Atomic and Molecular Absorption Spectrometry for Pollution Monitoring

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Lecture – 24
Instrumentation

Continuing our discussion on the instrumentation for fluorescence, there is lot of similarity as I told you earlier with respect to fluorescence and absorbance methods, and the same thing holds true for sample handling also. Usually measurements are made with rectangular cells and as I have told you earlier the measurement has to be done perpendicular to the incident radiation. So, the in normal spectrophotometric cells the opposite sides are polished and the remaining two sides are somewhat glazed to properly handle the glass cuvettes or quartz cuvettes.

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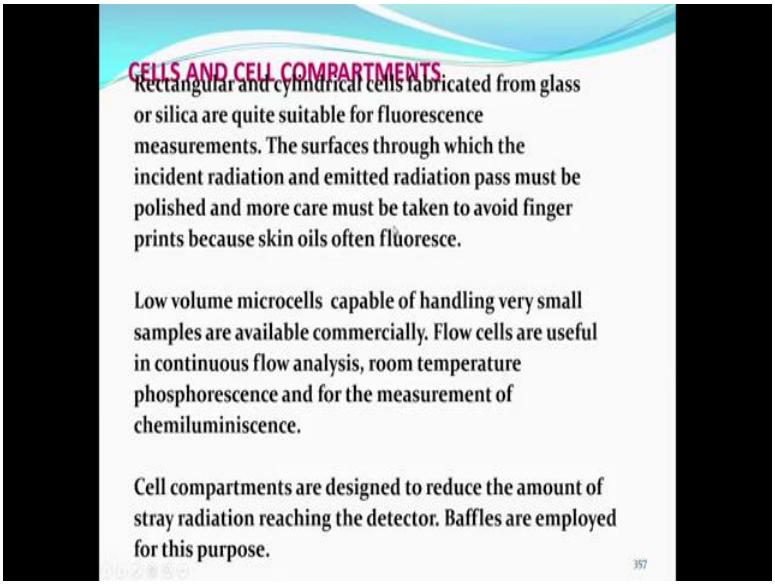

But here, in this case the adjacent sides of the cells are polished, and the remaining two sides are not polish sometimes you will also come across the cells which I have where the sample where the sample radiation bottom of the cuvette is polished to collect the fluorescing radiation. So, we had to take care that whenever we are handling the cells for fluorescence. So that we should not leave any mark on that, and low volume microcells are capable of handling very small samples they are available commercially, we can even

use flow cells that are useful in continuous flow measurements and then room temperature phosphorescence and measurement of chemiluminescence illuminations etcetera all these low volume microcells are quite capable.

So, cell compartments are also need to be designed slightly differently compared to the spectrophotometer and so that the radiation reaches the detector properly, and sometimes we employ baffles sometimes we need to avoid all the spray radiation generated from the box sample box as well as from the stray radiation generated from the excitation processes.

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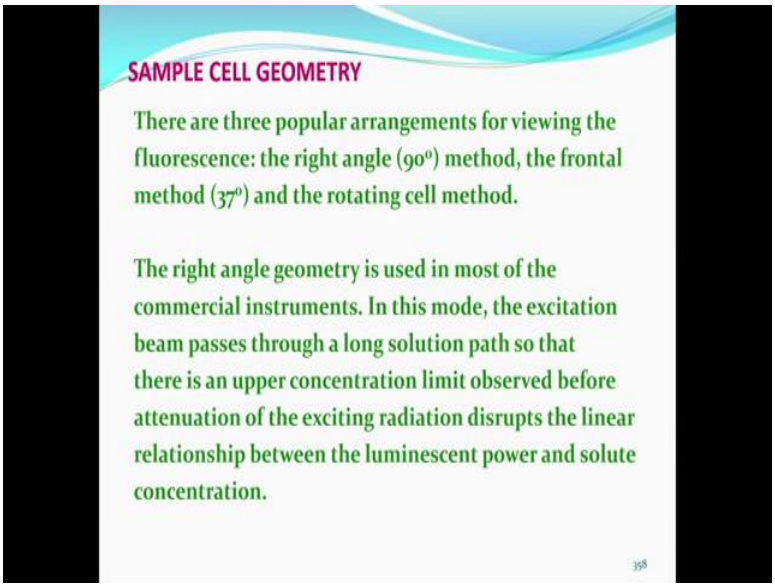

So, sample cell geometry approximately is something like what I have already told you the right angle geometry is used in most of the commercial instruments. So, excitation pass beam passes through a long solution path, so that there is an upper concentration limit observed before attenuation of the exciting radiation that disrupts the linear relationship between the luminescent power and the solute concentration.

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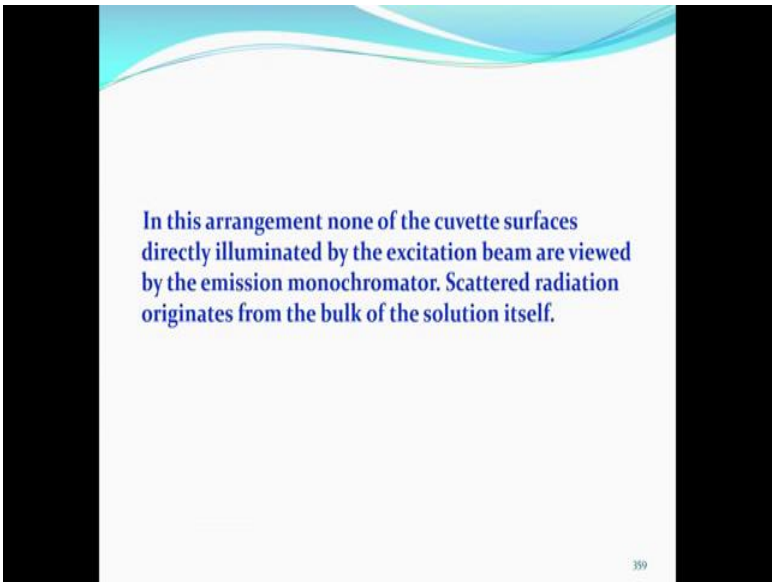

So, in these arrangements some none of the cuvette surfaces directly illuminated by the excitation beams are viewed as I have told been telling you repeatedly. So, scattered radiation originates from the bulk of the solution itself therefore, it is very important for us to determine the solid angle geometry; it is the important for us to measure it at right angles as well as fix the slit to an optimum level depending upon the concentration of the substance which we want to measure.

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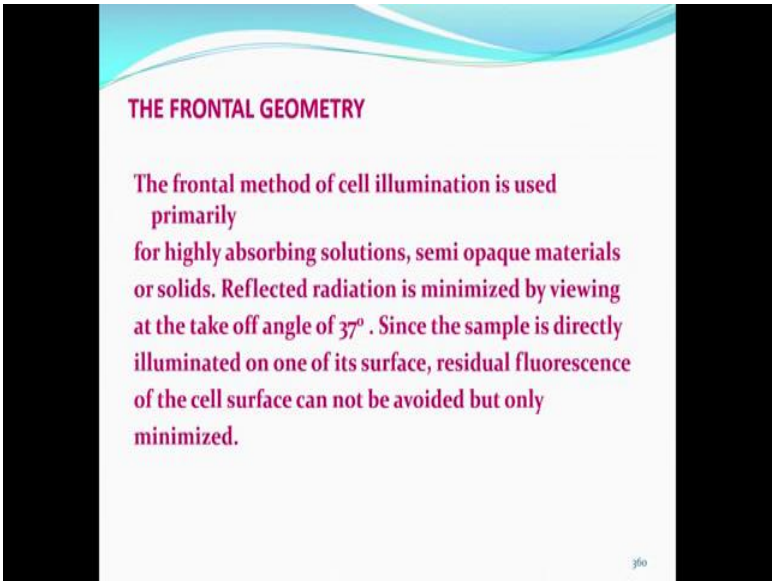

So, in the frontal geometry, there is a cell elimination itself is used primarily for highly absorbing solutions and semi opaque materials or solids also.

So, reflected radiation is minimized by viewing at the take of angle of 37 degrees, since the sample is directly illuminated on one of its surface residual florescence cannot be avoided, but only it can only be minimized. So, this automatically places some sort of a restriction on the major florescence measurement.

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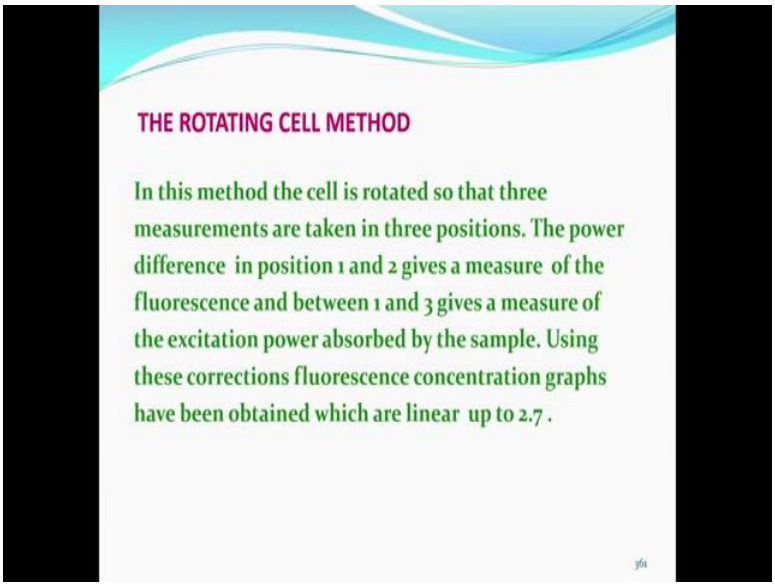

Sometimes to avoid this, what we do is we rotate the cells. So, in the rotating cells what we do is the three measurements are taken in three positions. So, the power difference in position one and two gives a measure of the florescence, and between one and three gives the excitation power absorbed by the sample. So, these corrections will help increase the order of concentration through which the linearity can be increased by up to 2.7 order almost 3.

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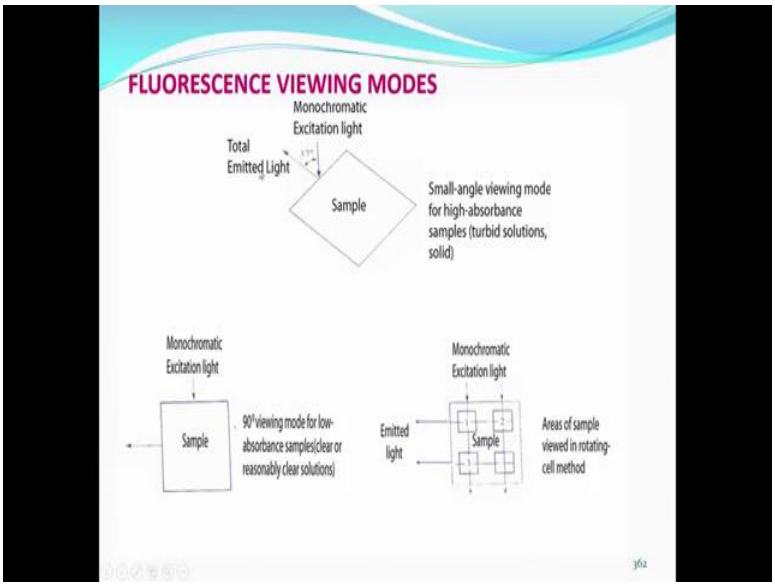

So, this is a fluorescent these are the fluorescents viewing modes on the top I have shown you total emitted light this is viewing angle 37 degrees, and this is a small angle viewing mode if I place it here and then in 90 degrees viewing mode this is the sample schematic description, and here it is monochromatic excitation 1,2,3,4 and here this is the emitted light and these are areas of sample viewed by the rotating cells. So, 1, 2, 3 are the preferred depositions. So, all this can be adjusted using the sample compartment liver.

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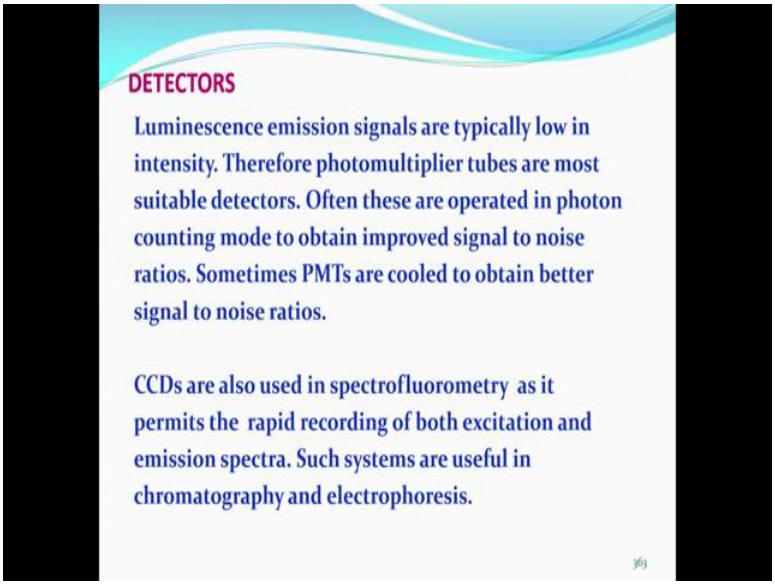

So, we come to the detector, basically detectors are essentially same there is not much to be told about the detectors because photomultiplier tubes are most suitable detector, so far in florescence as well as absorbance or in many other instruments also.

Often these are operated in photon counting mode to obtain improved signal to noise ratio. Sometimes PMTs are cooled to obtain signal to noise ra better signal to noise ratio. So, they are also you CCD is also used, in spectrofluorometry as it permits the rapid recording of both excitation and emission. So, for such systems what we need is a quick scanning method. So, they are useful in chromatography as well as in electrophoresis. So, in chromatography the detector part is a fluorescence detector basically, and it electro fluoresces also once the compounds are separated from the mixtures, they can reach the spectrofluorometry detector if the substance is fluoresce.

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So, data handling again we have a standard correction for blank, and corrected excitation corrected emission spectra all these things we had discussed earlier, and then we can also have something like difference and derivative spectra peak detection and processing deconvolution, 3 dimensional luminescence.

So, many possibilities are there whenever we are handling the data. So, calibration plots can be obtained on the computer itself I can do some curve fitting and then statistical parameters I can handle, and then smoothing of the spectra and many other features that

including kinetic measurements etcetera can be performed, if I have a good spectrofluorometer.

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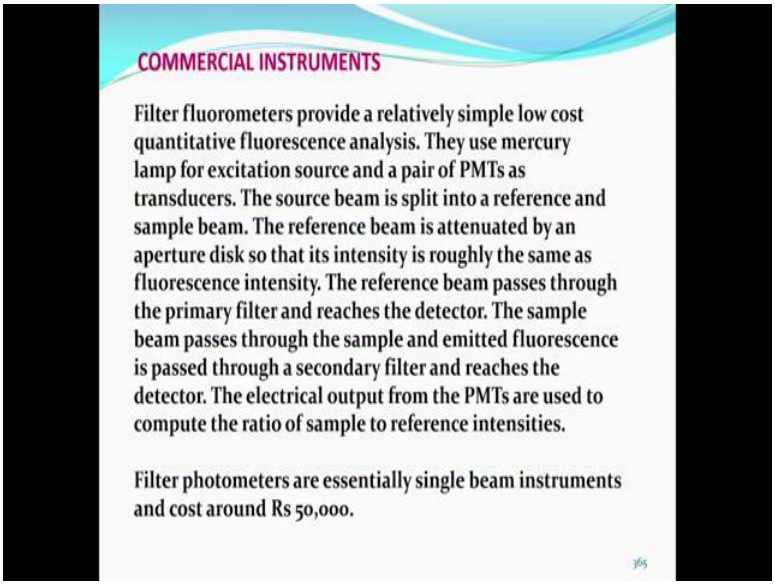

So, among the commercial instruments normally whenever I have to talk about a commercial instrument, the idea is to get an fluorescence spectra corresponding to the corresponding excitation and corresponding emission spectra.

If the work required is of very high standard, then we have to go for spectrofluorometer with the monochromators or gratings any one of those things we will do, and for less in the quantifying for less exact work I can go for a mercury for filter photometer of fluorescence meters and a pair of PMT is will be good enough for the requirement. Normally reference beam is attenuated by an aperture disk that also can be adjusted manually depending upon the requirement. The reference beam normally passes through the primary filter and reaches the detector and the sample beam passes through the sample and emitted fluorescence is collected at right angles etcetera, and the electrical output from the PMT are normally used to compute the ratio of the sample to the reference intensities.

So, if we take a look at the instrumentation essentially you will find that filter photometers are essentially single beam instruments, and these are for routine commercial purposes that is where routine analysis is done may be in industries or something like that, where organic dyes and organic compounds are processed and which

are used for quality control and production purposes. But if you want to more exact work then you should go for spectrofluorometers.

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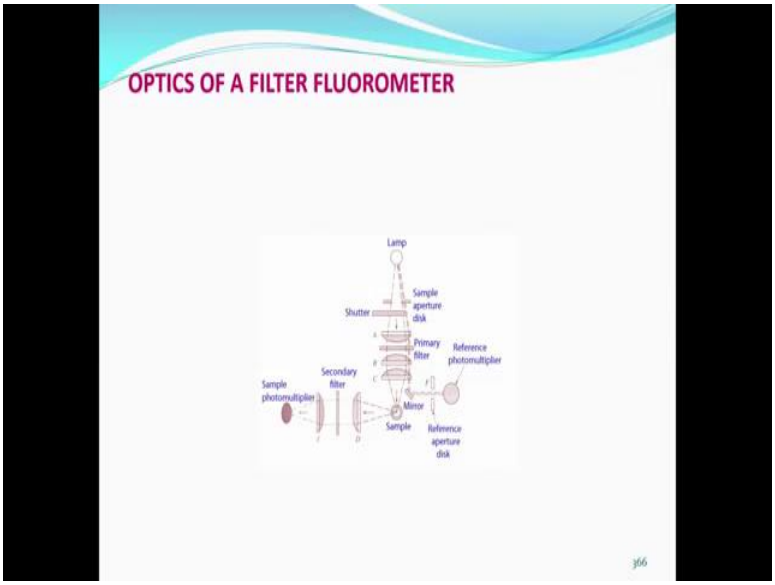

So, these are the optics of a sink filter fluorometer what I have here is I have a lamp and then radiation is there sample aperture, I have a sample and then this get etcetera primary filter and then I have the mirror and then sample is there, then at right angles I have the collection secondary filter and this gets etcetera and photomultiplier tube that is the detector.

Here I have a reference photomultiplier. So, I place a mirror here and take it there and take the ratio alternately that is using an ac current.

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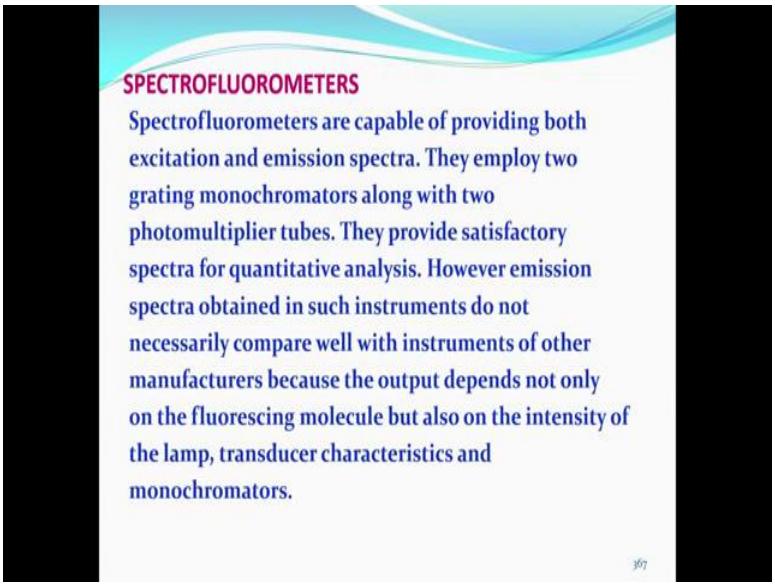

So, fairly simple schematic diagram and spectrofluorometers are capable of providing both excitation and emission spectra separately also, the employ two grating monochromators and two multiplier photo photomultiplier tubes, they provide satisfactory spectra for quantitative analysis, especially if you want to know the water quality or water flow in a river spectrofluorometer is best way, because what we do is we put a solution of a fluorescent material in the river and then collect the sample down the river and see what is the fluorescence intensity change over a distance and you can compute the flow of the water and quantity of the water that is flowing etcetera depending upon the dilution of the fluorescing material.

So, such instruments do not normally compare well with instruments of other manufacturers, because output depends not only and the fluorescing molecule, but also on the intensity of the lamp and transducer characteristics etcetera. Most of the fluorescence dyes are used for such purposes for example, rhodamine 6 g, rhodamine b and fluorescent quinine sulfate such compounds are very easily fluorescing and there are also compounds like amino compounds, azo dyes several hundreds and thousands of compounds which are fluorescing can be monitors by using simple fluorescence spectrometers.

So, spectra obtained in such instruments are referred as uncorrected spectra. So, they can still be used for rough laboratory purposes.

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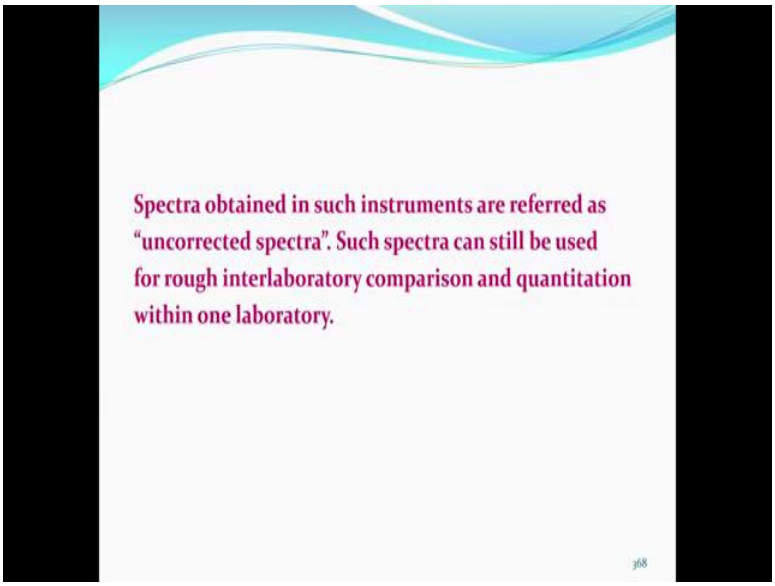

But, the thing is it you can be used only for quantification or comparison not more than that not high grade scientific work.

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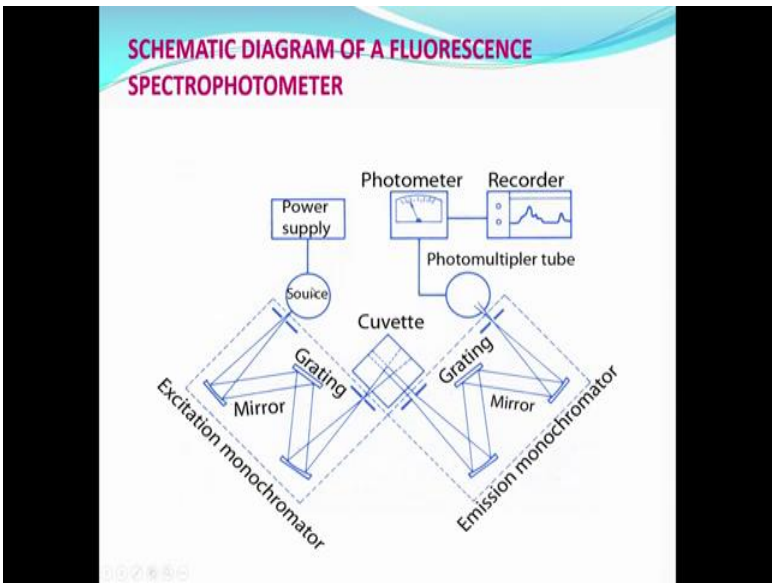

So, schematic diagram of a fluorescing spectrophotometer I am showing here it is a very simple system again this is a source power supply source and excitation there are couple of mirrors and gratings, and then I have a cuvette another collection mirror here and then one more grating this is for you can see that again this is perpendicular, and then another mirror photomultiplier tube and photometer and recorder and associated optics.

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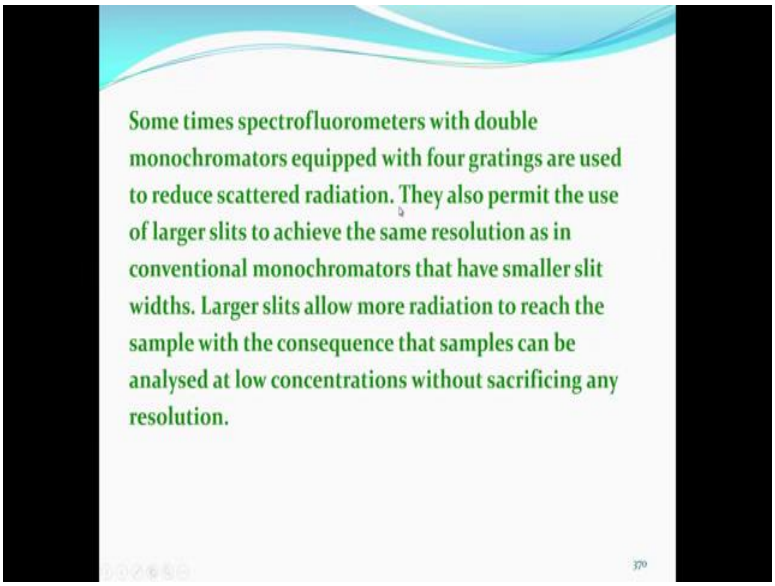

So, this is the schematic diagram of a simple for spectrophotofluorometer. So, double monochromators are also equipped with about four gratings, and they also permit the use of larger slits to achieve the same resolution. So, larger slits normally allow more radiation to reach the sample; with the consequence that sample can be analyzed at very low concentrations without sacrificing any resolution.

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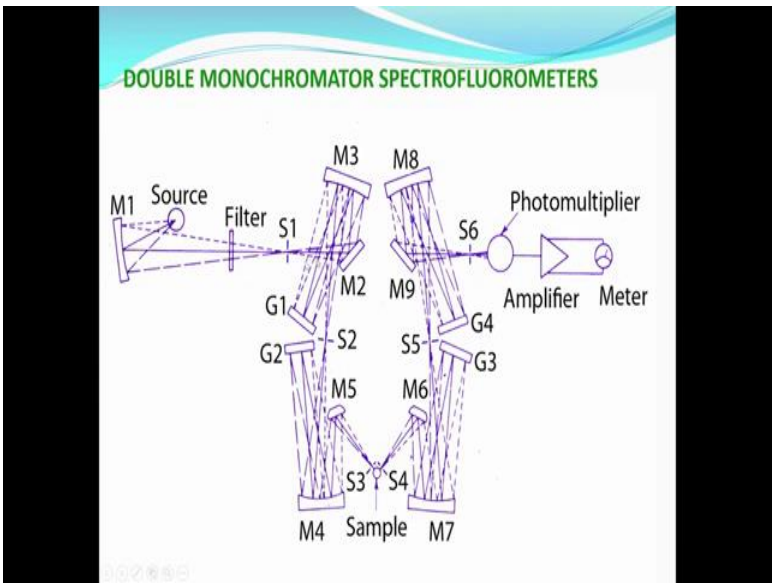

So, double monochromator is slightly more complicated, but it is not so complicated that you cannot understand. See it is all very simple here if I you follow the cursor in the on

the screen first it is source, mirror then to render them parallel, and then a filter one slit and then more couple of mirrors and then sample, then collect it right triangles and again I have go through similar procedure, and then the final slit and photomultiplier amplifier tube.

Very simple basically, but the design is complicated by the use of number of mirrors and the gratings that is all the difference.

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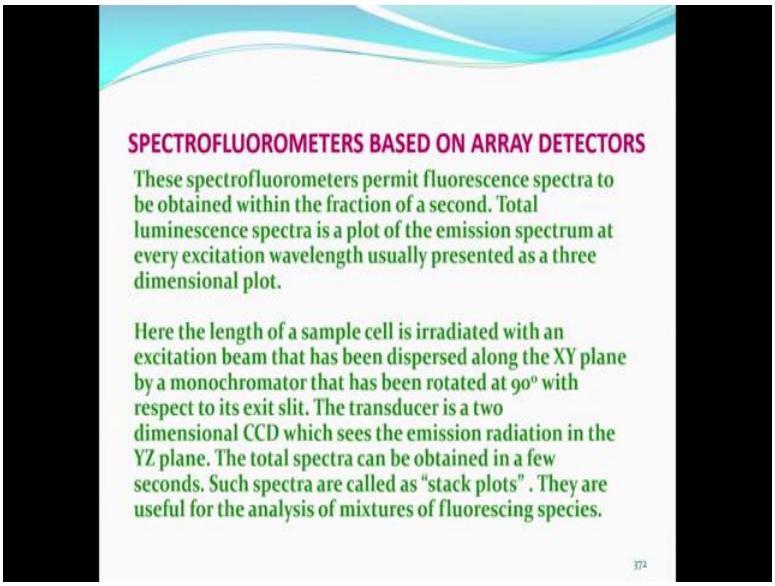

So, we also have couple of spectrofluorometers based on array detectors, which permit fluorescence spectra to be obtained simultaneously at different wavelengths. So, this is a fairly good advance because I do not have to scan it through number by number wavelengths. So, all of it can be done in one shot and at a fraction of a second the whole spectra is obtained. So, that if the two substances are decomposing or something like that we can always take a look at how the sample spectra can be obtained before it decomposes. So, the length of a sample cell is irradiated with an excitation beam that has been dispersed along the x y plane by monochromator that is rotated at 90 degree as usual.

There is nothing very special difference about this, but the total spectra can be obtained we can obtain number of spectras, and such spectras are called as stack plots. Stack plots are useful for the analysis of mixture of fluorescing species, because if there are more than one there will be some movement of difference between the each stack.

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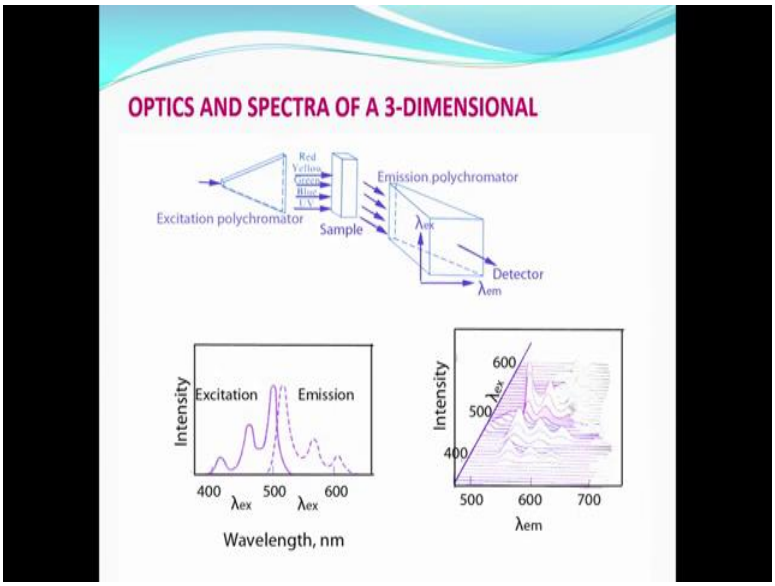

So, we can also go for three dimensional fluorescence spectrophotometers, and the schematic diagram I am showing you here and 3-D spectra are obtained are shown here and we can see that they have a particular usefulness in special sub circumstances not for routine analysis ok.

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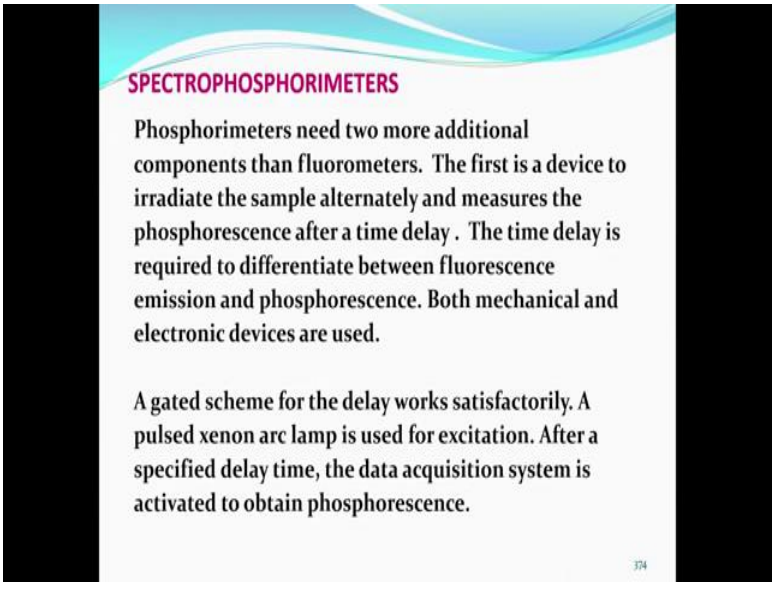

So, now I move on to spectrophosphorimeters; there is not much to tell with respect to spectrophosphorimeters, because essentially the only difference between fluorescence and phosphorescence is the delay in the measurement, because of the delay in the return

to the ground state. So, the first ray is a device what I need is the device to irradiate the sample and measure the phosphorescence after a time delay.

So, there has to be a time delay component included in the instrument. So, this is required to differentiate between fluorescence and phosphorescence. So, if there is no time delay fluorescence is good enough, but if there is a time delay and separate spectrum for phosphorescence, I need to introduce a time delay. So, both mechanical and electronic devices can be employed. So, a grated scheme for the delay works satisfactorily. So, a pulsed then on lamp is used for excitation all those things are fairly should be fairly clear to you now, and data acquisition system must be activated after a time delay to obtain phosphorescence.

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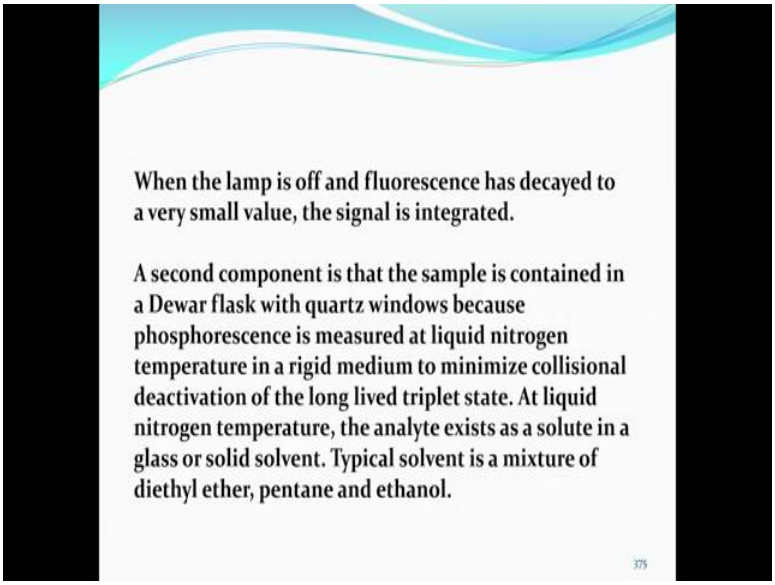

So, the when the lamp is off and fluorescence has decayed to very small volume value we generate one signal and second component is that the sample is contained in a Dewar flask with quartz windows because phosphorescence is measured at liquid nitrogen temperature, it does not happen at room temperature. So, I need a device which where I can hold the sample like liquid nitrogen temperature, so that is a slight instrumentation complication that is required in such cases. So, the here the analyte exists as a solute in a glass or solid solvent, in a glassy form or solid solvent and special solvent is a mixture of diethyl ether pentane and ethanol and several others solvents can be used for phosphorescence.

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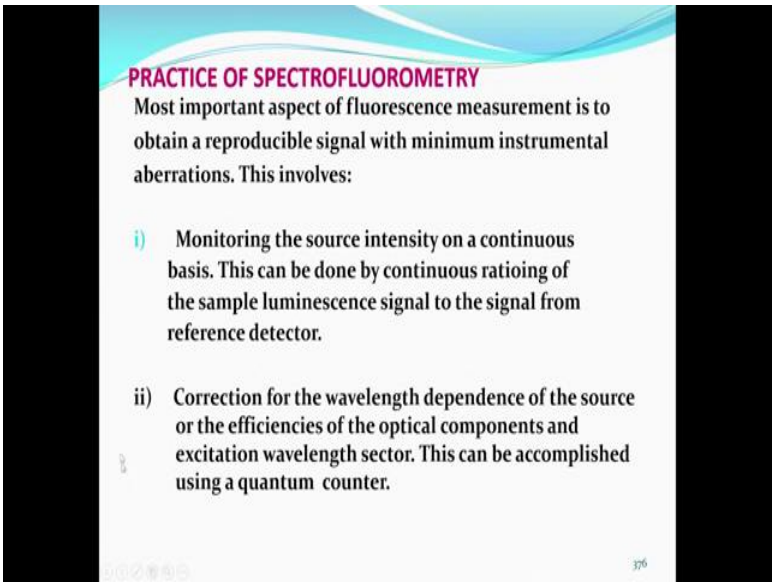

So, whenever we want to practice spectrofluorimetry what I need to do is I have to optimize the things monitoring the source intensity, this can be done by continuous rationing of the sample luminescence to the signal from reference detector, and then I have to correct for the wavelength dependence of the source or the efficiencies, this I can do it by using a quantum counter.

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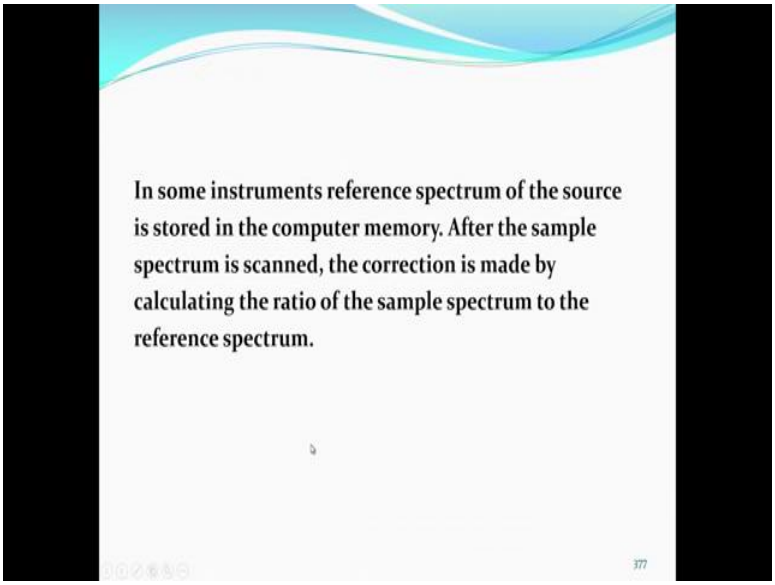

And then in some instruments is reference spectrum of the. So, source is stored in the computer memory, you know all electronic advances make the job very simple for

routine job routine performances. So, after the sample spectrum is scanned the correction is made automatically by calculating the ratio of the sample spectrum to the reference spectrum.

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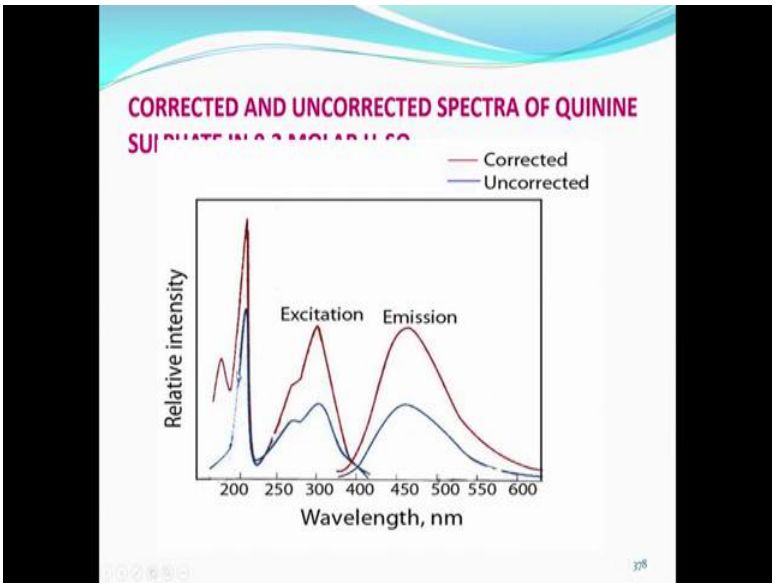

So, here I am showing you the corrected and uncorrected spectrum, here it is excitation and then this is the emission and corrected spectrum are having are shown in the red, uncorrected spectrum are shown in the blue form.

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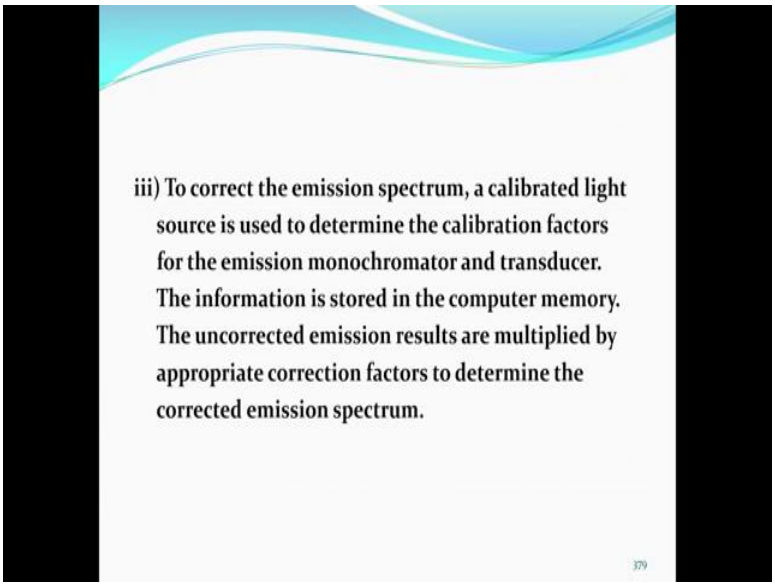

So, we to correct the emission system a calibrated light for the emission monochromator and transducer is required. So, the source information is again stored in the computer memory, uncorrected signals are multiplied by a factor appropriate to the correction and then it is determined to correct the emission spectrum also, same thing can be applied to the emission spectrum.

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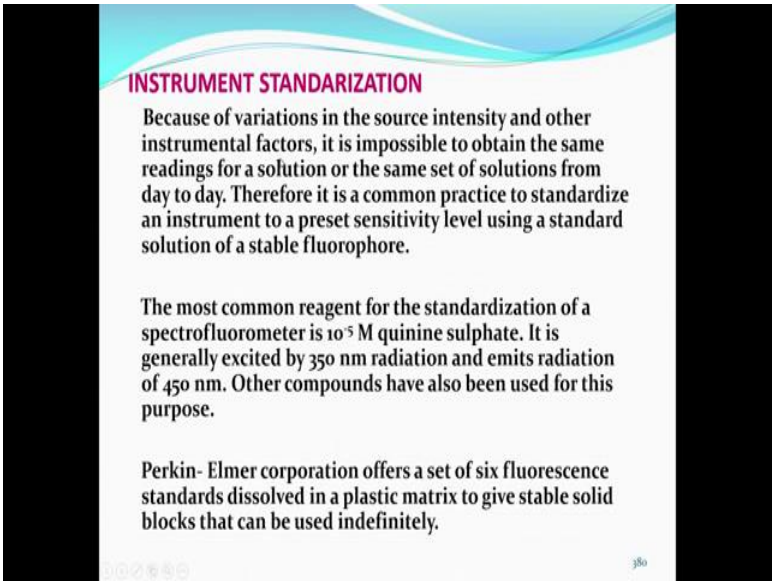

So, the instrumentation standardization is because of the variations in the source intensity all these instrumentation needs to be standardized, it is impossible to obtain same readings for the solution of the same set as usual. So, it is we have to standardize the instrument and the most useful reagent for standardization of a spectrofluorometer is 10 raise to minus 5 molar quinine sulfate.

It is generally excited at 350 nanometer and emits radiation of 450 nanometer; whenever you by a compound whenever you by a spectrophotofluorometer the supplier will give you a standard cuvette also nowadays, which is fluoresce quinine sulfate solution in a sealed cell or it can be dispersed in a glass and made into a cuvette and once that that is available you can standardize all the wavelengths etcetera using the quinine sulfate standard. Sometimes you can make your own standard also of quinine sulfate it is a very easily available across the shelf, and you can always go to the reference whenever you publish a paper or something like that, whenever you want to validate a data what you should do is you must always compare it with a standard quinine sulfate solution.

So, other compounds also I have been used for this purpose, but quinine sulfate seems to have acquired the favor of most of the analyst. So, Perkin Elmer corporation what they do is they offer a set of 6 fluorescence standards, result in a plastic matrix different wavelengths. So, it gives stable solid blocks that can be used in definitely. So, once you have that it is easier to standardize the instrument, and if they you do not get the standard value what you should do is increase the slit of the emission or excitation to get the some same standard value, so that the results can be reported corresponding to a particular standard this is very important in all fluorescence and phosphorescence methods.

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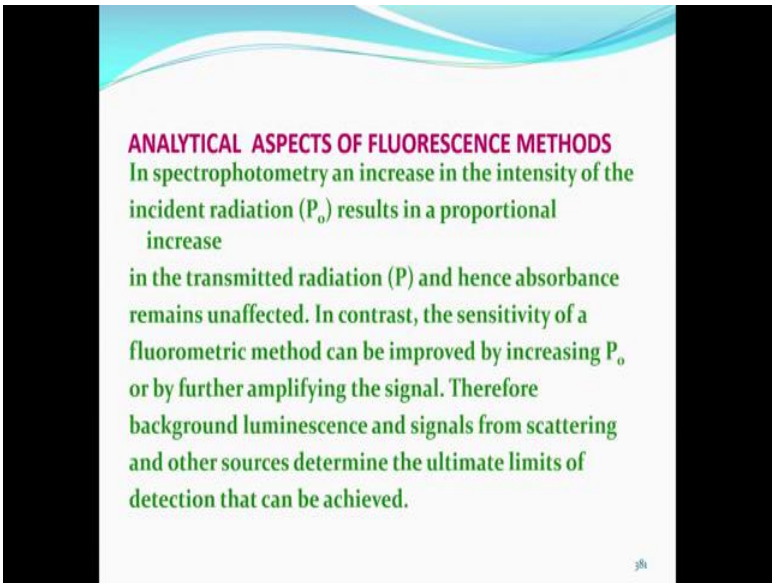

So, in analytical aspects of fluorescence because I have been telling you that in most of the fluorescence equipment are available as a modification or a special module for a spectrophotometer. We can increase the intensity of the incident radiation resulting in a proportional increase in the transmitted radiation also, but absorbance remains unaffected because it is only the difference between the initial and final.

So, the sensitivity of a fluorometric method can be improved by increasing P naught or by a further amply amplifying the signal. So, background luminescence signals from scattering and other sources determine the ultimate limits. So, this point also I have explained to you a quite a few times, and the precision is of course, limited by the source flicker noise and drift.

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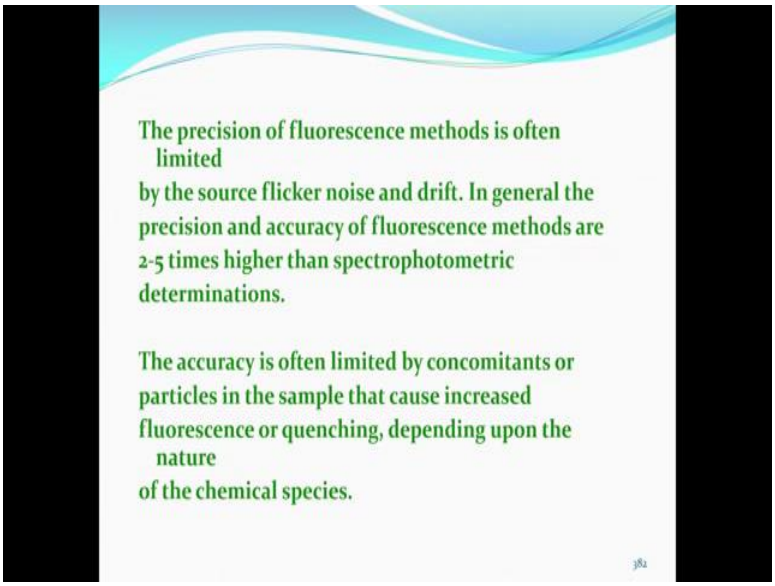

So, if you want to go to very lower level of detection and determination, the flicker noise and drift becomes more important. Normally we go for three times the noise as a standard for difference in the measurement. So, if the noise itself is about 3 sigma, then we cannot have it have a signal that corresponds to that corresponds to the sample. So, anything higher than three sigma yes, then it is possible for us to consider it has a specific signal. In general the precision and accuracy of fluorescence are 2 to 5 times higher than spectrofluorometrics spectrophotometric methods.

So, the accuracy is always dependent upon the particle sizes that are present in the sample. So, it is very important for us to have a very clear solution, probably you should use the 0.45 micron filter to remove all the solid particles, otherwise quenching to may take place and then sometimes the scattering also may play a very important role in such systems.

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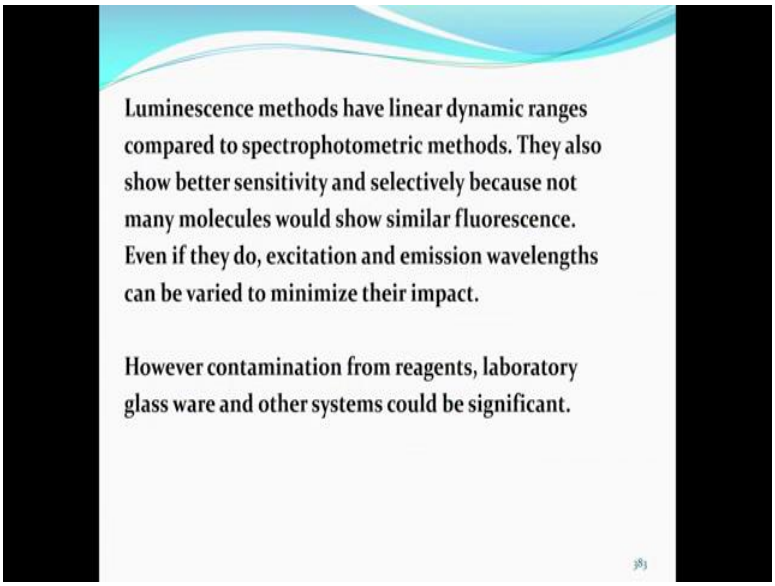

So, luminescence methods normally as I have already told you that they have linear dynamic ranges compared to spectrophotometric methods. They also show better sensitivity and selectivity, because not many molecules would show similar fluorescence this is very important. So, most of the fluorescence methods become almost specific unlike the spectrophotometric methods; so even if they show similar fluorescence sometimes the excitation and emission wavelengths can be varied to minimize their impact; that means, the interference of substances which can affect the accuracy of analysis, can always be minimized if I choose some other emission wavelength.

Again it there may not one or two wavelengths it may match, but it would not match definitely the whole spectrum. So, the limit of determination is always from the contamination of the reagents, laboratory glassware and other systems that becomes more important. Noise, electrical systems and then signal strength and then reagents laboratory glass and other systems of the other systems means concomitant matrix components.

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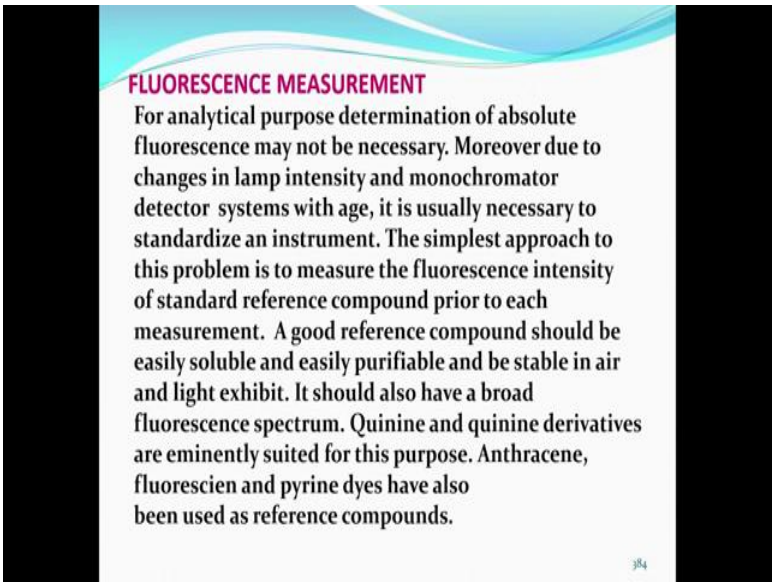

So, fluorescence measurement we will normally what we do is the whenever we want to make a measurement, there will be some amount of change because of the lamp aging. So, it is necessary to standardize the instrument, simplest approach is to go for standard reference material which I had already told you which is a 0.1 molar quinine sulfate.

So, a good reference compound should be easily soluble and easily purifiable. So, for this quinine and quinine derivatives are eminently suited for this purpose, other dyes are Anthracene, fluorescein and pyrene dyes they have also been used as reference compounds.

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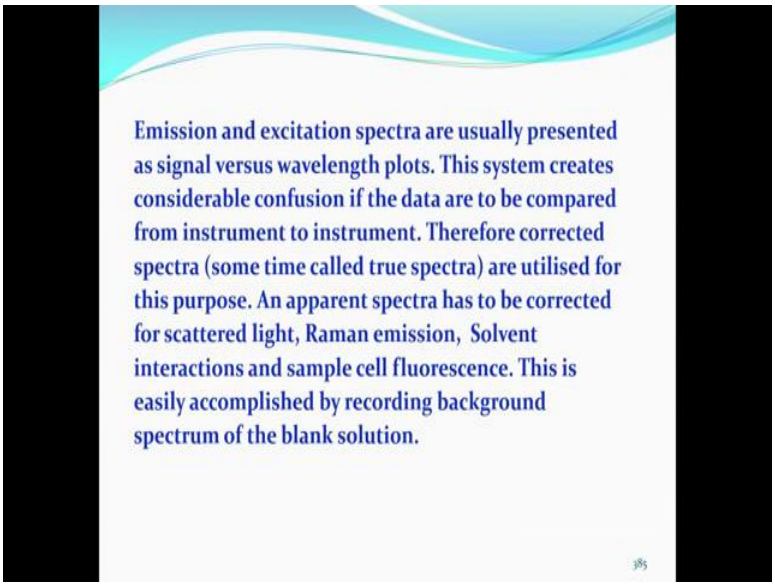

So, we will stop here, and then continue our discussion about how to give a finish to a fluorescence method and then spend some time on chemiluminescence methods, because that is also part of the environmental monitoring system nowadays, and we will definitely move on to the actual analytical methods for the determination of pollutants using these absorbance methods.

Thank you very much, we will continue our discussion in the next class have a good day.