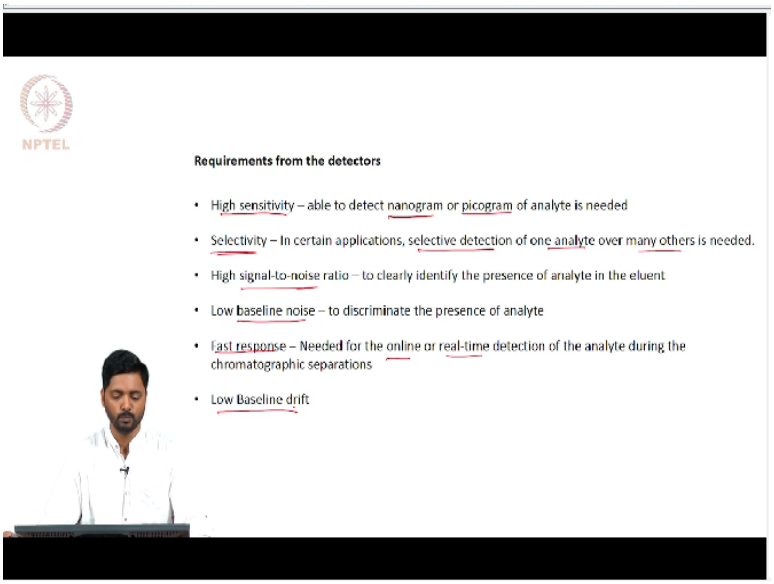


So, of course, we can think of our eye as one of the detectors but as instrumentation progress, we required more and more sensitive detectors which would be able to sense the presence of analyte in really tiny amounts. So, the first requirement for any detector will be high sensitivity to the presence of analyte. So that we will be able to detect really minute quantities of analyte that is eluting out of chromatographic separation.

So, this becomes especially important when our chromatography is what is known as an analytical chromatography, where we simply need to analyze the presence of the analyte a contrasting protocol is when we do what is known as preparative chromatography, where the

idea is to isolate the analyte in good enough amounts So, that further investigations can be undertaken.

So, coming back to detectors, we would want our detectors to be able to detect nanogram or even picogram of the analyte that is coming out of the chromatographic column. Of course, in some applications, we also require the detector to be selective and indeed there are certain detectors that respond to certain unique properties of the analyte. And these for example, include what are known as the electron capture detectors and the fluorescence detectors.

So, these detectors allow selective detection of a particular analyte over many others, for example, fluorescence detector will only respond to molecules or compounds that emit photons and not to other analytes that are coming out of the chromatographic column. Definitely one major requirement for a good detector is that it should have a high signal to noise ratio. That means, when the analyte is coming into the flow cell where the detecting its presence, it should be able to identify it very clearly.

For this we require a low baseline noise. And this helps us to discriminate the presence of analyte and we will talk about the baseline noise in a little detail subsequently in this lecture. Another requirement for our detector is that there should be minimal lag time from the moment the analyte enters the detector to what the detector sense the presence of analyte. So, we require a very fast response from our detector.

nd this is especially needed if we are doing what is known as the online or real time detection of the analyte during chromatographic separations. And finally, our requirement from the detector is also of having low baseline drift, we will also talk about what this baseline drift is.

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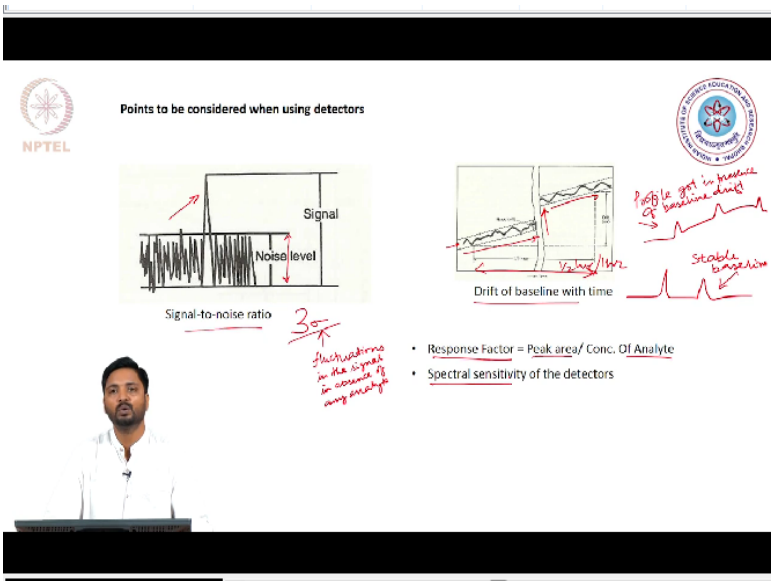


So, let us move ahead and see what are the points that we need to consider with regards to the detectors, I already told you that we need to have a high signal to noise ratio. And typically, the minimal signal that you can detect with good confidence is what is known as the 3 sigma value of the signal and the sigma here corresponds to the noise that is inherent in our detector even in the absence of the analyte.

So, as you can see that this noise is actually fluctuation in the detector response even in the absence of analyte and this fluctuation is characterized in terms of sigma. So, sigma characterize the fluctuations in the signal in absence of any analyte and what we want is that one whenever analyte comes in contact with the detector or is detected, the signal that it will generate should be at least 3 times this typical fluctuation in the noise that that is observed in the detector.

And the drift of baseline with time is another important criteria. What happens is that when the detector is kept on for a long time, there will of course be the baseline noise that will be generated from the detector. But more importantly this baseline noise can undergo a significant change during the course of time. For example, this is a time period of say about half to half hour or 1 hour.

And during this course of time we already see that there is a significant change in the baseline of the detector with time. So, this results in profiles of chromatographs which are not very

horizontal. So, instead of getting a profile like this what we would want, we will start getting profiles like something like this. And this obviously requires further manipulation or analysis of the data to derive useful information from this.

So, just to rephrase this is the profile that we get in presence of baseline drift. And an ideal case scenario is when we have a stable baseline so that the analyte generates very response, we have already in one of the classes discussed about the concept of response factor, which is nothing but the area under the peak divided by the concentration of the analyte that is coming out and this response factor can indeed be different for different analytes and this needs to be kept in mind.

Now, another very important point that needs to be kept in mind when using detectors that respond to light radiation or electromagnetic radiation or the spectral sensitivity of the detectors. Now, the photons of different wavelength will have different response on the detector, and the detector may be more sensitive to the photons of a particular energy or wavelength. And this point or this aspect needs to be kept in mind and a normalization of the detector needs to be done when using the detector for real life applications.

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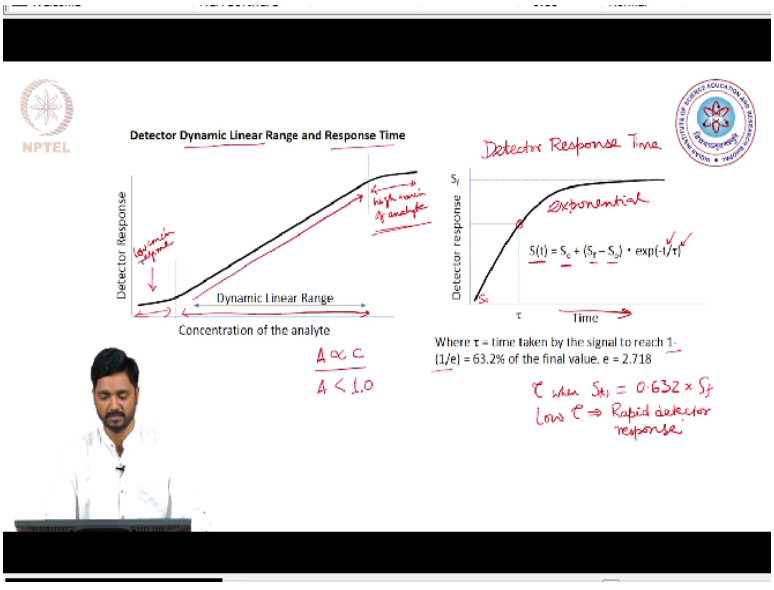


Two other major points that we need to remember are what are known as the dynamic linear range as well as the response time of our detector. Now, dynamic linear range is effectively the concentration range of the analyte where the signal is linearly changing with the change of the

concentration of the analyte. So, what is definitely observed for most of the detectors is that in the very low concentration regime the detector response is very poor because we have a very low signal to noise ratio.

So, our ability to detect analyte at this concentration regime is very restricted. But, as the concentration of the analyte is increased into the element, we start seeing that the detector responds almost linearly to the presence of the analyte. Now, this range is what we call as the dynamic linear range, as the concentration of the analyte is further increase which is this regime of high concentration of analyte.

It is observed that the detector response saturates and that may be related to the electronics or the way the detector senses the presence of analyte. And this is again a regime which will be not of particular interest for us, one common example for this is the absorbance spectra that is generated using UV-vis detectors. So, what has been observed or what one can remember as empirical rule of thumb is that analytes that give absorbance values of greater than 1 will not follow the Beer Lambert law, which indicates that the absorbance of analyte is proportional to its concentration.

So, if we want to get or satisfy this particular condition of absorbance being proportional to the concentration, our absorbance values should always be less than 1.0. Because beyond this, we will be hitting the high concentration regime, where the detector response will no longer be linear. Now, we also talked about the detector response time. So, this is nothing but how the signal that is being generated at the detector changes with change of time.

So, what we observe is that for many of the detectors, there is almost an exponential increase in the detector response as the time progresses. And this is described by this equation here, where the detector response with respect to time is equated as the initial detector response, which will be this value plus the final detector response minus the initial detector response into an exponential value of time versus something known as tau.

And tau is actually indicated here. And this is the time that the signal takes to reach the value of 1 minus 1 by E that is equal to 63.2% of the final value. So, tau again is when S t is equal 2.632 into S f and this number needs to be kept in mind and obviously, we want low tau values for our detector to respond quickly. So, a lot of value will imply rapid detector response, which is a favorable characteristic that we are seeking in our detectors.

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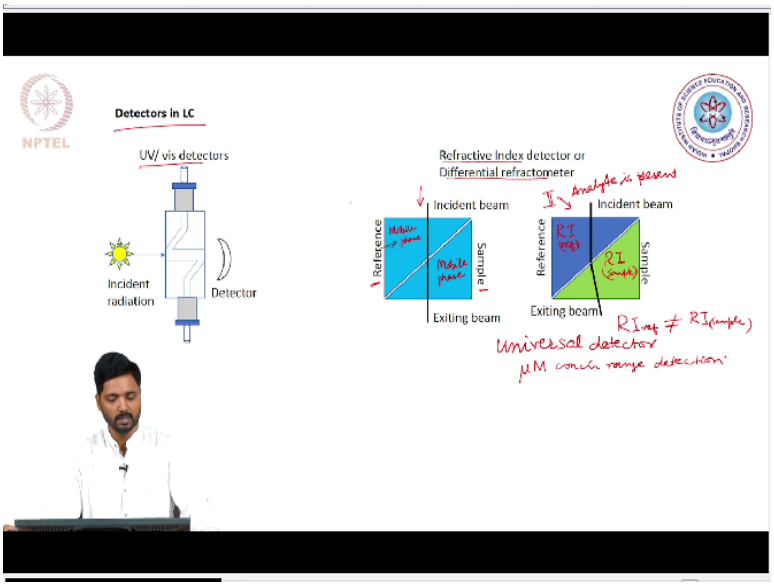


Now, in the previous classes I have already discussed about the detectors that are commonly employed in liquid chromatography, we discussed about the UV-vis detectors, we have also indicated that fluorescence detectors can be used and the other detector that is often used is the refractive index detector, which is also called as the differential refractive meter. Now, what does this indicate is that, it is trying to plot the difference in the refractive indices of the reference and the sample.

So, we have cells through which an incident beam is being passed. And these cells are filled either with the reference which is simply our mobile phase. And the sample which when no analyte is eluting, will be same again as the mobile phase. So, when an incident beam passes through these 2 cells it essentially experience a very constant or very similar refractive index of the medium and hence will undergo negligible change in its direction.

On the contrary, when eluent contains the analyte the situation is indicated here, in the second case, where the analyte is present. In this case, the reference cell will have different refractive index. So, it will have a RI ref. And similarly, the sample cell will have the RI sample which will be different from each other. So, RI ref will not be same as the refractive index of the sample cell.

And this results in the bending of the incident beam as it passes through the sample chamber because of the changes in the refractive index. This is very easy to figure out this change or this spending of the incident beam and that can be plotted with regards to change in time. Of course, we understood that a key advantage of this detector that it works even for analytes which will inherently not absorb any electromagnetic radiation.

So, in that sense, it is what is known as a universal detector. But it is response or sensitivity is poor. So, it can detect materials only or analytes only in micro molar concentration range.

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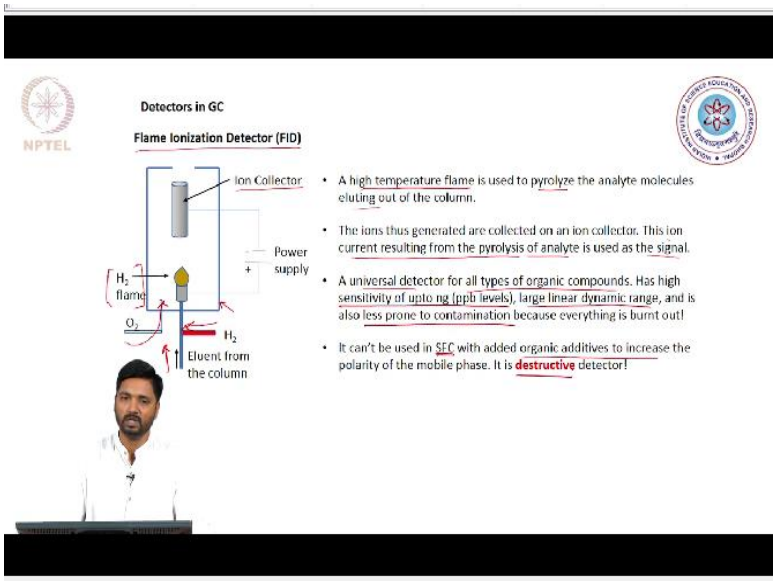


Now, the detectors that are used in gas chromatography are somewhat different from the liquid chromatography because here we are dealing with a gaseous phase of both the mobile phase and the analyte that is eluting out. So one of the most common and almost sort of universal detector is the flame ionization detectors, especially in laboratories where organic molecules are being analyzed.

And we have already seen that gas chromatography is indeed more suited for low molecular weight organic molecules or biological metabolites. So, what we have here is in the flame ionization detector, there is a high temperature flame that paralyzes the analyte molecules that are eluting out of the column. So, if we have the eluent from the column going into the detector, we also mix some hydrogen gas with this eluent.

And we burn this mixture in a chamber which is now resulting in generation of certain ions. So, we use a hydrogen flame because of the high temperatures that we can use with it and for that we have this oxygen inlet valve as well. So, hydrogen and oxygen are mixed in this chamber and hydrogen flame is generated which burns out the analyte that is eluting out and this generates and ion current and this current resulting from the pyrolysis of the analyte is used as the signal.

So, for that the simple way it is done is that we have an ion collector, which is a sort of a cylindrical electrode which is appropriately biased and this collects the ions that are being generated from the pyrolysis of our material or our analyte. So, this detector of course, is considered to be universal incense that it is able to detect the presence of all types of organic compounds.

It of course, will not work for analytes that do not undergo burning for example, water vapor or even hydrogen sulfide will not be detected or carbon dioxide, carbon monoxide rather the detector does not respond well to the presence of these analytes. However, for organic compounds which undergo rapid burning especially in the high temperature flame of hydrogen, it is very straightforward to generate an ion current.

So, because we are generating an ion current, the detection of current is very, very sensitive and as a result, the sensitivity of this detector can go up to nanogram levels or part per billion levels. And it also has an advantages point that it has a very large linear dynamic range. So, a very wide ranges of concentration of the analyte can be detected with the help of this detector and it is also less prone to contamination because essentially we are burning out everything.

So, it is only producing gaseous exhaust which results in low contamination of the detector. However, and the key disadvantages with regards to this detector is that it cannot be used with supercritical fluid chromatography, in which we have added organic additives to increase the polarity of the mobile phase. In the previous lecture, we noticed or we learned that sometimes we add organic additives such as methanol and acetone night trial to our supercritical carbon dioxide to increase its polarity.

However, if we add these additives, the use of flame ionization detector cannot be employed. Now, another key disadvantage with regards to this detector is that it is a destructive detector. And what this means is that we are essentially burning out the analyte that is coming out of the gas chromatography and we will not be able to collect or isolate the analyte if we are detecting its presence through flame ionization detector and everything gets burned out in this detector. So, what solution people came out was thermal conductivity detector.

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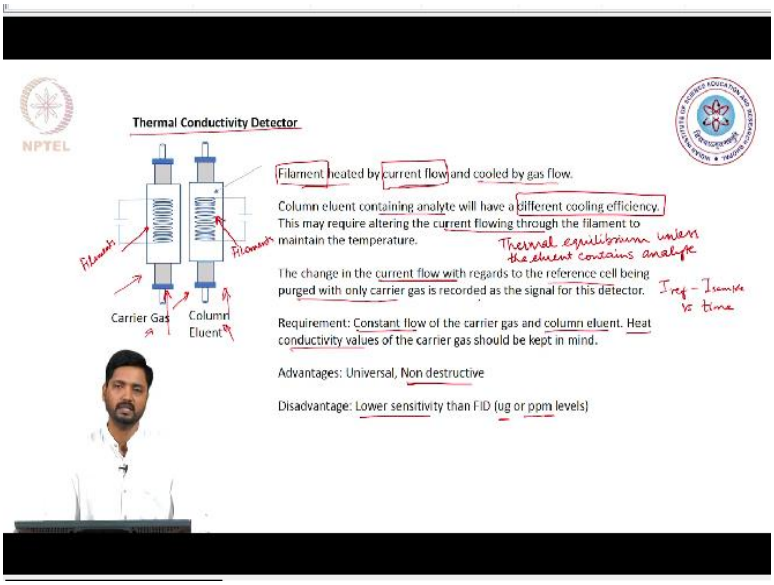


So, in thermal conductivity detector, we have 2 cells in which the carrier gas or the column eluent flows through and these cells will have a filament which is heated by the flow of current and another aspect that controls the temperature of this filament is that the filament is being cooled by the flow of the gas which is passing through the thermal conductivity detector cells and the column eluent, which contains analyte will result in a different cooling efficiency.

And this may require altering the current that is flowing through the filament to maintain its temperature. So, the idea here is that we maintain the temperature of these 2 filaments. So, these are our filaments in the reference and the sample cells and they are maintained at a very constant temperature by flow of current as well as the flow of the mobile phase which maintains a thermal equilibrium.

So, we continue to have a thermal equilibrium unless the eluent contains analyte. Now, when the eluent contains the analyte it results in different cooling efficiency for this heated filament and that results in the requirement of flowing different amount of current through the sample cell compared to the reference cell. So, this change in the current flow with regards to the reference cell which is being parsed only with the carrier gas is recorded as the signal for this detector.

So, essentially we will be plotting the I ref minus I sample versus time in this detector and for this detector our requirement is a very constant flow of the carrier gas and the column eluent both of them should flow at a very constant rate, so, that a thermal equilibrium can be maintained very properly and we also need to be aware of water the heat conductivity values for the carrier gas as well as the analyte.

Because that will dictate how efficient will be the cooling of our heated filament. The advantage with this detector is that of course, it is a non destructive detector plus, it is also a universal detector in that it will respond to all kinds of analytes. But, the minor disadvantage here is that it has a lower sensitivity than the flame ionization detector and it can only detect the analytes in microgram levels or parts per million levels.

While previously we saw that the flame ionization detector was able to detect things in nanogram or parts per billion levels.

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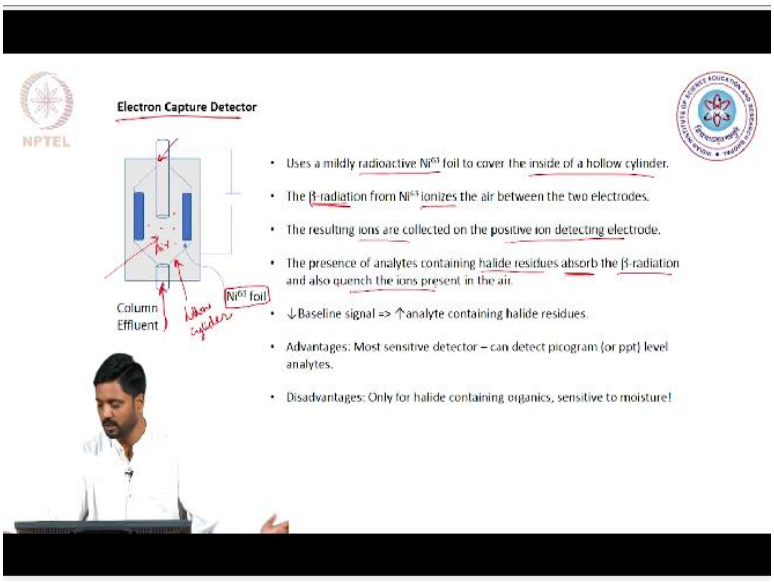


Now, the third and final detector that we will talk about in this lecture is unique detector which is known as the electron capture detector. Now, the unique aspect of this detector is that it uses radioactive nickel 63 foil as a source of beta radiation and this nickel the 63 foil is covered or coated inside the hollow cylinder which is shown here. So, this is the hollow cylinder through which our columns column effluent is flowing.

And we have this nickel 63 foil that is present inside the hollow cylinder and this results in production of the beta radiation and as a result, the air inside this chamber or this cylinder is ionized and as a result we generate a current flow. So, the ions resulting from the ionization of the air molecules by the nickel 63 radiation are collected on the positive and detecting electrode which is shown schematically as a hollow cylinder.

So, the current that results from the ions that are being collected is plotted here as a function of time as the detector signal. So, the presence of analytes especially those containing halide residues, or those which are highly electronegative will result in the absorption of the beta radiation, so, if we have sample molecules that are also present in this column effluent, they will absorb the beta radiation and quench the ions present in the air. Let us see how this happens. So, we go to the board.

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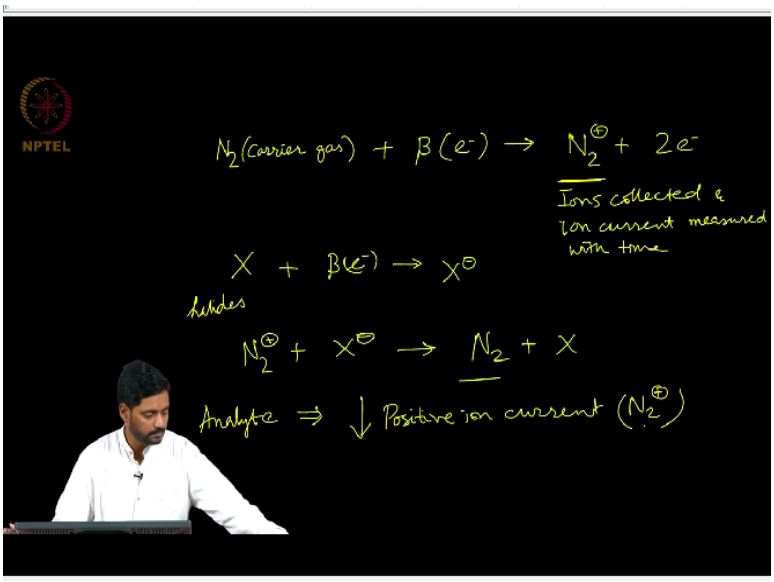


So, what originally is happening is that we have the nitrogen from the carrier gas and this reacts with the beta radiation which is nothing but a source of electrons and this results in generation of the nitrogen cation and 2 electrons and the nitrogen ions are collected and ion current measured with time. Now, if our sample is present in the carrier gas and specifically the sample that contains halides.

Then these electronegative analytes will absorb the beta radiation and give rise to X minus and subsequently this X minus will combine with the n 2 plus to quench the N 2 plus and regenerate the analyte and what that results in is that in presence of the analyte we get decrease in the positive ion current coming from N 2 plus and this decrease in the positive ion current is what gives rise to the signal from the analyte.

So, what I have written here is that the decrease in the baseline signal or the baseline ion current is indicative of the increase presence of the analyte containing halite residues. So, the key advantage of this detector is that it is amongst the most sensitive detectors that are available to us and it can easily detect picogram or parts per trillion presence of the analytes. And the minor disadvantage with regards to this detector is that it is a very selective for halide containing organics.

But is also sensitive to the presence of moisture because moisture can also absorb this beta radiation and result in reduction of the ion current signal. So, the column effluent that needs to pass through is to be of a very dry nature. So, it needs to be devoid of any moisture. So, as we get the maximum possible detection. So, I hope that this lecture would have given you a good basis of understanding how different detectors work in the chromatographic systems.

And the choice of the detector will depend upon the properties of the analyte and the response of the detector to the change in the properties of the analyte vis a vis the properties of the carrier gas. So, this brings us to the end of this week's lecture and we have covered the practical aspects of chromatographic separations this week. Thank you.