Modern Instrumental Methods of Analysis

Prof. J. R. Mudakavi

Department of Chemical Engineering

Indian Institute of Science Bangalore

Module No 03

Lecture No 14

Atomic Fluorescence Theoretical Aspects

Welcome to the next section of our discussion on the fluorescence methods we continue our discussion on the fluorescence measurements.

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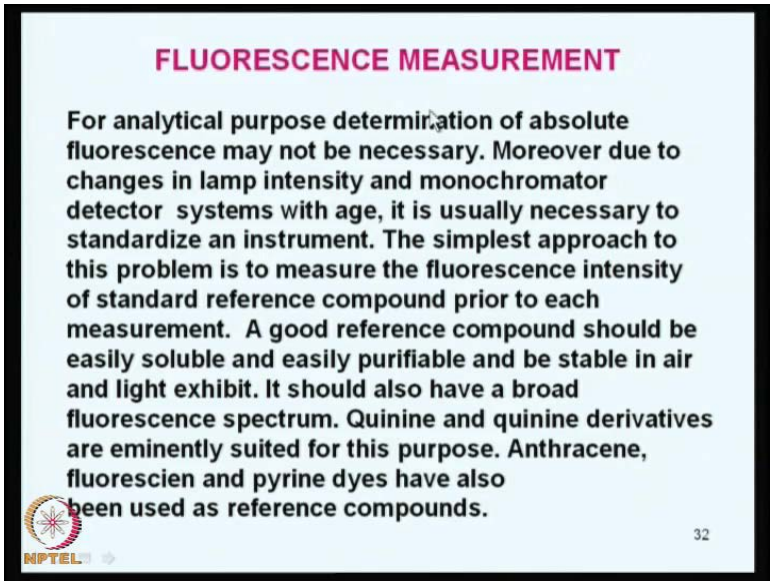

For analytical purposes determination of absolute fluorescence may not be necessary. Moreover, due to changes in the lamp intensity and monochromator detector systems with age, it is usually necessary to standardize an instrument. The simplest approach to this problem is to measure the fluorescence intensity of a standard reference compound prior to each measurement. A good reference compound should be easily solvable it should be purifiable and be stable in air. And it should be light stable it should also have a broad fluorescence spectrum.

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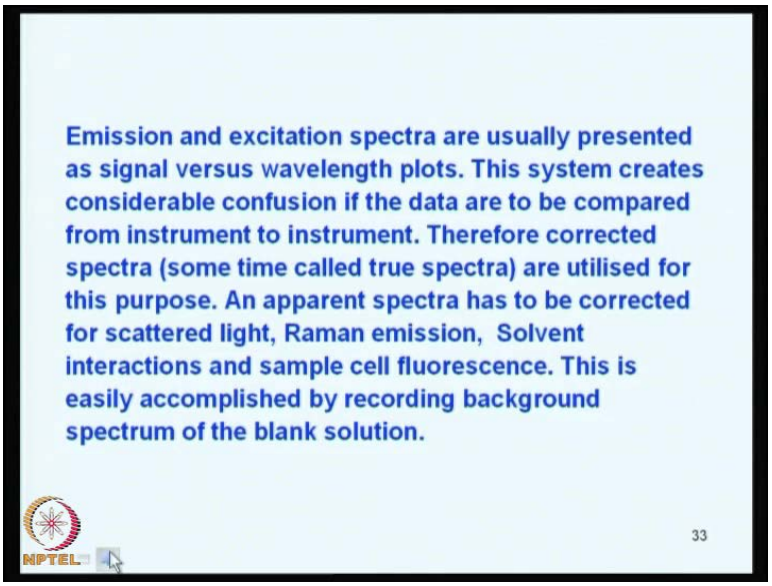

Quinine and quinine sulfide derivatives are eminently suited for this purpose. Anthracine fluorescein and pyrene dyes have also been used as a reference compounds. Emission and excitation spectrum are usually measured and presented as signal verses wavelength plots. This system creates considerable confusion if the data are to be compared from instrument to instrument. Therefore, corrected spectrum sometimes they are called as true spectrum are utilized for this purpose. An apparent spectra has to be corrected for scattered light, Raman emission, Solvent interactions and sample cell fluorescence. This is easily accomplished by recording the background spectrum of the blank solution and then you can subtract the sample fluorescence from the blank solution.

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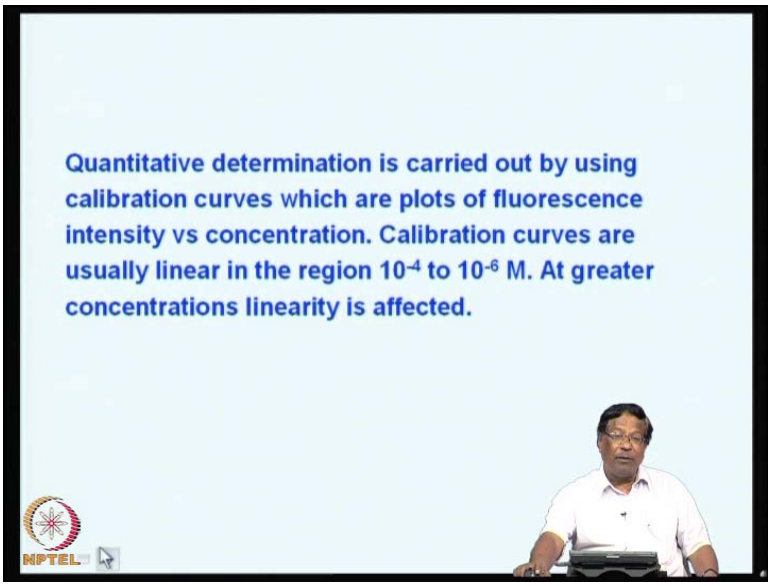

So, quantitative determination you can usually carried out by using calibration curves just like in spectrophotometry which are nothing, but plots of fluorescence intensity fluorescence intensity verses concentration. So, calibration curves are usually linear in the range of 10 raise to minus 4 to 10 to rise minus 6 molar. At greater concentrations; obviously, linearity would be affected. And you should not be working if the linearity is compromised because of the difficulties in reproducing the fluorescence measurements.

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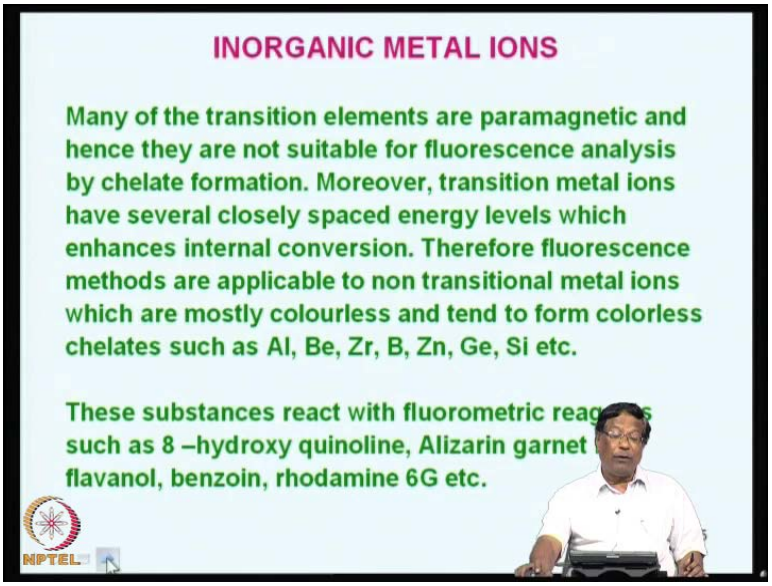

Many of the transition elements are basically paramagnetic and hence, they are not suitable for fluorescence. You remember that, we had discussed the diamagnetic substances are suitable for fluorescence and definitely paramagnetic substances do not form fluorescence species by chelate formation also. Moreover transition metals have closely spaced energy levels, which enhances internal conversion. Therefore, fluorescence methods are applicable mostly to non transition elements metal ions, which are mostly colorless and, but they do; they must, they also tend to form colorless chelates what are such compounds? Basically aluminum, beryllium, zircon, boron, zinc, gallium, germanium, silicon etcetera.

These substances react with fluorometric reagents, such as 8 hydroxy quinoline, alizarin, garnet tri r, flavanol, benzoin, rhodamine G, 6 G, rhodamine B, rhodamine 6 G etcetera. Crystal violet and there are several other fluorescing agents, which can react with such elements to give fluorescing substances, which can be measured and.

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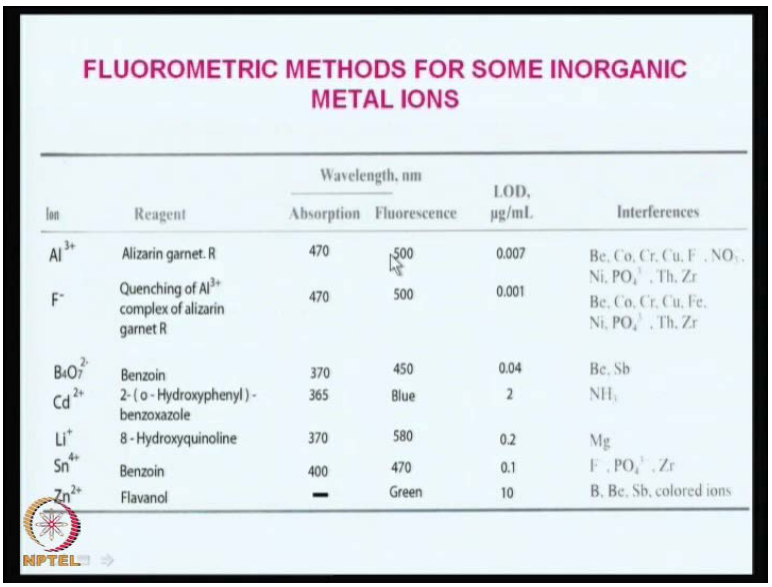

Now, I will show you a list of methods fluorometric methods, for some inorganic metal ions. And here you can see then I have listed on the left side elements here. So, here I am showing you the elements, like aluminum which can be determined using a alizarin garnet r. Its absorption wavelength is 470 and fluorescence occurs at 500 nanometers. And limit of detection is 0.007 micrograms per milliliter that is almost 7 PPB parts per

billion. But they can see, there are some interferences like this beryllium, cobalt, chromium, copper, iodine etcetera some of the element.

Another method fluorescing fluorescence technique is for fluoride and here, the reaction of quenching of aluminum complex of alizarin garnet r is utilized as a fluorescing analytical method. And absorption is again at four seventy and fluorescence occurs at 500 limit of detection you can see is 0.001 microgram per milliliter. Now, you can see that these limits are much better than absorption methods. Of course, there will always be some amount of interferences in all the methods. For example, this boride reacts at benzoic to produce fluorescence around 450 nanometers and the limit of detection is approximately 0.04. In this case only beryllium and antimony interfere.

Similarly, cadmium can be determined using two ortho hydroxyphenyl benzoxazole and they gives blue fluorescence absorption, wavelength is 365 and limit of detection is 2 mill microgram per milliliter, only ammonia interference in this case. Similarly, there are some of other methods for lithium with 8 hydroxyquinoline and then tin with benzion zinc with flavanol etcetera.

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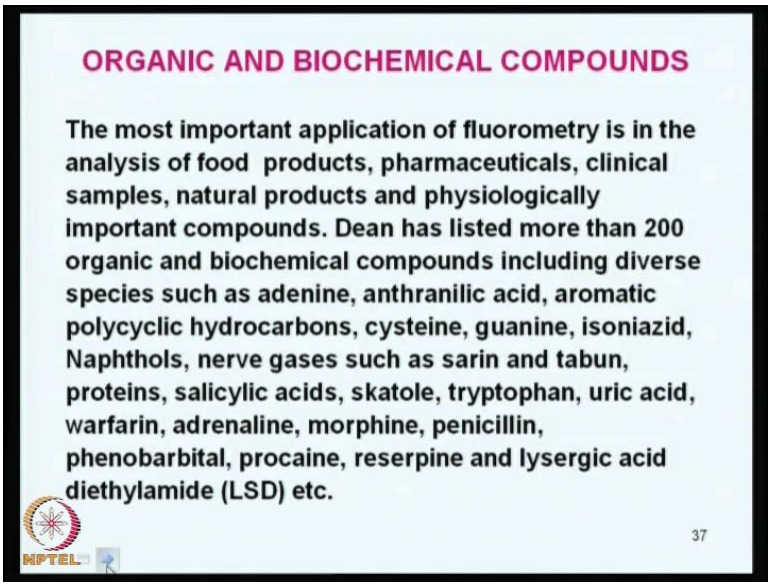

Generally, what happens is most of the fluorescence methods are available for about 30 to 35 elements. And these the elements can be determined the they are available in the data base, if you look up the data base for spectrophotometry or fluorescence. You would see that, most of these elements are listed along with their methods and you can get the

analytical conditions for their determination in PPM FPPB levels. The most important application of course, for fluorometry, is in the analysis of food products and pharmaceuticals, clinical samples, natural products and physiologically important compounds like our body fluids plasma etcetera.

Dean has listed more than 200 organic and biochemical compounds including diverse species such as adenine, antaranilic acid, aromatic polycyclic hydrocarbons these are known as PAH ploy aromatic hydrocarbons, they are present in the air and as pollutants. Cysteine, guanine, isoniazid, these are all some compounds of amino acid compounds. Naphthols, nerve gasses such as sarin and tabun, proteins can be determined. Salicylic acids, skatole, tryptophan, uric acid, warfare and several other elements like this adrenaline, morphine, penicillin, Phenobarbital many of the drugs procaine reserving lysergic acid diethylamide etcetera can be determined using fluorescents methods.

And these methods are available in the data base one can look up the specialized books which will list such reactions.

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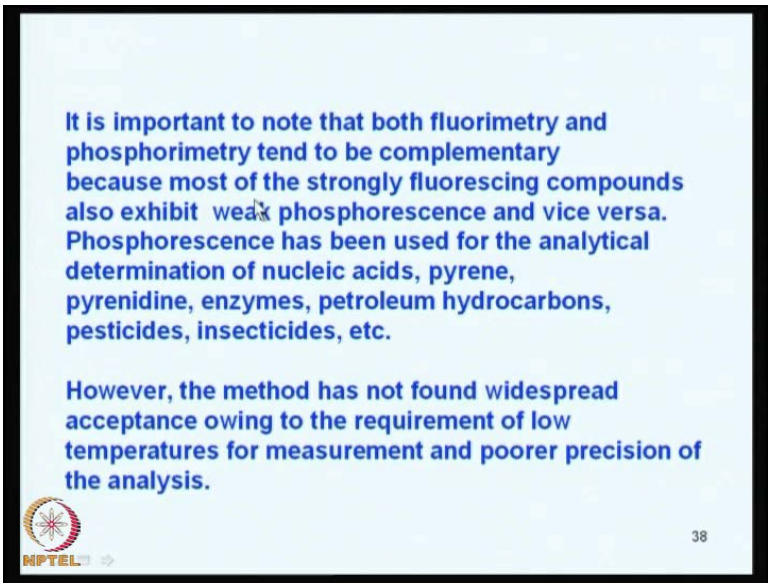

And it must it is important to note that fluorometry and phosphorimetry tend to be approximately, complimentary because most of the strongly fluorescing compounds also exhibit weak fluorescence and vice versa. Phosphorescence has been used for analytical determination of nucleic acids, pyrene, pyramiding, enzymes, petroleum, hydrocarbons, pesticides, insecticides etcetera. However, the method has not found widespread or

acceptance owing to the requirement of low temperature for measurement and poorer precision of the analysis. Therefore people usually do not go for phosphorescence as an analytical technique, but it can be done; however.

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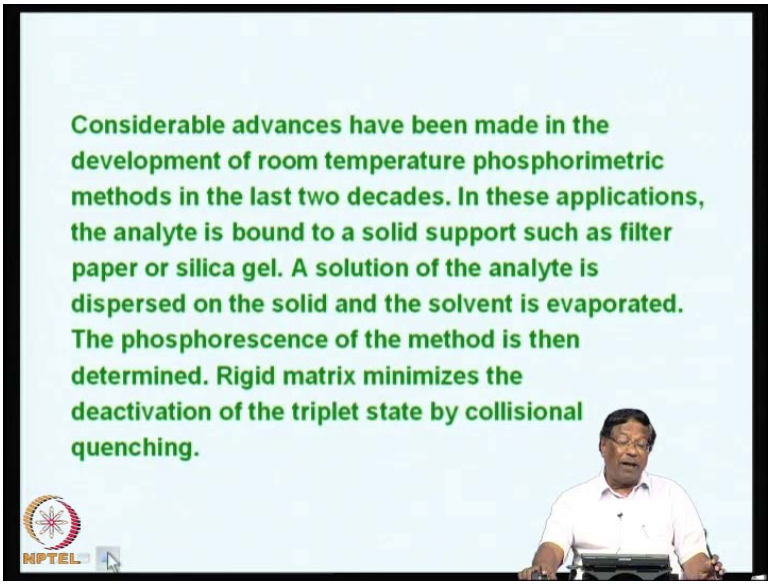

So, considerable advances have been made in the development of room temperature fluorescence phosphorimetry in the last two decades. Because otherwise you will of any phosphorimetric measurement have to be done at liquid nitrogen temperature if you remember the discussion we had earlier. In these applications, the analyte is bound to a solid support as a filter such as, a filter paper or you can use silica gel etcetera. Then what you do? A solution of the analytic is dispersed in the solid.

And the solvent is evaporated. So, you get a substance solid substance and the phosphorescence of the method is then determined. So, basically what you are achieving is a rigid matrix, that minimizes is the activation of the triplet state, deactivation of the triplet state by collision quenching that is important. So, you can also an incorporate the analyses into a core of missiles. So, you know what is missiles? Missiles are basically surface active agents like, our detergents which form hydrophilic, which are long molecules substances. Which have got hydrophilic bounding at one end and hydrophobic tendency at be other end of the molecule.

So, they trend to curl, along molecule if you take it wont stands still it will train to curve and you can incorporate the analyte in such missiles which also serves to protect the

triplet state. Similarly, donate shape polymers such as cyclodextrins have been used for the measurement of phosphorimetry. In most of the room temperature experiments heavy atoms such as thalim, lite, silver and lead ions are used to promote intersystem crossing because we know that such system promote phosphorescence. So, fluorescence major measurements are now a days again we are the switching our discussion back to fluorescence.

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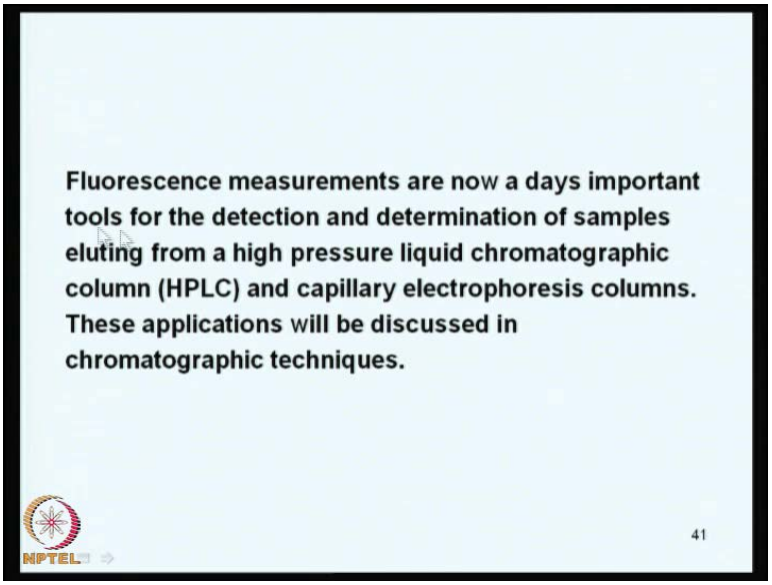

So, fluorescence measurements are now a days important tools for the detection and determination of samples eluting from a high pressure liquid chromatographic column will study about the chromatography later. And these fluorescence equipments are used as detectors in fluorescent detectors and it is also useful as detector in capillary electrophoresis columns. These applications will discussed in the chromatographic techniques.

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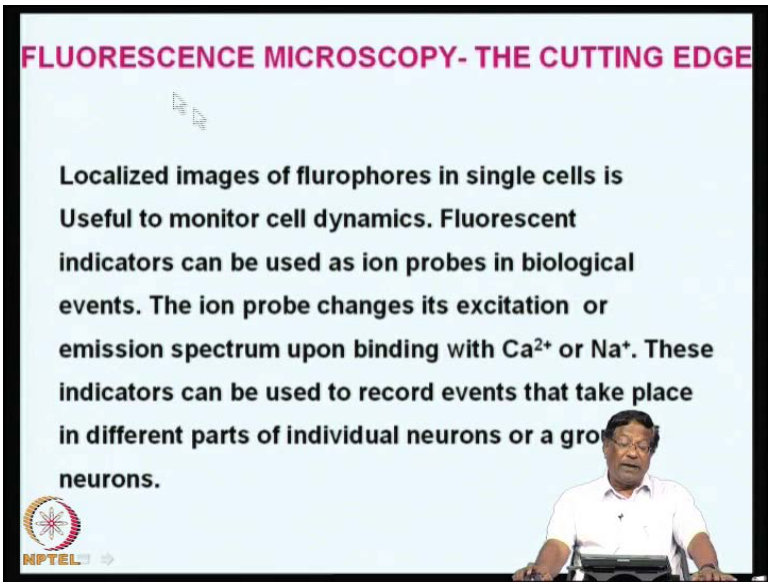

Now, you can imagine that fluorescence is a not only important at PPB levels of the determination of metals and a organic and inorganic compounds biochemical compounds etcetera. But you can also imagine that, it can be used for a cutting edge technology development. One of them is a then to obtain localized images of fluorophores in single cells in the human body now, to monitor the cell dynamics. For example, fluorescent indicators can be used as ion probes in biological events one such ion probe changes its excitation or emission spectrum upon binding with calcium or sodium ion.

And these ions are present in our body. So, these indicators can be used to record events that take place in different parts of the individual neurons or a group of neurons. I have selected this technique to introduce you to the new system of thinking or following biological events in the human body. For example, the dye fura two to, it is a trade name readily available in the market.

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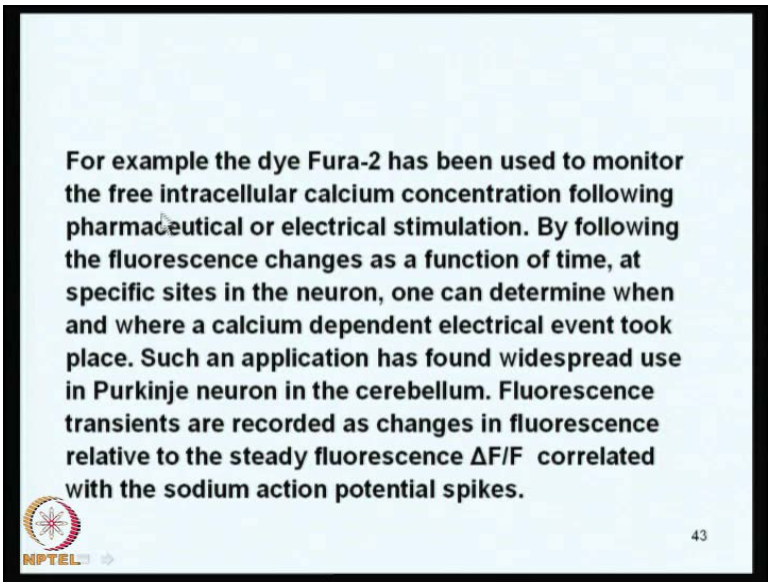

It has been used the monitor free intracellular calcium concentration following pharmaceutical or electrical stimulation. What you mean by that? You are suffering from something we give you a pharmaceutical drug. And then we want to follow what is happening at the cell level in presence of this drug in the human body. So, by following the fluorescence changes as I function of time at this specific site in the neuron, one can determine when? And where a calcium dependent electrical event took place?

So, such an application finds widespread use in purkinje reaction neuron in the cerebellum and fluorescence transients are recorded as changes in fluorescence relative to the steady fluorescence delta f by f correlated with this sodium action potential spikes and very exciting application of fluorescent. Now, I would be failing in my duty if I am do not talk to you about chemilunesecne methods. Now, you know that we have discussed luminescence in this; in the last three four lectures we have discuss that luminescence includes fluorescence, phosphorescence and chemiluminescence as well, but we are not discuss about chemiluminescence so far.

So, now, I would like to discuss with you chemiluminescence because in the last two three decades, chemiluminescence as a acquire lot of importance as a analytical tool. The application of chemiluminescence in an analytical science is a recent development. Of course, the phenomenon has been known since quite long time. So, what we are trying to observe in chemiluminescence is that, several compounds in the environment react with

chemical species, to give chemiluminescence. The attractive features of chemiluminescence phenomena include its simplicity. Very simple reaction you just take two reagents let them react and they react to give product which will generate an electromagnetic radiation which can be monitor.

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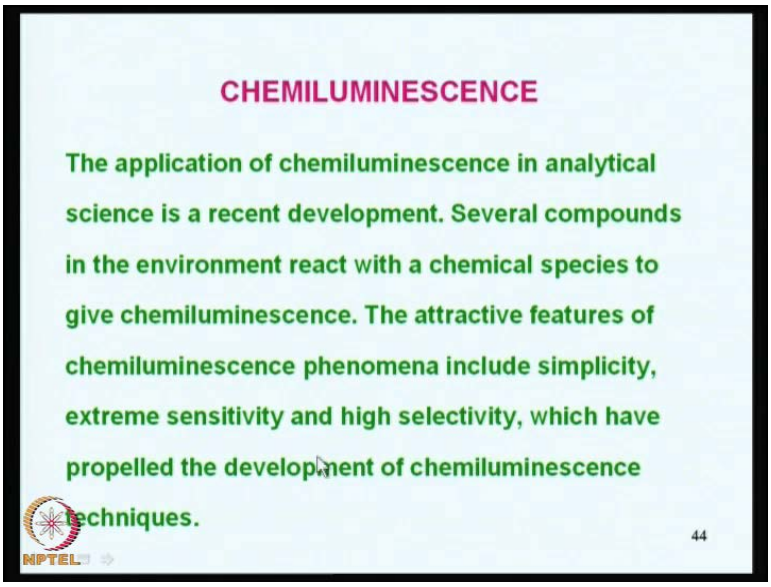

And because of its simplicity and extreme sensitivity and high coupled with high selectivity have propelled the development of chemiluminescence techniques and.

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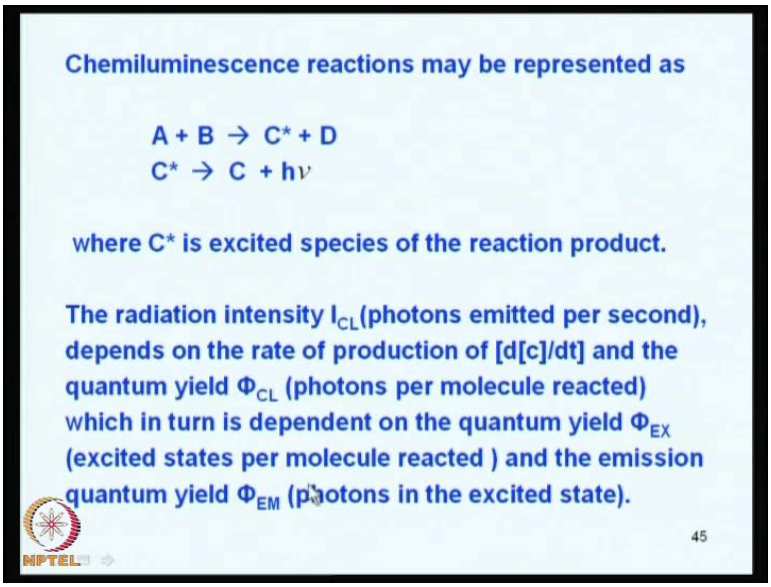

Basically you can represented chemiluminescence reactions as a simple system like A plus B going to C star plus D. These are reactants A and B reactive give you a product C star and D. And this C star is excited species and it goes to C plus h nu; that means, the conversion of C star to see is accompanied by the emission of radiation. And this radiation as to be specific to this reaction A plus B going to C star plus B. Therefore, the total reaction is nothing, but a plus B going to C plus h nu.

So, the radiation intensity I CL that is nothing, but photons emitted per second. It depends upon the rate of production that is d c by d t conversion of c and quantum yield phi CL. That is the number of photons per molecule reacted which in term is dependent upon the quantum yield phi E X which is nothing, but excited states per molecule reacted and the emission quantum yield phi E M. So, this phi I CL is a basically related to all these things.

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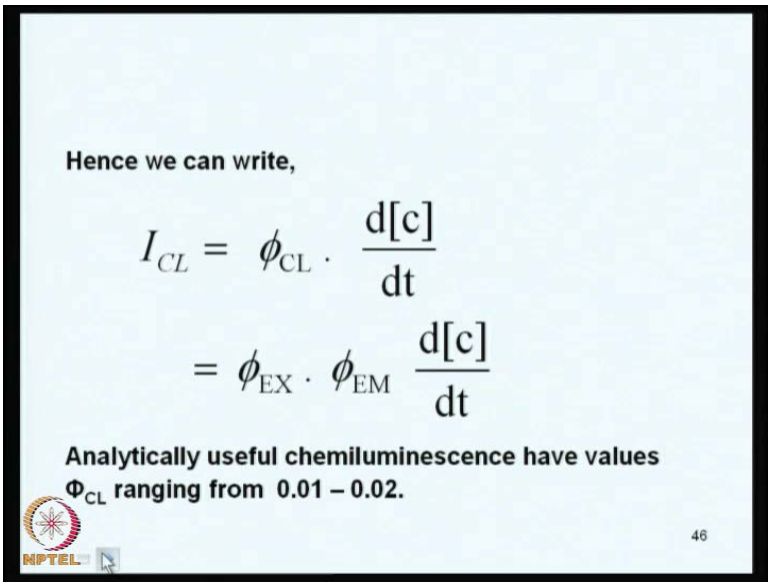

And we can express the reaction like this I CL is equal to phi CL into d c by d t and phi CL is nothing, but the product of excitation and emission followed by d c by d t. So, the analytically useful chemiluminescence, have values of phi CL ranging from 0.01 to 0.02. That means, in this equation where a put 0.01 to point phi 02 and the reaction can be monitored successfully using these reactions.

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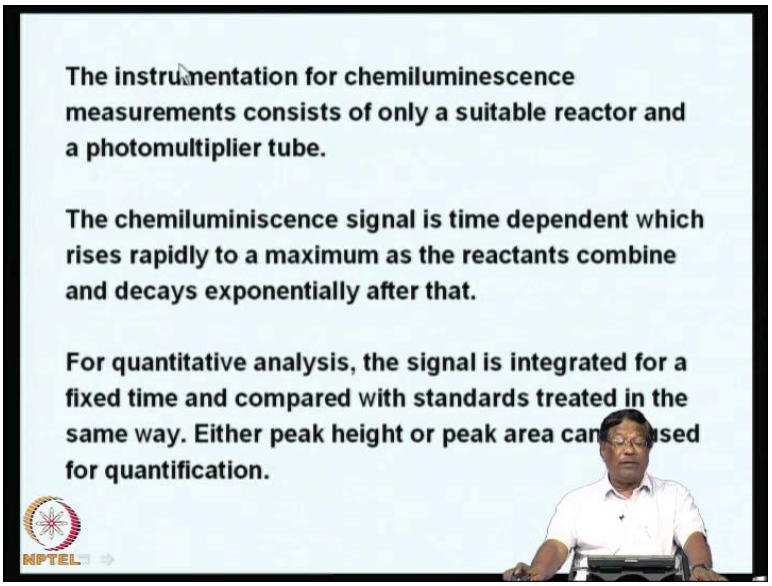

So, the instrumentation for chemiluminescence measurements basically, what it contains? It contains the reactor system in which the reactants are mixed and then you have silt to collect the radiation, that is coming out and then a monochromator to collect radiation at a particular wavelength and measure at related to concentration. So, the instrumentation for chemiluminescence measurement consist of a suitable reactor followed by a monochromator and a photomultiplier tube very simple reaction.

The chemiluminescence signal as to be time dependants, because in a given Dutch reactor there is always for certain amount of the reactant available and the reactions system as it proceeds it will reach maximum concentration and then it will decay. So, the optimum concentration and the signal corresponding to the optimum concentration as to be measured. So, the chemiluminescence signal is basically time dependant one which raises rapidly to a maximum as the reactants combine and decays exponentially after that I will show you a figure subsequently after this.

So, for quantitative analysis the signal as to be integrated for a fixed time and compared with a standard treated in the same way. So, either that you can use either peak height or the peak area for this purpose.

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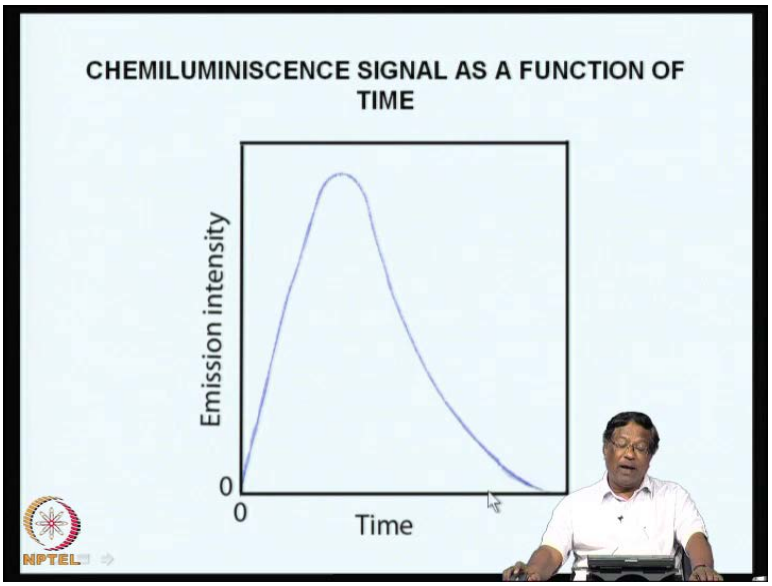

Now, I here I am showing you figure of chemiluminescence signal as a function of time. Here you can see the excess its time and the other signal is emission intensity. You can see that as the reactants continued to mix react producing C star it will reach a maximum here. And then it will be quiz exponentially as the reaction goes to completion. So, you have to measure the signal at this height, you can use either peak height or peak area define by time, that is they have to integrate the signal it will tend to time limits.

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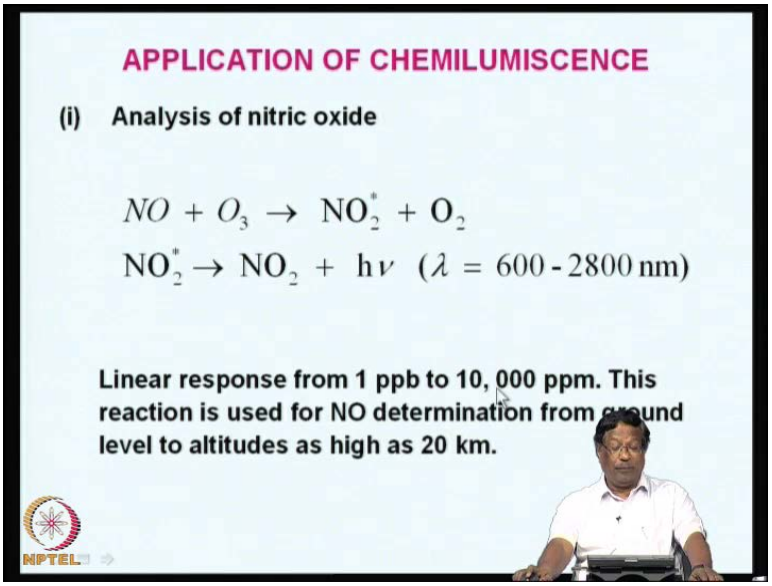

So, the theory is very simple, applications are very simple, but the beauty is they can be use for hertz per billion level of an analysis. Now, I am giving you an example like this analysis of nitric acid oxide. So, the basic reaction is nothing, but nitric oxide is the reacting with ozone to produce NO2 excited state, plus O2. And the NO2 will an revert back to nitric oxide, unexcited molecule along with the emission of electromagnetic radiation. And this lambda is somewhere between 602 and 2800 nanometer, we can use any radiation in this range.

And make sure that you are collecting the radiation of an only a particular wavelength. So, linear response in this reaction is from 1 ppb parts per billion to, 10000 parts per billion ppm not billion ppm and. This reaction is use for the determination of nitric oxide from the ground level up to in status fear, up to altitudes of about 20 kilometers also; that means, not only in the ground level, but also in the at high altitudes. And such reactions are important to us to study the variation of nitric oxide as it is exists in the stratosphere.

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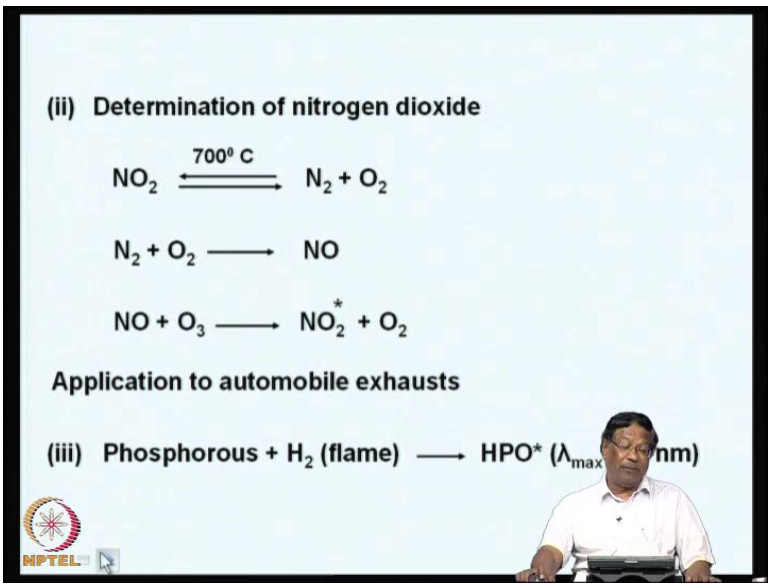

Another reaction I want to show you here, that is NO2 nitric oxide decomposes at about 700 degree centigrade to nitrogen and oxygen. And this nitrogen and oxygen can also combine to form nitric oxides. And nitric oxides again combine with ozone to produce NO2 excited molecule and oxygen. And such reactions occur in the vehicles, where we have the petrol or a diesel burning in presence of air and nitric oxide, nitrogen dioxide

NO X, nitrogen oxide, nitric oxide all this things can form. And what you can do? Is to collect part of the emission gasses and allow them to react with ozone to produce NO2.

So, this has got applications to automobile exhausts where you can see the concentration of nitrogen oxides which is actually an environmentally pollutant. Similarly, phosphorous can be reacted with hydrogen flame, hydrogen burning and phosphorous can be introduced in a burning hydrogen flame which produces HPO star molecule. Which is luminescence wise very active and it has got lambda max for 526 nanometer. So, you may ask what is the importance of such reaction? The importance of such reaction is that many of the phosphorous compounds are used in substances like pesticides, insecticides, DDP and several other environmentally damaging compounds.

And we do not have easy means or simple means of the determination of such compounds at ppb levels. And believe me at ppb levels the damage is done, not in ppm or per milligram level. Even at ppb levels such a substances can cause damage to the cells and one of the very important reaction for the determination of phosphorous in environment. And the next example I have chosen is the determination of ozone now, you know about ozone, most of the ozone is there in a our on the earth at a height of about 11 to 60 kilometers. And ozone layer everybody has heard of and ozone is suppose to absorb the ultra violet radiations coming from the sun and protect us.

And all of you must have heard about ozone hole, where ozone is being consumed to be a part of the atmospheric reactions at high altitudes. And the ozone concentration deplete over particular area in the space, this is known as ozone hole. Now, how to determine the ozone gas? And that itself is in ppb levels and the ozone can be determine by reaction with Rhodamine B. It is absorbed on silica gel from 1 ppb to 400 parts per billion. And it can also be determine by reacting with ethylene, but that is not such popular reaction. But ozone reacting with Rhodamine B always gives you a beautiful fluorescence spectrum, chemiluminescence spectrum not even fluorescence and the both these reactions are specific for ozone.

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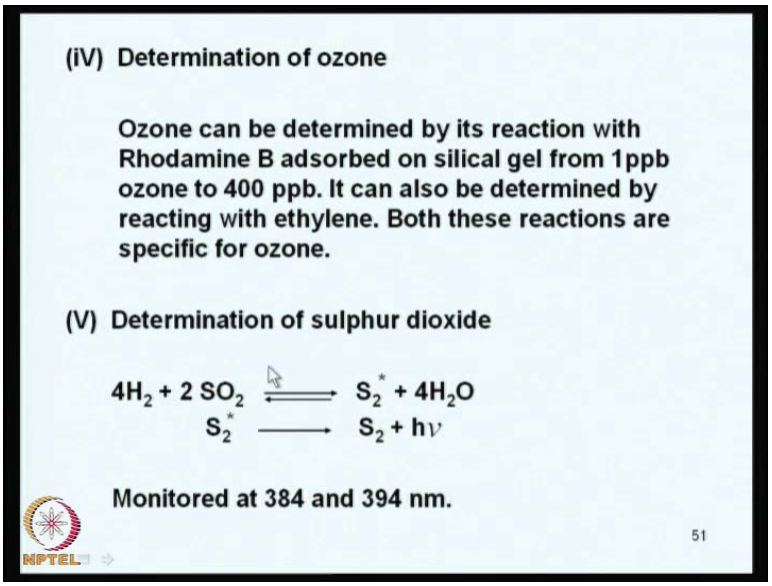

Now, another reaction is determination of sulphur dioxide that is the again I want to stress here, that the determination of sulphur dioxide is a very important part of our environmental cleanup procedures. Because sulphur dioxide is emitted by thermal power stations and several other industries metals melting etcetera and also by almost all the vehicles that we use they all contains sulphur. So, the reaction which sulphur, when the petrol burns, the sulphur reacts with oxygen to form sulphur dioxide. And the atmospheric sulphur dioxide concentration goes up during whenever, the vehicle is passing by.

And at the concentration of sulphur dioxide follows diagonal pattern in city then lot of people are going for work, there concentration increases. And then, when the people are in there work places and vehicle population is less, than the concentration decreases. And again when the substances; when people start coming back from the office again the vehicles will be starting petrol will be burning and sulphur dioxide will being generated and the concentration of sulphur dioxide in the environments goes up. So, the concentration of the sulphur dioxide in the atmosphere describes a di urinal pattern; that means, 2 maxima if you plot the concentration of sulphur dioxide in the air verses time.

So, the determination of sulphur dioxide is also a very important part of environmental management especially, because it is coming from the transport vehicles and you all known how important transport is to our modern society? So, the reactions of sulphur

dioxide with hydrogen produce and excited sulphur molecule and water of course, and this excited sulphur molecule luminescence giving you a radiation at 384 and 394 nanometers.

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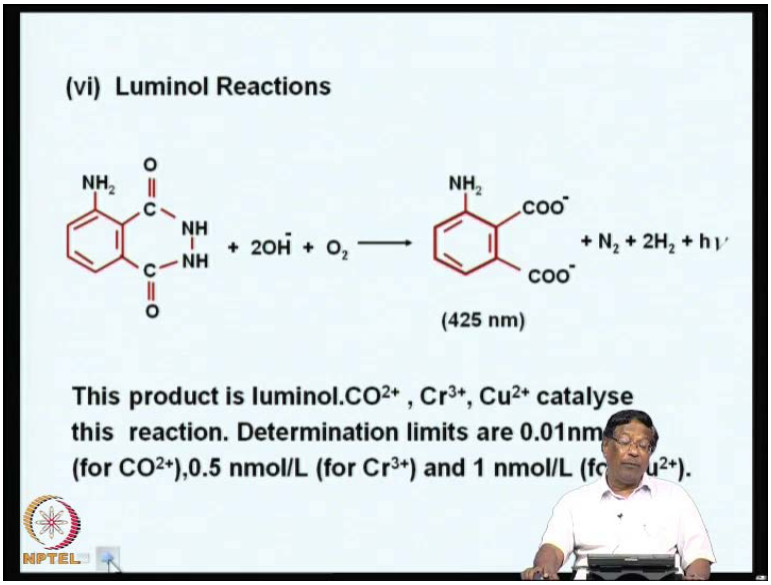

So, this is another reaction that can be used for the determination of sulphur dioxide by chemiluminescence method. Now, here I am showing you one more example, that is the a compound like this with 2NH groups CO and it reacts with a hydroxide in presence of oxygen to form luminal. This is a phthalic anhydride phthalic acid anion and plus nitrogen etcetera, this luminescence at 425 nanometers, the product is known as luminal. Now, carbon monoxide chromium 3 and cobalt 2 plus chromium and copper etcetera such metal ions catalyze this reaction.

And the concentration for catalyze are very very minimal. So, they you can determine the concentration of a substance by the production of luminol by using, very low concentrations. And the detection limits obtained for this are 0.01 nanomoles per liter for cobalt and 0.5 nanomoles per liter for chromium and 1 nanomoles per liter for Cu2 plus that is copper. So, many such metal ions can catalyze this reaction including hydrogen peroxide.

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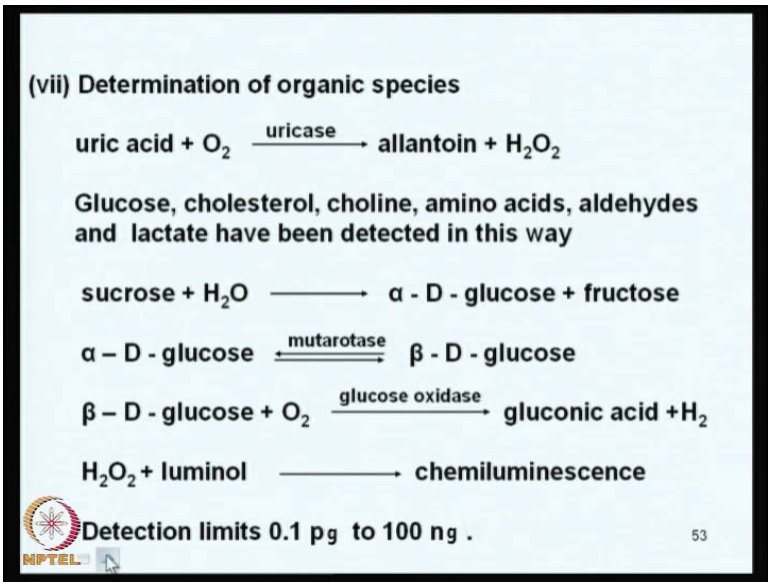

And then, there are other determinations by chemiluminescence and these include uric acid and oxygen these are enzymatic reactions I am collecting the some of the examp les. And a uric acid reacts in presence of uriccase to produce allenton and hydrogen peroxide and hydrogen as you known hydrogen peroxide catalyses luminol reaction. So, you take uric acid reacted with oxygen and you get H2O2 as byproduct and permit this H2O2 to react with luminol and chemiluminescence is obtained. So, a similarly reaction the similar reaction can be obtained by using glucose and then cholesterol and then chlorine can use amino acids you can use aldehydes and lactates all this have been detected in this way.

You can take sucrose and water that leads to hydroxylation of sucrose lead to alpha d glucose and fructose. And alpha d glucose can react with enzyme called as mutarotase to produce beta d glucose. And beta d glucose can react with oxygen in presence of glucose oxidase enzyme to produce gluconic acid and H 2. And H 2 O 2 can be react with luminol to give chemiluminescence and. So, you can correlate all this reactions and get back to the original concentration of the reactant and the detection limits obtain in this way or from 0.1 ppm to hundred ppm 100 ppm.

So, this is what I wanted to teach you about a luminal chemiluminescence reactions. And now I would like to an take you to another aspect of fluorescence, that is X ray fluorescence. I have decided to include this along with fluorescence actually like X ray

fluorescence and X ray absorption all almost all X ray analytical techniques are grouped into separate category X ray absorption esca owshe and X ray fluorescence etcetera. But I have decided to include X ray fluorescence in this discussion because it has got a similar phenomenon occurring whenever we are using X rays and atoms; that means, fluorescence occurs.

So, it is a in the fitness of things that we should discuss X ray fluorescence spectrometry also, but we since we are or not discussing any other X ray analytical techniques I thought it is prudent to include X ray fluorescence techniques. So, now, I would like to before I proceed further I would like to take you to some of the important aspects of X rays you should understand. So, as you know we have while studying in the electromagnetic radiation we have stated that X ray are part of the electromagnetic radiation, which vary from which I have got wavelengths in a 0.1 to 100 strong angstrom units.

Now, you should also know a little more about the nature of X rays before we talk about X ray fluorescence. In this way I have thought, I will give small short introduction to the X rays what we are handling.

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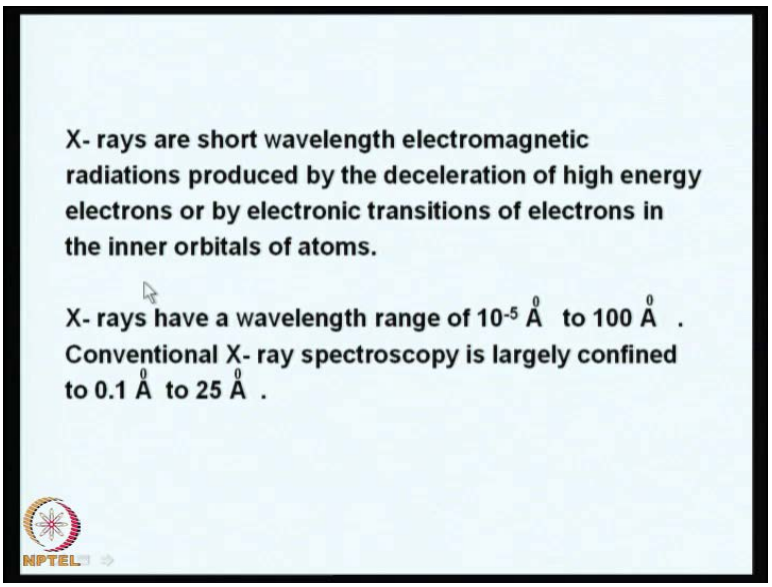

In general X rays are a short wavelength electromagnetic radiations, they are produce by the deceleration of high energy electrons or by electronic transients of the electrons in the inner orbitals of the inlet atoms. Now, what we mean by this is that X rays you take

the electrons allowed them to bombard on target using acceleration. And when the electron beams strikes a target some of them will hit the middle atoms, instead of the empty space around the around the nucleus.

Sometimes they electrons will also hit the electro; inner orbitals, inner orbital electrons knock them off from the orbits of the atoms. So, when an electron is removed from the atom from the k shall, l shall you may remember most of the things, that you have discussed earlier that electrons are arranged in the in an atom basically nucleus around it. There are k electrons, l electrons, l shall, m shall etcetera. From the k shall suppose, an electron is knocked off by the incoming electron beam then an I m is excited atom is produced. And the vacancy created in the k shall is filled by another from l shall or m shall or n shall etcetera to fill that vacancy.

And during this process the deceleration of the electron beam generates X rays. So, what we I have talking about is basically what are X rays? X rays are short wavelength electromagnetic radiations, they are produce by taking high beam, high energy electrons produce from cathode and directed on to the anode using electrode high electrode potential. And when they hit atoms electrons are now knocked off from the inner orbitals and this space created by the incoming radiation is filled by l shall or m shall or n shall etcetera and that wavelength corresponds to the X rays.

So, will if you study this slide I have put together some of the thoughts that I have explained to you, that they are produced by the deceleration of high energy electrons are by electronic transition of the electrons in the inner orbitals of the atoms. So, in general X rays have wavelength of 10 raise to minus 5 angstroms to 100 angstrom units. Conventional X ray spectroscopy; however, is largely confined to 0.1 angstrom to 25 angstrom we do not deal with other X ray wavelengths, but confining general to 1 angstrom to 25 angstrom units X rays. In this we have soft X ray and hard X rays etcetera soft X ray are the one which are used in our day today medical applications.

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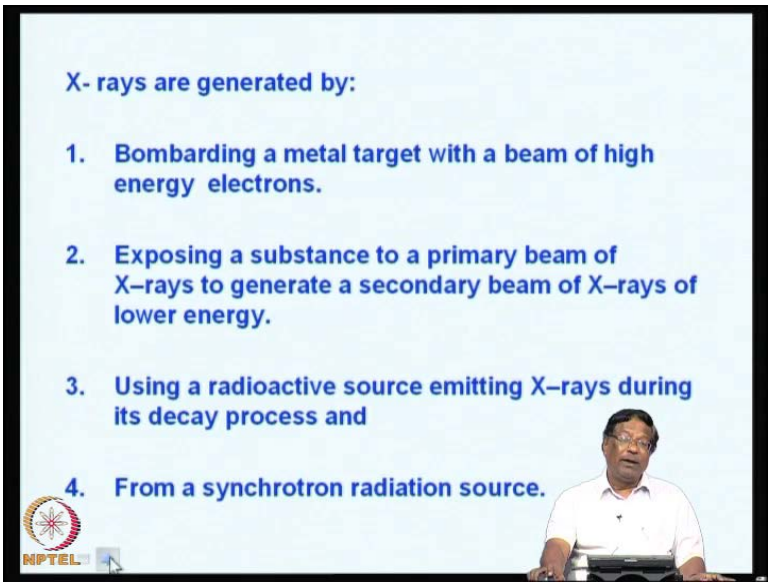

And such as a bone structure determination whenever you have an accident, a bone is broken and all those things are images are done by X rays. And they are known the X rays used in such cases are known as soft X rays. Hard X rays are still smaller wavelength which I have got more energy, better penetrating power and they can be use for studying the changes in the atomic structures. And before we with this introduction I want to tell you that X rays are generated by bombarding a metal target with a beam of high energy of electrons.

And you can also generate X rays by exposing a substance to primary beam of X ray you take the X rays produced in one case and allow them to fall on a secondary on another metal atom. So, you can get another set of X rays. So, you either use electrons to generate X rays or X rays themselves produce by the electrons to generate secondary X rays. Then there is a another source, X rays can also be used a produced by using a radial active source. And you as have all known a radial active source generates X rays emits X rays during its decay process continuously. And many such isotopes are known and those X rays also can be used for analytical purposes.

And of course, there is another way of producing from sicrontron radiation source, but this approach is not always very useful because sicrotrons high energy high capital intensive systems which are not easily available for day today purposes. They are mostly research equipments and the research facilities created by the governments in particular

places. And in a X ray tube both X rays are produced in X rays tubes; that means, you have to have the cathode we have to have anode both of them to be put in a bulb black structures. And the electron b measures to be generate from the cathode and they have to be targeted on to the anode where they would to be accelerated by the potential difference.

So, the electrons are produced at the heated cathode usually made of tungsten. So, this tungsten is a metal target cathode and they are accelerated towards another metal; obviously, that is known as the target. And that is they electrons produce at the cathode must be accelerated and they should heat the target at very high speed. So, the use of about 100 k v potential difference is almost mandatory and most of the X ray tubes. And upon colliding with a anode part of the energy of the incident beam is converted into X rays. And the continuum X ray spectrum we get two types of spectrum X ray spectrum, one is continues X ray spectrum and another is a line spectrum.

So, continuum spectrum you are all familiar because we have seen such several such spectra in our discussion, in a molecule fluorescence molecular absorbance and chemiluminescence etcetera. Most of the things what we are discuss so far they are molecular fluorescence. And now we are discussing about atomic fluorescence based on the pure metals. And of course, we will be discussing about they will be discussing about the production of X ray not only from pure metals, but also from some of the chemical substances compounds also. But whatever be the way of production of X rays, the spectrum of the X ray what you get out of it, consist only of two types one is a continuum spectrum another is line spectrum.

So, the continuum spectrum means, there are no gaps and they exhibit valid define short wavelength limit lambda zero, which is the characteristic of the applied voltage instead of the nature of the compound. So, what we are saying is? The acceleration and the acceleration given to the electron beam by the voltage is more important in the production of X rays and that will leads to continuum X ray in continuum spectrum. And this is the continuum spectrums from an X ray tube with tungsten target.

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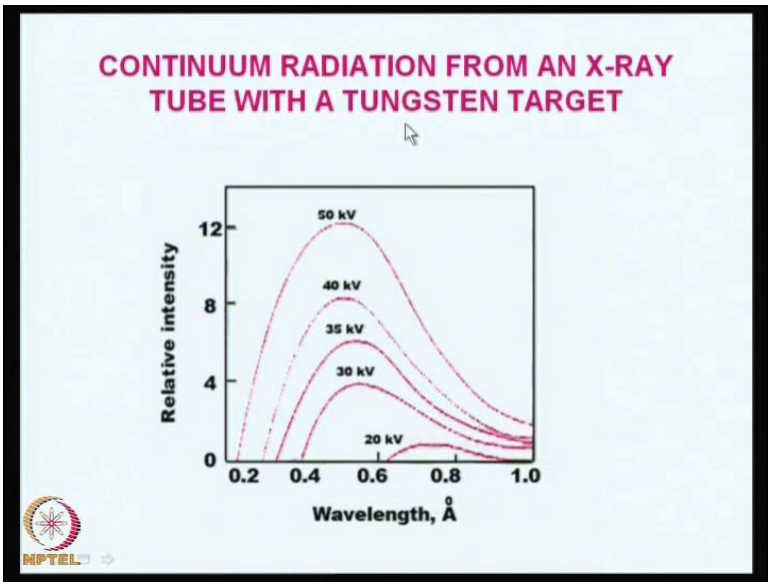

So, what you have here? We have here wavelength and this is the relative intensity have plotted at is 0 4 8 12and then wavelength is between 0.2, 0.4, 0.6, 0.8 and one angstrom units that is 10 raise to minus 8 centimeters. And you can see the energy of the spectrum at a 0.2 is around this is produced around 50 k v and it reaches a maximum and then it falls down. Same is the case if you use 0.3 a 40 k v if you use 40 k v acceleration, it does not start from 0.2, but it starts from 0.3. And then if you use 35 k v potential difference, it may start somewhere from about point approximately 0.28 or something.

And then 30 k v another spectrum starting from 0.4, 20 k v it start from 0.6. This type of spectrum is irrespective of the target; that means, it is only a function of the applied voltage, that is 20, 30, 35, 40, 50 k v what you have employed in our discussion. So, this is ah with a tungsten target generated from an X ray tube.

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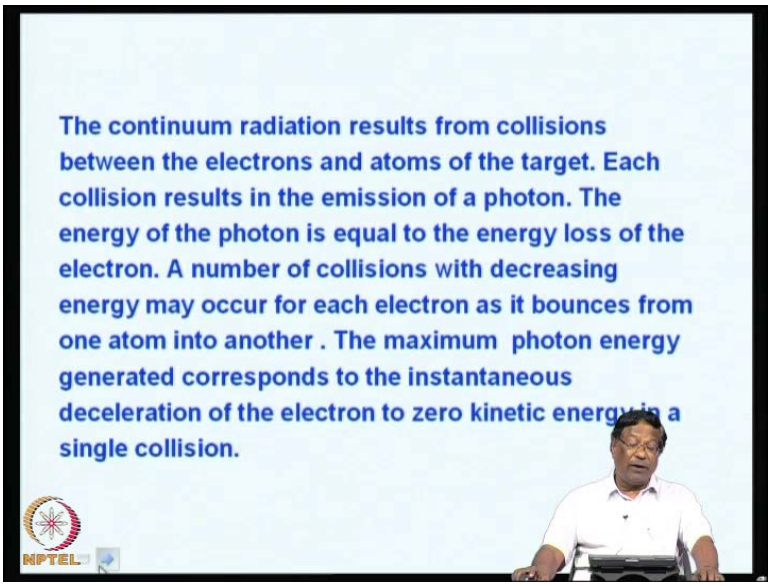

So, the continues radiation results from collissions between the electrons and the atoms of the target, each collagens results in the emission of photon. So, the energy of the photon is equal to the energy loss of the electron beam. You have an electron beam coming and hitting a target the electron beam, looses its energy and then the short wavelength beam produced and this the bat. Therefore the energy of the photon must always equal to the loss of energy of the electron present in the electron beam.

So, a number of collisions with decreasing energy may occur because once the beam collisions with one atom and it decelerates and then part of its energy is reduced. And again here, they reduced energy electron beam may hit another molecule; another atom. Again its energy is reduced and then it may hit another atom. So, the energy of the beam keeps on continuously reducing, falling down that is what we saw here that the energy keeps on coming down. This what? We have seen here, the as more and collisions occur the energy keeps on coming down this is irrespective of this is irrespective of the target. It is a function only of the voltage 20k v to 50 k v, what we have employed here in this picture.

So, the number of collagens with increasing energy may occur in each case as it bounds from one atom to another. Now, the maximum photon energy generated corresponds to the instantaneous this deceleration of the electron to zero kinetic energy in a single collision. Suppose, a singly collagens happens and the energy is totally lost, then we

have maximum photon energy because the photon energy must be equal to the loss of the energy, kinetic energy of the electron beam. So, the maximum must correspond to the loss. So, this leads to continuum energy.

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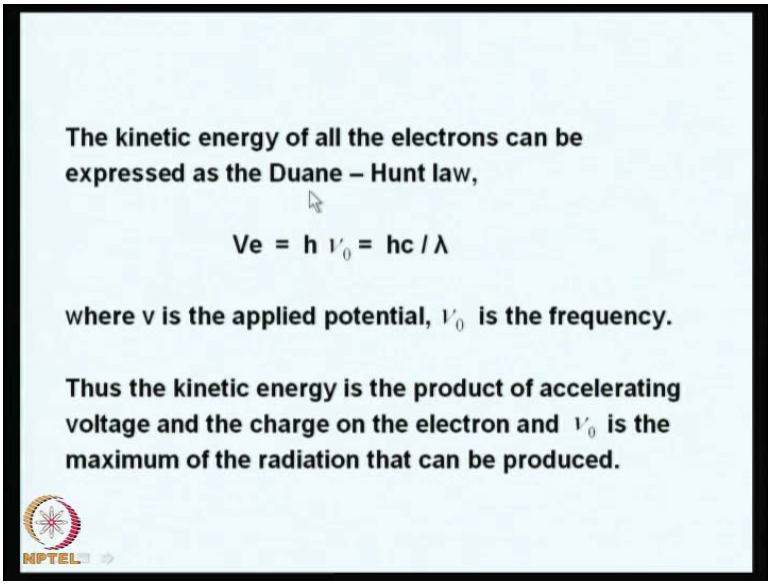

So, the kinetic energy of the all the electrons can be expressed as Duane Hunt law. For example, V into e it is a product of the applied potential and where the electronic charge corresponding to h nu zero. That is frequency into Plancks constant which is nothing, but h c by lambda. That is we are replacing nu zero by the various velocity of the light and the wavelength. So, this is very standard equation that is known where v is the applied potential and nu zero is the frequency and we all know that frequency is expressed as c by lambda. So, v e is equal to h c by lambda therefore, the kinetic energy is the product of accelerating voltage and the charge on the electrons and nu zero is the number of maximum of the radiation that can be produced.

Now, X ray line spectrum now, so far we have discussed about X ray continuum spectra. Now, will discuss about X ray line spectra. So, what is X ray line spectrum? They are all the results of electronic transition in the inner most atomic orbitals like I telling you that if they hit an electron they knock off the electron from the orbiting atom from the nucleus not form the nucleus, but from the inner orbitals k shall l shall m shall etcetera. From k shall and electron is knocked off the energy is so high, that it is completely

knocked off from the system and a excited ion is produced. And to fill the vacancy another electron as to fall and that energy the corresponding to that wavelength is X ray.

So, X ray lines spectra result from electronic transition in the inner most orbital atomic orbitals. They occur in the longer wavelength range of 4 to 6 angstrom units. In the previous in the previous continuum spectra, we have seen that inner wavelength is only up to 1 angstrom units here, in the continuum spectra. But in the line spectra we have the radiations occurring at longer wavelengths and the longer wavelength than 0.1 and angstroms.

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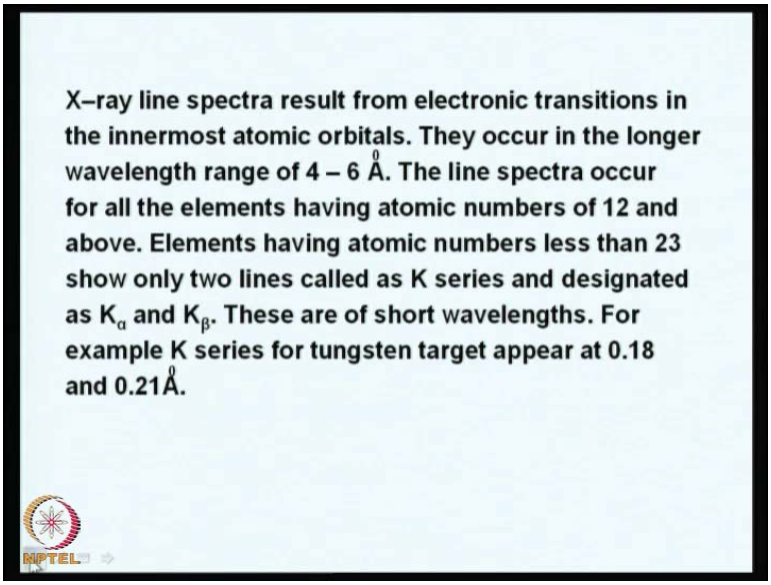

So, the range is 4 to 6 angstrom units the line spectra occur for all elements having atomic numbers of 12 and above. And elements having atomic numbers lees than 23 show only 2 lines. And these 2 lines are called as k series and each line is designated as k alpha and k beta. These are of short wavelength. For example, k series for tungsten target appear at 0.18 and 0.218.

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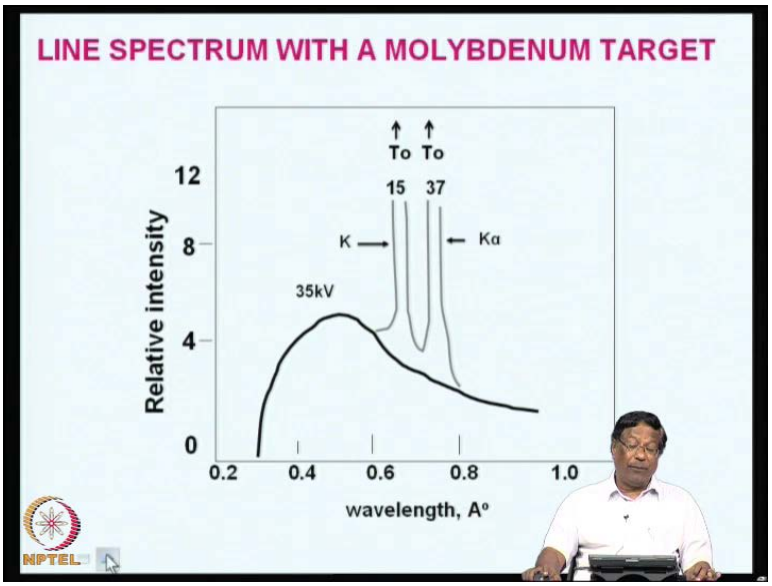

Now, you can see here the line spectra with a molybdenum target I have presented. It is basically relative plot of wavelength verses relative intensity. And the wavelength varies from zero to 1angstrom units and around 35 k v line, if we use we get spectrum like this. This is similar to what I have shown you earlier as a continuum spectrum. But in addition when you use a molybdenum target you get an two lines, one is around 15 intensity another is 37 .And this occurs k line, this is k alpha and this is k beta and this the energy corresponding to this is about 0.7 and this is a 0.62 or something and this is around 0.7. So, you would appreciate that only two lines spectra are obtain with respect to molybdenum.

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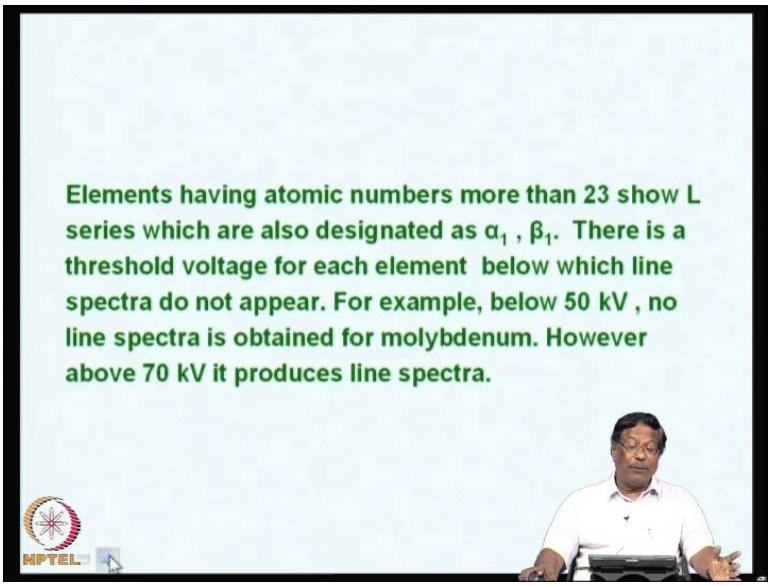

So, elements having atomic numbers more than 23 show another series known as L series. And there are also designated as alpha 1and beta 1 there is a threshold voltage for which for each element below which line spectra do not appear. So, to get line spectra, you must use a minimum accelerating force of about 50, 35 etcetera depending upon each element. For example, below 50 kilowatts, no line spectra is obtained for molybdenum. And if you use acceleration voltage below 50, no line spectra you will get only the continuum spectra; however, above 70 kilowatts it produces line spectra.

So, this is the discussion what we are having about understanding of the properties of X rays and then will continue our discussion in the next class about the properties. And then we can see how we can use this instrument? The these properties for the X ray fluorescence because X ray fluorescence analytical technique is a wonderful technique for the determination of most of the inorganic elements, that you can even study the surface properties. So, that is why? It is so important for us to understand what is X ray fluorescence. And before that we have to know more about X rays so, will continue our discussion in the next class.