Atomic and Molecular Absorption Spectrometry for Pollution Monitoring

Dr. J R Mudakavi
Department of Chemical Engineering
Indian Institute of Science, Bangalore

Lecture – 25
Chemical analysis, applications

Greetings to you, we were discussing the fluorescence measurement techniques. For analytical purpose what is the important is absolute determination of fluorescence is not necessary that is because it is always measured with reference to a standard. So, changes we will occur in lamp intensity etcetera. This I have already covered yesterday and there will be monochromatic detector systems also age and it is usually necessary to standardize and instrument.

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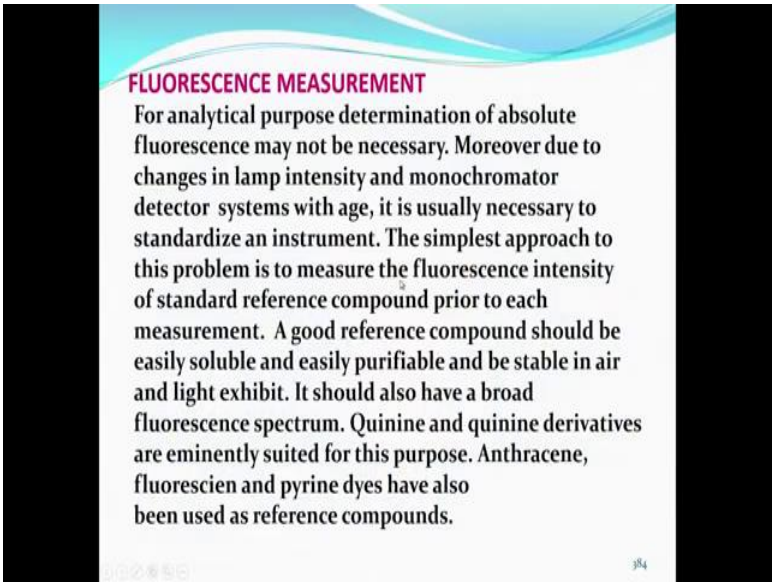

The simplest approach to this problem is to measure the fluorescence intensity of standard reference material this we have already discussed that quinine and quinine sulphate derivatives etcetera are suited, but I have also said that anthracene, fluorescien and other dyes, where in dyes have been used for this purpose as reference compounds.

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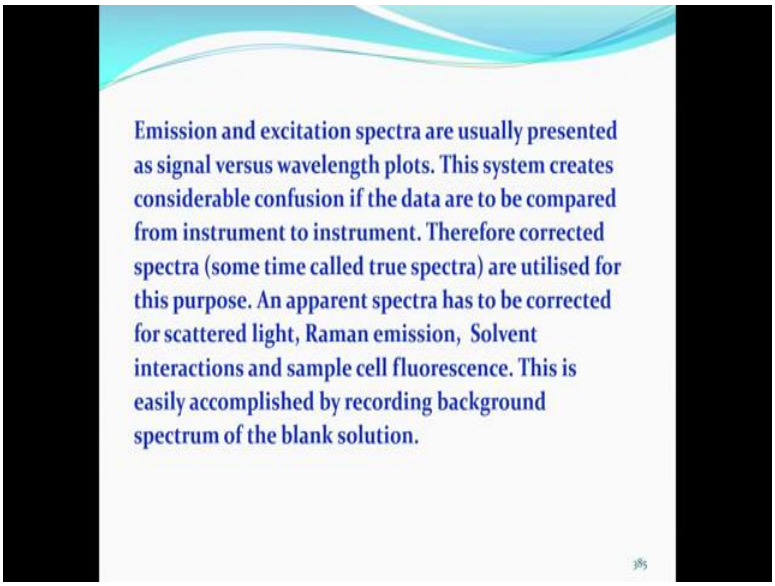

So, usually in any research paper if you take a look, emission spectra and excitation spectra are usually presented separately and they are presented a signal versus wavelength plots. So, this system creates con considerable confusion, why? Because if the data are to be compared from instrument to instrument, from this lab to another lab, from this country to another country, if we do not give the full details of the reference and standard and absolute data it will be very difficult for the researcher to compare the data, also to reproduce the data because you may you might have use 0.2 mm slit weight, but somebody else may use 0.3 mm slit weight or 0.7 mm slit weight then what happens? You will definitely get a different reading.

So, sometimes we do present corrected spectra and an apparent spectra needs to be corrected for scattered light Raman emission solvent interactions and sample cell fluorescence. In spite of all this what you need is a blank solution is required. So, blank solution is important sample flow spectral correction is important and the instrumental settings are important. So, all the three are very important if you want to do some laboratory inter laboratory or inter straight comparison with respective to fluorescence. Suppose you can say you collaborate with potassium with quinine sulphate, somebody else may calibrate with pyrene again there maybe differences in the actual data with respect to each same system, but in different settings.

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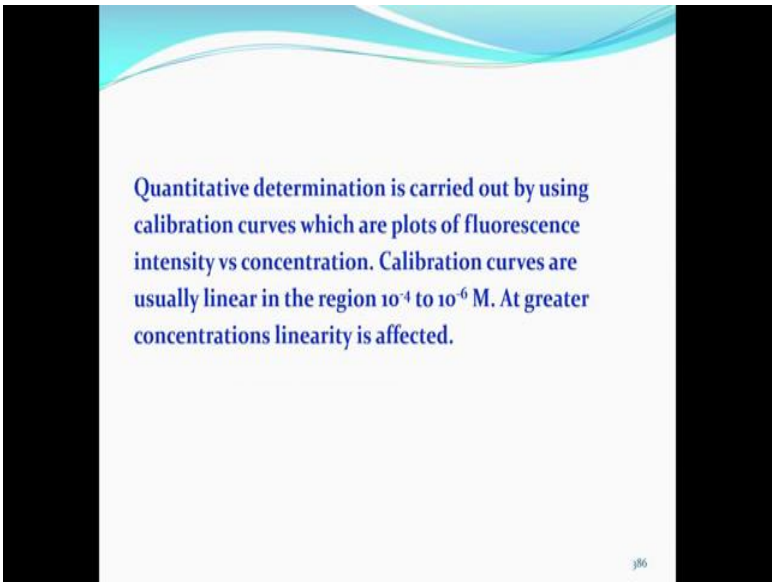

So, this is one problem especially with respect to the fluorescence measurements. So, what we do is we normally determined as usual by calibration curves which are plot of fluorescence intensity versus concentration it is not absorbance. So, we do not have a sandal sensitivity as such, but absorbance versus co intensity versus concentration is good enough for fluorescence measurements. Sometimes calibration curves are usually linear up to 3 orders, 2 orders this also we had discussed yesterday, but what is important to remember is that higher concentrations linearity is affected, it bends towards the x axis and for any reading there may be two different concentrations one of which is correct and another one is wrong.

So, this also we have seen yesterday in one of the figures, what I had presented with respect to fluorescence intensity versus concentration because when they are curve bends down towards x axis there will always be two numbers for the same concentration. So, one has to be extra careful with respect to such information.

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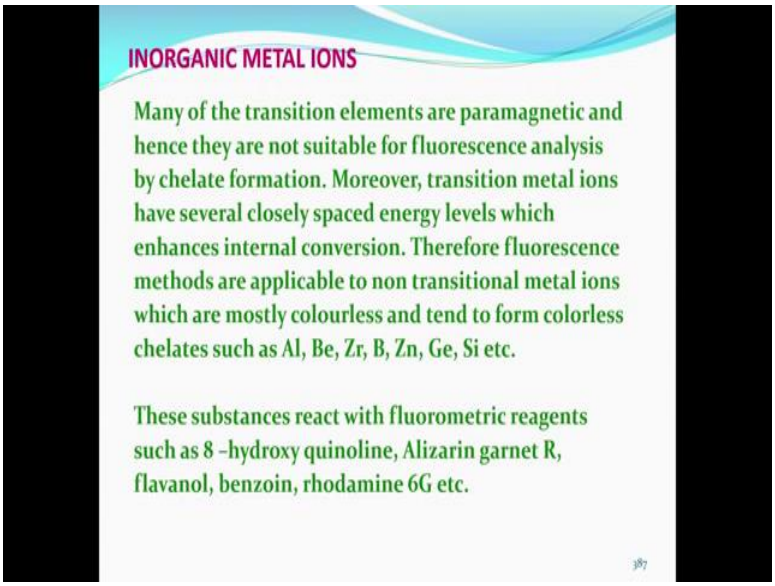

Now what do we do can we determine the elements using fluorescence metallic elements? The answer is yes, many of the transition elements are paramagnetic as we know and hence they are not suitable for fluorescence analysis by chelate formation. More over transition metal ions have several closely spaced energy ready energy levels the orbitals which are very closely spaced that enhances internal conversion. So, fluorescence methods are applicable to non traditional methods rather than transition metals.

So, this distinction one can make very easily because whenever we need a fluorescence we need the electrons to move to some other vibrational energy level with energy difference, but if the energy levels are very closely spaced then you may not see much difference in fluorescence or colour or any of those parameters which we normally look for in the absorption. So, we can straight away rule out may metal ions which are mostly colourless and they those which 10 to 10 to for colourless chelates also we can ruler because actually measurement of fluorescence is a measurement of colour in the final analysis, if the complexes are not coloured and then obviously, the question of fluorescence does not arise at all.

In this class we can include aluminum, beryllium, zirconium, boron, zinc, germanium, silicon etcetera. So, these substances the, but what I can do is I can make them reactor with fluorometric reagents just like complexing agents such as a 8 hydroxy quinoline is

one and alizarin garnet R is one, flavanol, benzoin, rhodamine 6G, rhodamine B many of the fluorescing complex reagents fluorometric reagents are available, but these things can form fluorescing compounds with some of this ions which are coloured also, so that may give raise to fluorescence. So, for such elements you do need a fluorometric reagent, but on their own their do not fluoresce.

Of course if you compare with actual metals you can get fluorescence you may get the metals to fluoresce that is with very high energy that is atomic fluorescence we call it and, but that range is not covered by spectrophotometry.

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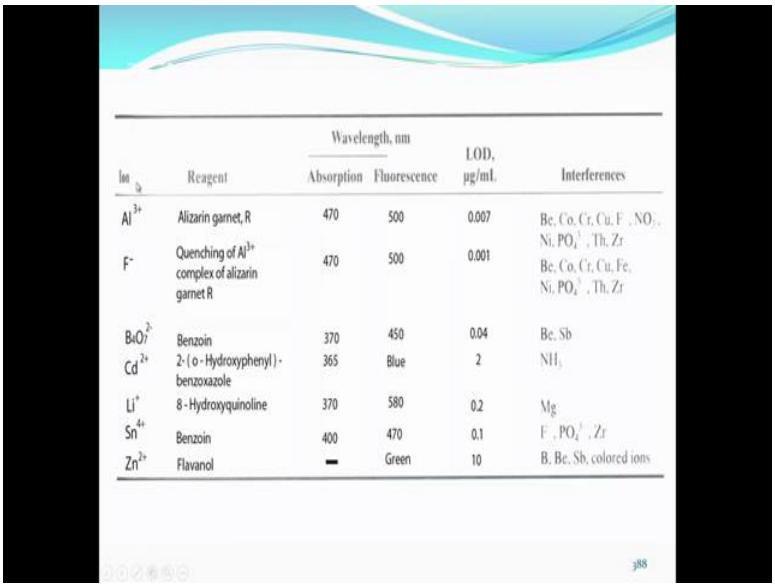

So, for here I have a table for you that shows many metal ions here and then the typical reagent fluorometric reagent absorption fluorescence limit of detection and some amount of interference is are always expected because many of the metal ions could be coloured also they may react with the reagent under the typical determination conditions.

So, for example, you can take a look at aluminium, aluminium reacts with alizarin in garnet are that is a name of the fluorometric reagent it is a dye and absorption maxima occurs around 470, but fluorescence occurs around 500. So, there is a very clear cut difference of about 30 nanometers and limit of detection using this method is approximately 0.007 micro gram per ml, that is about 7 pico grams, 7 nanograms, 7 nanogram per ml is the determination limit. So, normally whenever we handled the metal ions by fluorescence we talk of nano grams per ml, n g per ml or milligram per kg

whatever you may prefer, but still the range is in the determination in the range of nano grams.

So, it is about 10 raise to minus 3 times less than the absorption methods. So, fluorescence is definitely an advantage over absorptiometry, but you can also see that just like spectrophotometry there will be interference from beryllium, cobalt, chromium, copper etcetera nitric cover nitrates are also interfere in this process, but there are ways and means how to get read of this interferences and make it specific for aluminium. Same thing is true with respect to other elements also, an elements as well as anions for example, you can take a look at the fluoride, fluoride normally alizarin in garnet also reacts with fluoride because here I have written it as a interferent - if it is an interferent the it means it reacts with the reagent. So, lot of people look at the interferences and then develop methods for the interfering element by removing the other interfering compounds.

So, the same fluoride reacting with alizarin garnet are gives you the almost same absorbance, same fluorescence, but they detection limit in this case is 0.001 nanometer. Here what happens is aluminium garnet is red coloured and if you have add fluoride known quantity the fluorescence intensity decreases. So, that decreases monitored here and the concentration range is almost 1 nanogram. We normally talk whenever we talk of pollution control etcetera we talk about 1 milli, 1 ppm. Now you are talking about 1 nanogram, 10 raise to minus 3. So, it is; obviously, very good method for the determination of alizarin, not as alizarin for fluoride the same method if I add fluoride the fluorescence will be quenched.

Similarly, but not exactly similar there is another reagent fluorescing reagent before o 72 minus and that ray on borate very important compo constituent of the soils and it also as the micronutrient and it reacts with benzoic to give you absorptions my maxim around 370 and fluorescence is around 450, detection limit is approximately 0.04 and that is microgram per millimeter, that is approximately 40 nanograms. So, interfering substances are only beryllium and selenium here. Similarly you can work out at the other elements like cadmium which reacts with two hydroxyphenyl benzoxazole and detection limit is two, interfering iron is only ammonia because ammonia forms a very strong complex with cadmium and then similar lithium, you see the problem with lithium is it

cannot be determined by spectrophotometry because the element is very small. So, there very few spectrophotometric methods.

The only way to determine lithium in a given sample is by flame photometry, in flame photometry people do you determine sodium, potassium, calcium, lithium and all these things, but in general lithium is not normally determined by spectrophotometry or something, but we do have a reagent which will give you detection limit of about 0.2 nanometer and the 0.2 nanograms that is that is very nice. So, this is still lower than cadmium, but you can see they can take hard from the fact that it is free from magnesium interference usually lithium is always associated with magnesium. So, if here people look for methods where the magnesium interference is minimum in the determination of lithium.

So, then we also have thin 4 plus that is benzene is the reagent and 400 is absorbance 470 is this and 0.1 nanogram, 0.1 milligram that is about 100 nanograms interfering elements are also listed here zinc reacts with flavanol and if absorbance is not in the visible region, but it is in the UV range, but fluorescence is in the green range and detection limit of detection is about 10 nanograms. So, like that there are almost about 30 to 35 elements of the periodic table which can be determined using fluorescence. So, it is not that the elements cannot be determined, but compared to spectrophotometry fluorescence determination is slightly more involved, more sensitive and for this reason it requires more careful control of the reagents water environment and other contamination resend interferences. So, all these things one has to really take care and if you look up the literature and using Google and other data basis you will see that the there are methods which can be used for the determination of nearly 30 to 35 elements of the periodic table. So, that is a good progress.

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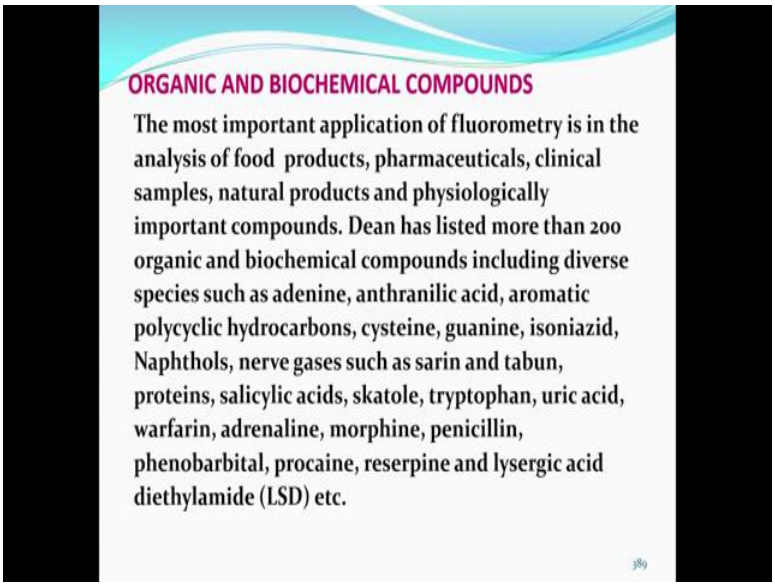

Now, the utility of fluorescence methods extends not just the elements in the periodic table, but to a vast number of organic and biochemical compounds. The most important application of fluorometry is in the analysis of food products pharmaceutical clinical samples, natural products, physiologically important compounds like that body fluid plasma and many other physiological important compounds are always determined by fluorescence techniques. Wonderful techniques have been worked out for all these compounds determination and almost every industry which is significant especially and active in chemicals they would like to have a fluorescence instrument in there laboratory.

Actually dean has listed more than 200 organic and bio chemical compounds including diverse species such as adeni, adenine, anthranilic acid, aromatic polycyclic hydrocarbons, these polycyclic hydrocarbons are all environmental pollutants. So, cysteine, guanine and isoniazid, amino acids, proteins, nerve gases naphthols and nerve gases such a sarin, tabun, proteins salicylic acid so many compounds are prone or are they are suitable for fluorescence measurements and its important concept in flow forensic science also, in forensic science also we need various organic compounds in the body fluids stomach contents urine etcetera and fluorescence is one of the most important and technique for many of these parameters.

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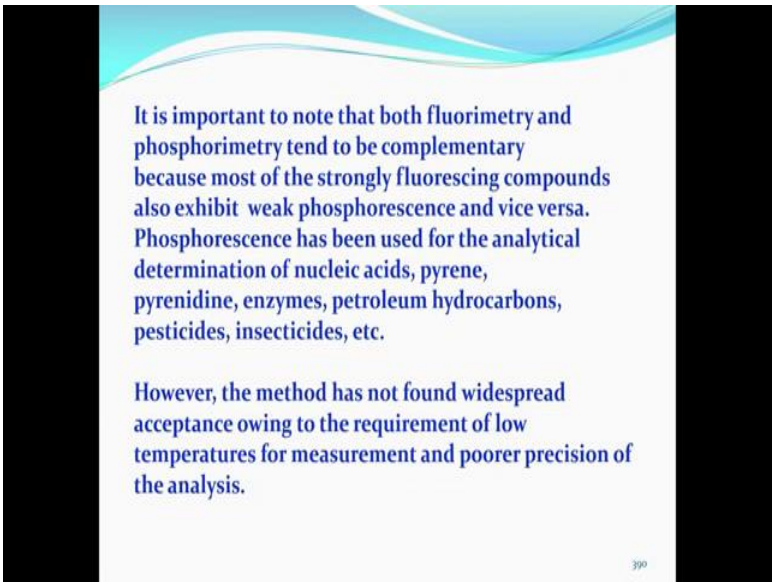

So, normally fluorimetry and phosphorimetry both tend to be complementary this I have already told you earlier because most of the strongly fluorescing compounds also exhibit weak fluorescence and vice versa this we have already discussed earlier. So, phosphorescence has been used for the analytical determination of nucleic acids, pyrene, pyrendine, pyrendine, enzymes petroleum hydrocarbons pesticides insecticides several other compound, but the method has not found wide widespread acceptance owing to the requirement of low temperature, cannot be done earn at room temperature. So, measurement and poorer precision of the analysis is a real risk because especially at low temperature slight variation in the temperature changes the flow phosphorescence.

So, one has to be very careful that is the reason why flow phosphorescence has not taken of like absorbance and fluorescence. Instrumentation also is a little tricky and costlier also.

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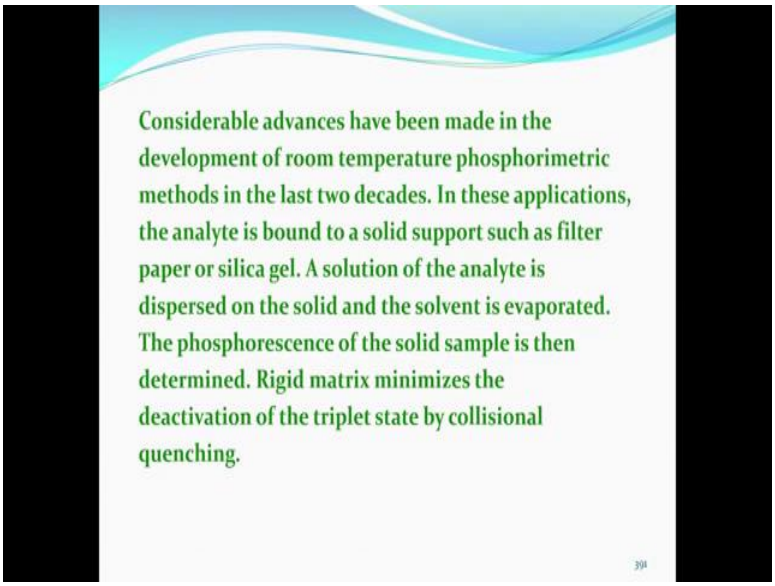

But there are considerable advances in the development of room temperature phosphorimetric methods also, in these applications the analyze it is what we do is we bind the analyte to a solid support such as a filter material, filter paper or silica gel or some other just would pulp you know we just bind it and then the try to dissolve it and reacted with a fluorescing material to get the fluorescence and phosphorescence. Normally a solution of the analyte is dispersed in the solid also I can take a plastic matrix and then dissolve it and then condense it to give a solid shape.

So, I can just evaporate it and so solid becomes available, phosphorescence of the solid sample can be determined this can happened at room temperature also. So, rigid matrix normally what happens is it minimizes the deactivation of the triplet state because there will not be any collision at all. So, collision quenching is minimum in solid samples that is why room temperature phosphorescence has taken off quite a bit in the last two decades, but still it has not found very wide spread appear acceptance in the laboratories.

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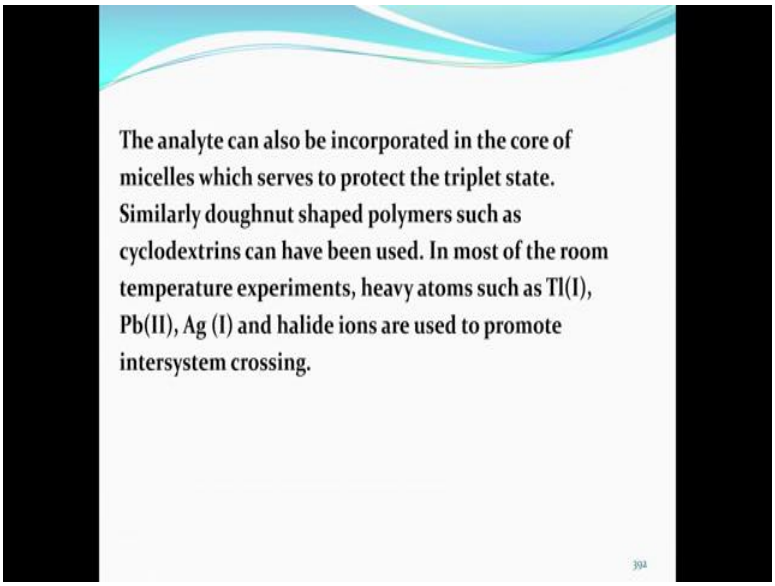

Or I can incorporate the analyte in the core of micelles, you know what is micelle a micelle is a dispersion of liquid in, this dispersion of liquid is done in a liquid medium or it can be done in solid medium what is important is water in oil, oil in water these are the typical dispersions, by oil we mean it may be a solvent.

So, water in solvent or solvent in water dispersion which is quite stable, but what I can do is I can transfer the fluorescing compound into a small (Refer Time: 21:50) of water in oil and then I can determine the fluorescence. I can have similarly donate shaped polymers such as cycloid strings these are all very commonly available and in most of the room temperature experiments we do have heavy atoms such as thallium, led, silver, and halide ions all these systems are used to promote intersystem crossing that helps us in the determination using fluorescence and phosphorescence.

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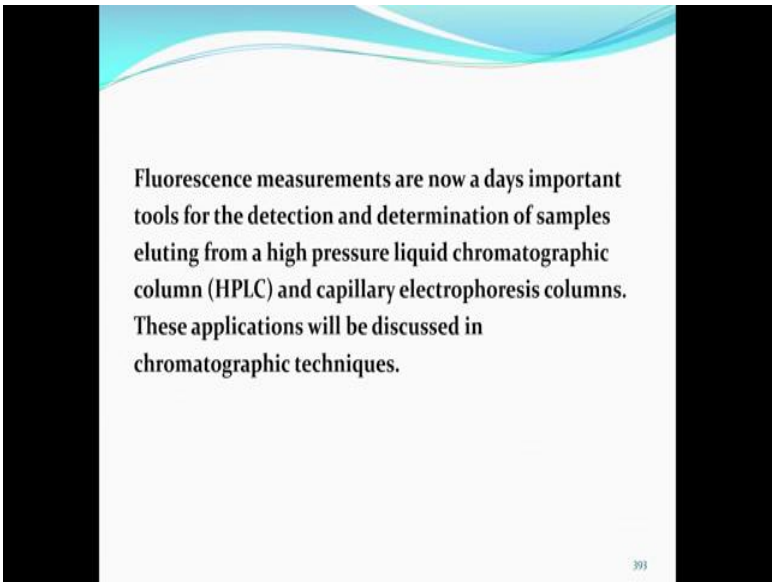

So, fluorescence measurements impercy they are nowadays important tools for the detection and determination of samples and they can whenever I want to separate some compounds in a chromatography basic principles you allow them to run through a column depending upon their partition coefficient they all separate out and then you run the caller, run through the column using your solvent and separated components will emerge from the column one after another depending upon their partition coefficient.

So, there are different kinds of chromatography and HPLC is one of them very important technique and capillary electrophoresis technique is also very important that also runs in the same fashion except that there will be some amount of electrical input to separate the components which are which are mobile under the electric field. So, the mobility depends upon the applied electric field and also in the characteristics of the substances. So, capillary electrophoretic columns are also useful, but what is important is application of fluorescence to the emerging compounds that is not you can not only separate the compounds, but you can also determine the compounds in a given mixture that is very important and I think many of you must be aware of how much important HPLC is in today's context, may be if time permits I will be teaching you about HPLC and atomic absorption also.

But for the being what I want to tell you is HPLC is one of the most accepted technique for; fluorescence is one of the most accepted technique for HPLC end of the pipe

treatment and determination of the emerging compounds. So, it is not just standalone equipment, but is a part of HPLC equipment where fluorescence can be one of the detectors, fluorescence detectors also permit you to determine compounds of the order of about 10 raise to minus 9 or 1 or 2 nanograms depending upon the HPLC column and other separation techniques used in the columns.

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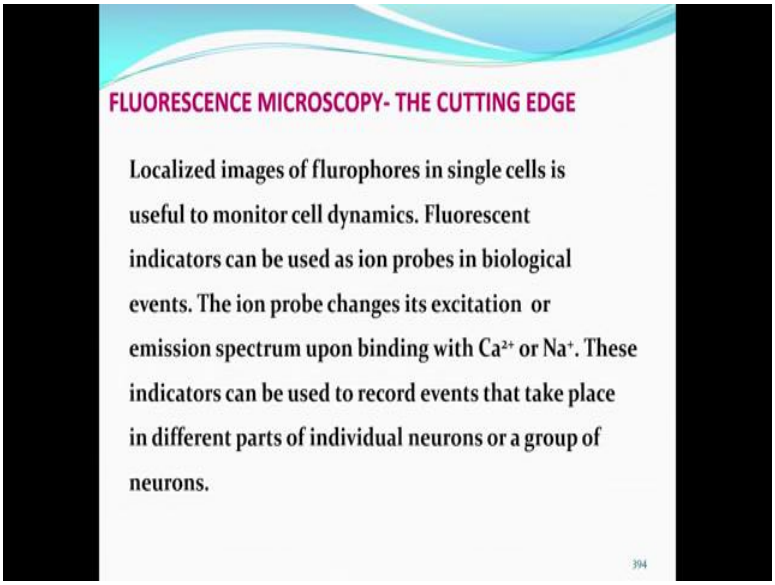

So, this is the cutting edge of the fluorescence microscopy what all we can do wonderful things which are normally not done in laboratory, but I feel compiled to each you about this fluorescence microscopy. What happens normally is localized image is a flurophores flurophores means compounds which can fluoresce which can induce fluorescence just like chromophore we are fluorophore. So, fluorophores in single cells is useful to monitor cell dynamics. So, fluorescent indicators can be used as ion probes in the biological events, the ion probe changes its excitation or emission, the spectrum depending upon binding with calcium or sodium.

Assume that I have a, assume that I have a some body fluoride and I want to determine particular compounds and when I add calcium or sodium ions they do it bind to the fluorophores and these fluorophores whenever they bind in a given time frame it can be used as a record of events that take place in different parts of individual body, it may a neuron or may be a group of neurons etcetera, but as and when I take a calcium injection or calcium tablet and I can monitor particular cells of my body the time taken by the

calcium and sodium substances to bind to the fluorophore either by increase and the result would be either increase in the fluorescence or decrease in the fluorescence depending upon the fluorophore

So, the real time record of the events happening in a given body is what we call fluorescence microscopy.

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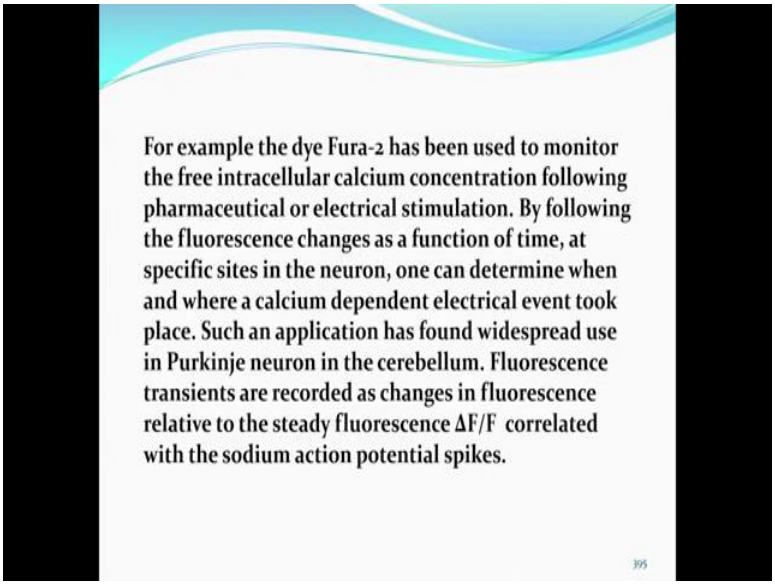

So, for example, you can go through this slide, in this slide what I am trying to teach is dye flora two has been used to monitor free intracellular calcium concentration following pharmaceutical or electrical stimulation. What we do actually, we electrical we take the particular body cell culture and add calcium following then I had the other come reagents and by following the specific sites in the neuron one can determine when and where calcium dependent electrical even took place. That means, when the signal goes down or up from the normal level we have a record of the event in the cerebellum. So, fluorescence transients are also recorded as changes inflorescence that is with respect to fluorescence it is just like a derivative spectra and delta f by f we can record and correlate with the sodium action potential spikes.

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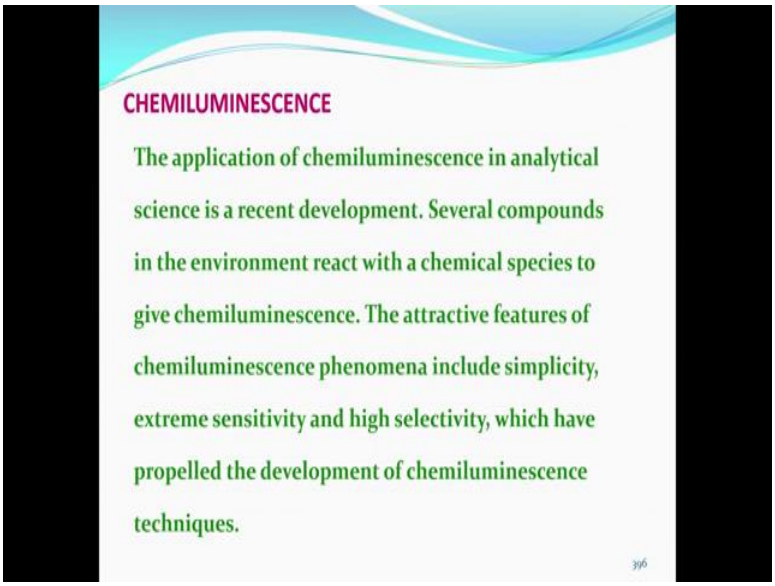

So, now I want to teach you that is a cutting edge example of what all beautiful things we can do with fluorescence.