Atomic and Molecular Absorption Spectrometry for Pollution Monitoring

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Lecture – 16
UV-Visible spectrophotometry, instrumentation-IV

We continue where we left of; that is now I want to discuss with you about the commercial instruments that are available in the country. Basically spectrophotometry is the low cost instrument. And it gives you an unlimited number of opportunities to determine all kinds of chemical with wide ranging applications. So, they are available you can by a spectrophotometer or colorimeter or something across the shelf, they are available.

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So, you should know more about the commercial instruments. Of course, lot of information is available in on google and internet, but still some basic information you are required to know.

So, number of calorimeter are available commercially their price is range from several hundred to several lakhs, depending on the type of analysis and sophistication. At the low end are filter photometers colour comparators and at the higher end are UV visible

near infrared spectrophotometers fitted with computer controlled instrument operations capability to hand the solids colours reflectance fluorescence etcetera.

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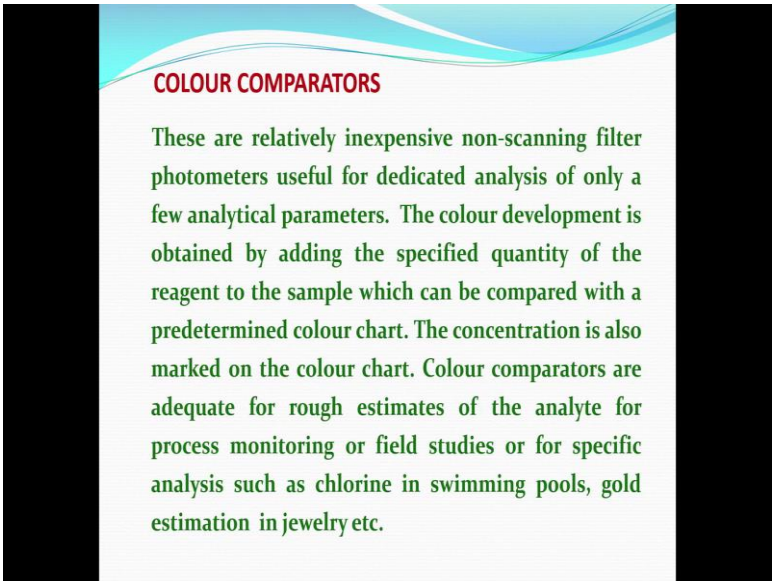

So, colour comparators are the very low end instruments. Basically they compete with our eyes. So, if you can prepare a series of standard solutions and look at their colour and then take an unknown, and if the colour falls in the range you yourself can approximately estimate the concentration depending upon your experience.

So, do you to you convert it into some sort of an instrument like measurement of colour with barrier layers' cell and small led and the simple filter glass filter or something like that, they become the low end instruments. So, the colour development, these are now essentially non-scanning filter photometer; that means, they will give a filter fixed wave length measurement may be some for blue some for red some for green some for yellow some for orange like that. And you make different kind of filters for each.

So, the colour development is obtained by adding the specific quantity of the reagent to the sample which can be compared with a predetermined colour chart also you can use. So, colour these are called as colour comparators. They are accurate adequate for rough estimates of the analyte concentration these are useful for process monitoring or field studies in you where you do not have to get very accurate analysis, but quite often we need pass or fail test. So, specific analysis, so for example, include in a swimming pool

somebody wants to determine chlorine, and how much is the residual chlorine. And somebody wants to determine how much of gold is there in jewellery.

So, all these things are very important, but at the same time you do not need a very accurate analysis.

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So, for such purposes colour comparators are useful. And these are the type of colour comparators. We can see the black box contains 2 cells which I have shown in the right side and then the filters are fixed inside at the bottom in a round plate these are the different colours which will give you specific wavelength absorbance. And these are the type of reagent which are kept in the form a tablet which you can dissolve, and then determine these are very low end and not meant for scientists.

You know for routine chemical operators in swimming pool or in a jewellery shop etcetera people use these things. And of course, they are very useful for quick and rough estimate. They approximate cost of such an analysis would be about 75 to 100 rupees, but if you know more about the scientific things you can reduce the cost to about 20 to 30 rupees if you know the chemistry of the systems.

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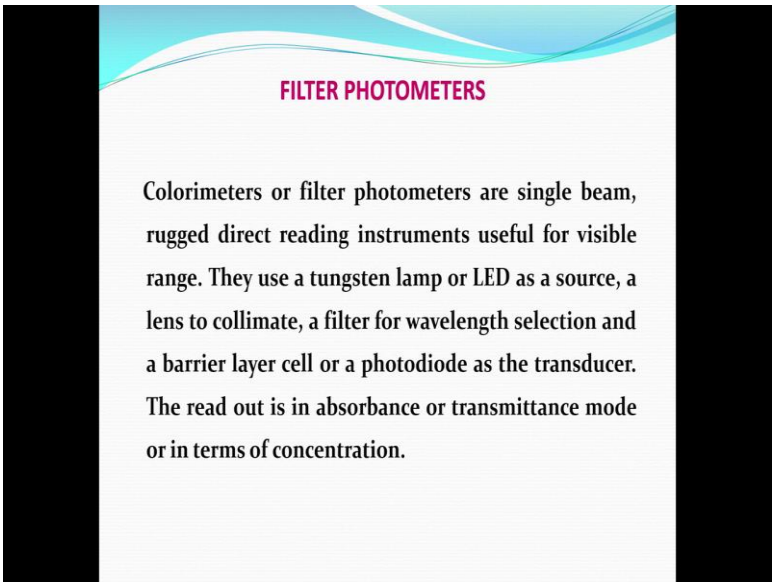

So, next higher end is filter photometers. So, filter photometers are useful for visible range that is 350 to 700 approximate ranges. Sometimes you may see 750 or 7-800 something like that. And filter photometers are essentially the same things what we have told using interference filters or glass filters and things like things like that presumes glass presumes because filter photometers are normally meant for visible. So, glass presumes or glass grating will do. So, it is either fitted with a barrier layer cell or photodiode cell as the transducer. You would not find photo multiplier tube in photo filter photometers.

Their also known as colorimeters. The wave length range would be approximately plus or minus 5 nano meters in such instruments. So, but the read outs read out also is quite either absorbance or transmittance mode or in terms of concentration in recent equipment. Sometimes theses most of the recent instruments they had concentration as a third parameter for determining waves, which avoids the requirements of a calibration every time or calculation for the concentration. So, these are such instruments will cost you about 20 to 25 thousand rupees and may be around fifty thousand rupees depending upon the sophistication.

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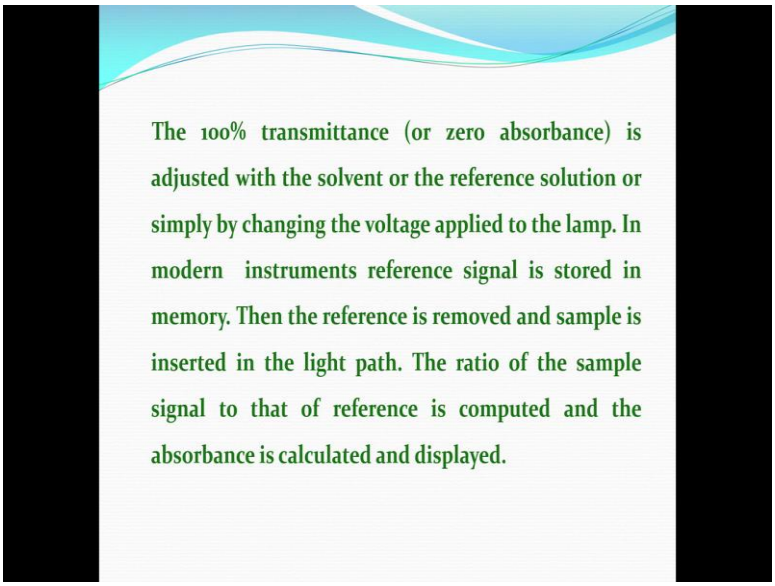

But they are useful for the low end laboratories where there is no requirement of the ultra violet measurements. So, they read out scale could be analogue or digital. So, in the analogue scale 100 percent transmittance is adjusted with the 0 percent absorbance. And 0 percent transmittance is absorbed it is adjusted with absorbance of 2. That is low negative log of log hundred is 2. So, the maximum absorbance is always 2 in most of the spectrophotometric measurements. So, by simply the 100 times percent transmittance for a reference you have to adjust with it either with the solvent or reference solution, but also by simply changing the voltage applied to the lamp. So, in modern instruments a reference signal is stored in memory.

So, they will give you only one slot for measurement of the system. So, the reference is always stored in the memory you just make your solutions and make the measurements and it will be compared with the store memory and then the calibration is computed and the absorbance is calculated and this displayed either as absorbance or transmittance or in concentration terms.

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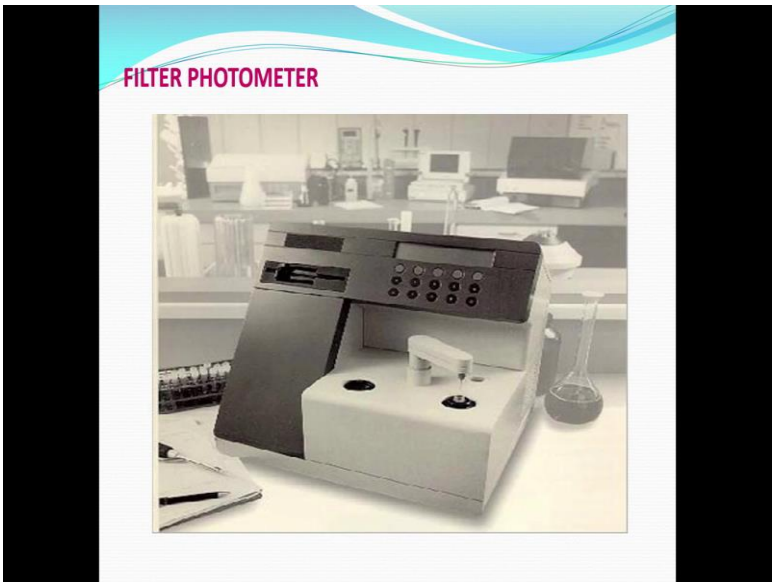

So, this is a some very popular filter photometer. Here you can see 2 slots one for reference one for sample. And then all other things are hidden in a box. And the sample compartment is somewhere here. And then the read out scale is here as electronics and optics is in this white box. And that also houses the radiation cell that is tungsten lamp or whatever it is in most of the filer photometers tungsten lamp only is only being used now a day there may be using led lights with white spectrum.

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So, this is a led based colorimeter pocket colorimeter, all you have to do is put your sample here in the middle and then measure the absorbance and it will show you as a readymade this thing. So, obtusely not because of the complicity complexity, but such things do cost quite a lot in the range of about 15 to 20 thousand rupees depending upon the requirements.

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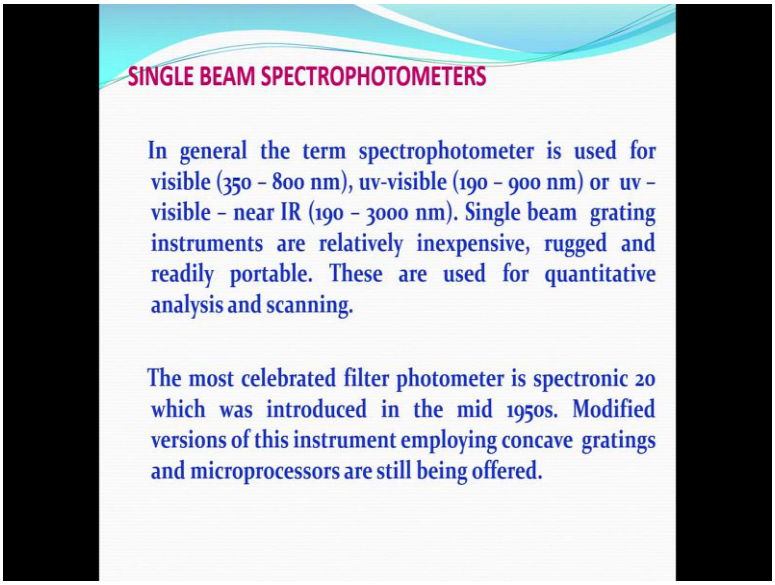

So, now we go to single beam spectrophotometers. So, what is main difference between spectrophotometer and a filter photometer? So, filter photometer is used for your visible range only. And spectrophotometer is used for a system which measures UV visible, ultra violet visible and or and slash or near IR also. That is 190 to 3000 nanometres that is the whole range. So, single beam grating instruments are normally employed here. Because as I have explained you earlier gratings can provide inexpensive rugged and readily make them readily portable.

These are used for in quantitative analysis. The most celebrated filter spectrophotometer filter photometer is spectranic 20, which the model is available even now and modified version of the same thing is available in at least of about 50 to 75 models available it is an originated origin originates somewhere around mid-50s, and modified versions of this instruments are available employing concave gratings etcetera are still being offered.

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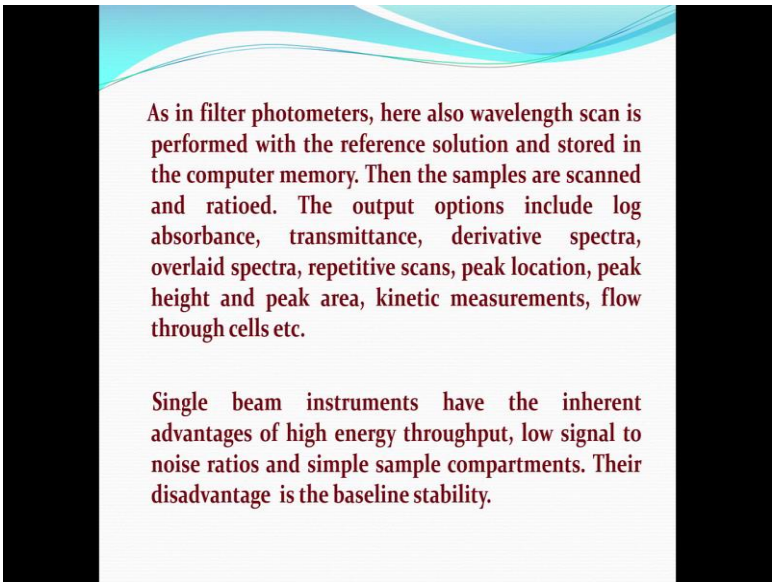

So, as in filter photometers here also wavelength scan is performed with the reference solution and stored in the computer memory.

The samples are scanned and ratioed the output options include log of absorbance logarithm transmittance derivative spectra overlaid spectra, repetitive scan you can do peak locations automatic search, peak height peak area kinetic measurements flow through cell, several kinds of the measurements can be made using such instruments. Single beam instruments have obtusely had the advantage of the high energy throughput because we do not have to remove the single cell the incoming radiation, the incoming radiation is coming in the in a single beam and in double beam I make it into 2 beams, one for reference one for sample. See if I have a single beam the intensity will be more. So, a single beam instruments always the sellers of single beam instruments always claim their instruments gives very high sensitivity, which is not really good because the intensity of measurement is always half halt in the refer in such cells.

So, the disadvantage is base line sensitivity. What happens is whenever I am doing some sorts of a measure voltage meant, there is always certain amount of drift the initial baseline is should be as study as possible, but if I store it in memory and in actual practice if there is an if there is a certain amount of certain amount of change in the electronics the baseline itself is shift, but that is not recorded in the memory because it is it is not an online continuous process. So, generally what we look for is continuous

online basement measurement along with the in original which is split into 2 at the cost of little bit of sensitivity. So, this is where the ingenuity of a wire is required exactly what is required in a given spectrophotometric cell.

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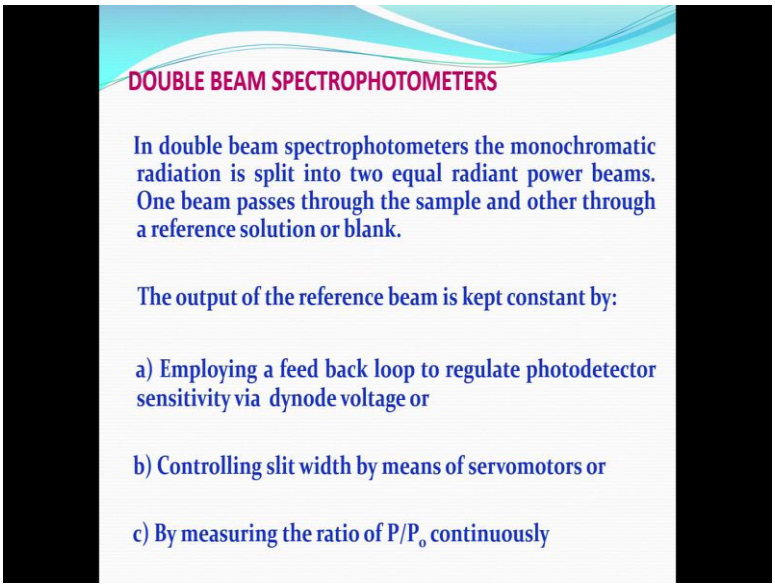

So, look at it like this, and then the double beam instruments are the next higher end which are the current state of the state of affair equipment. The range varies from 4 to 5 lakhs to 15 20 lakhs some instruments I have seen even up to 80 lakhs which can measure even very thin films of metals for reflectance absorbance etcetera. And then the output of the reference beam is kept constant, by employing a feedback loop to regulate photo detector; that means, any slide changing in the sensitivity is adjusted electronically for a fixed based baseline link strength.

So, employing a feedback loop to regulate photo detector via dynode voltage I can do that to or controlling the slit, I can control the slit itself by using a servo meter to increase or decrease the incoming light. And I can always measure the ratio, of the transmitted light and initial light in electromagnetic radiation continuously. These are some of the advantages for double beam instruments.

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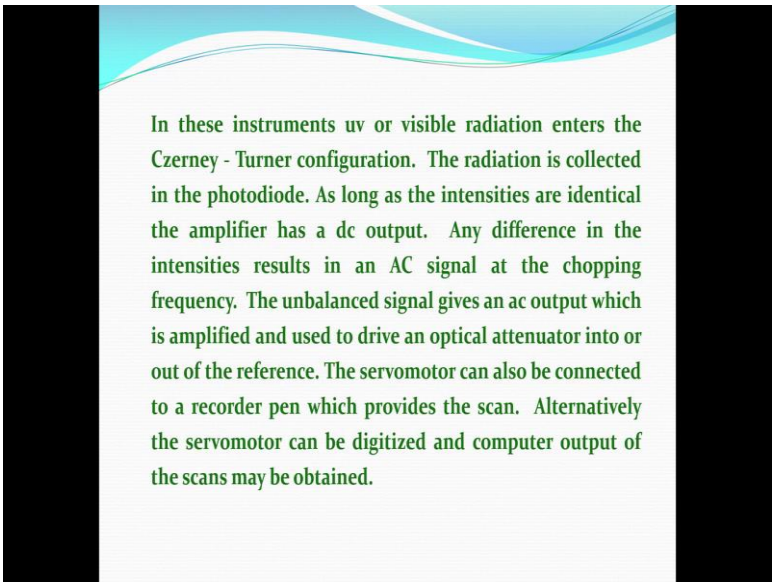

So, in these instruments UV or visible radiation enters the czerney turner configuration. 99- percent of the instruments which are coming in the market high end equipment use Czerny turner configuration which we have already discussed earlier. The radiation is collected in the photodiode. The given photo multiplier tubes are not. So, convenient as long as the intensities are identical the amplifier has a high DC output, but any difference in the intensities results in an AC signal. That is a big problem at the chopping frequency.

So, what happen is the unbalanced signals? It gives AC outputs which are amplified and used to drive an optical attenuator into or out of the reference beam self. So, the changes automatically occurring due to voltage fluctuation are taken care of, and they can also be used also be same thing can be achieved using servo motor connected to this connected to a recorder which provides scan also scanning and recording are automatic equipment automatically included in spectrophotometers, because the scanning can should be a continuous operation as far as possible. So, the servo motor also can digitise and computer output and etcetera excreta are quite possible in spectrophotometer that gives unlimited flexibility in the operation.

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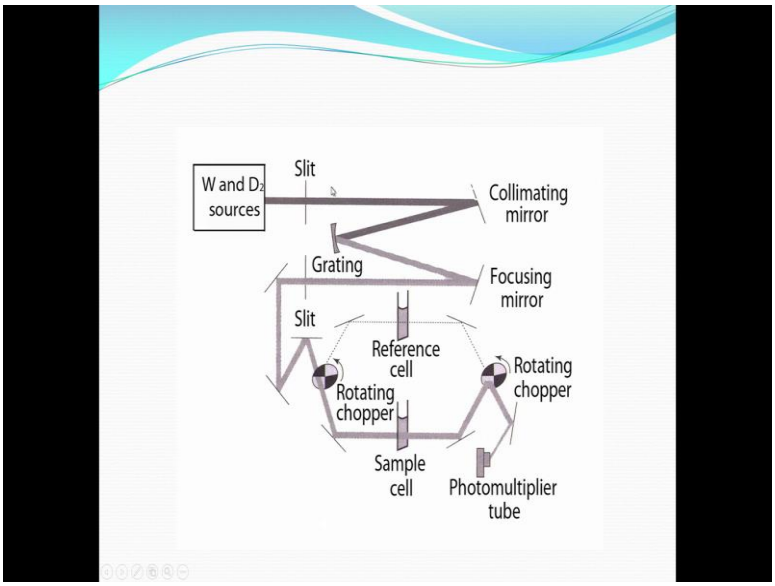

So, this is a kind of arrangement what we normally see in a spectrophotometer that is tungsten and deuterium source. And these gets alternated to 350 editorial source or then this whole thing rotates and tungsten comes to for the output is pass through slit a collimating mirror a grating a focusing mirror. And then a slit then rotating chopper this divides the incoming radiation in 2 one for reference one for sample and a chopper again and then photo multiplier tube and then to reader. This is only basically schematic diagram which you should be quite familiar.

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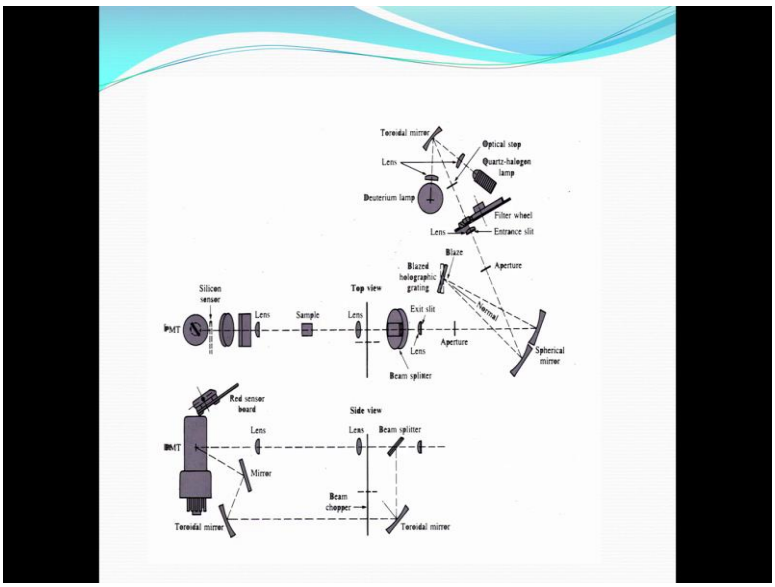

Now, actual instrument, you will see not just the number of in mirrors and other things which are I shown you in the earlier slide, but it will have at least about 20 to 30 additional mirrors concave mirrors convex mirrors and all other things mounted on an optical flat surface. So, that the signal handling and the optical movement of the electromagnetic radiation is ensured to reach the detector. Now it is very important that such an arrangement is a very delicate arrangement because sometimes the mirrors and other things are about 5 to 10 centimetre squares or concaveness etcetera; so 5 to 10 sorry millimetres not centimetres. So, 5 to 10 millimetres and the requirement of the radiation hitting the mirror a small mirror in a space is so, accurately required that it is almost impossible to replace the adjustment, if the due to any mechanical results the optical system is changed. So, what happens is in 99 percent of the instrument, the optical system is sealed from the user. So, if there is any requirement of the optical re alignment we will have to call this service engineer.

So, how do we know when to call a service engineer whether optical system is alright or not? That we do by changing taking a standard sample whose wave length lambda by x, is known whose wavelength is fixed. So, these are known as primary standards and in the primary standards potassium dichromate is one, potassium permanganate is one and there are several other chemicals which will give you a peak exactly at a known place and for known absorbance. So, if the movement there is also a holmium reference cell that is standard which you can insert take the spectrum, and compare a compare the spectrum to a standard one.

And if you see any changes it is time for you to call the service engineers normally the mirrors and lenses are never to be touched with our fingers. So, that is one of the requirement and here you can see in this figure how many pieces are there in lenses mirrors, and all other things are there you can marvel at the total number and the complexity of the system even though when we teach, or when we learn it does not look complicated, but instrumentation was becomes a very specialized subject with specific applications.

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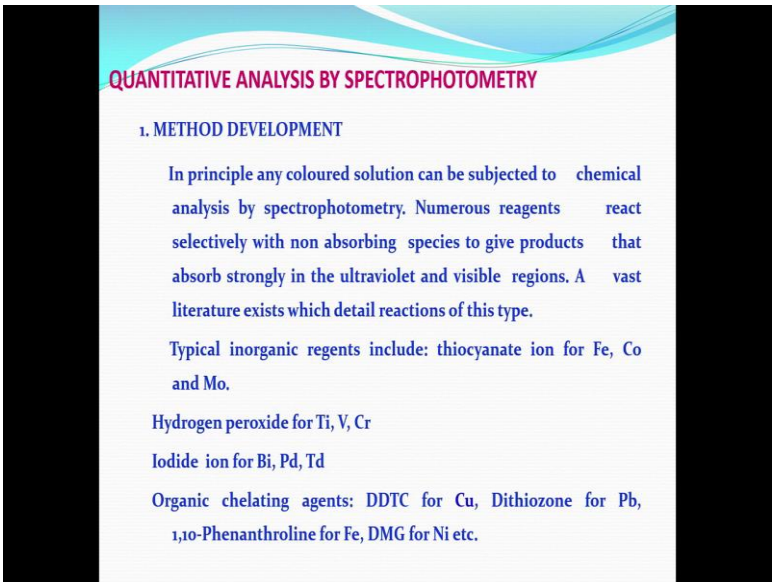

So, that is where I stop for the time being regarding the instrumentation.

Now, we go looking for what to do with spectrophotometry given a spectrophotometer. What all you can do with that is important. First things are to find out the unknown concentration that is number one you can do monitoring of the spectrophotometer in a flow end or something like that. We can use it for quality control denoting the absorbance of a good of a Fanta or some other drinks etcetera food and barrage applications etcetera, but the spectrophotometer is basically it covers the quantitative analysis.

So, how do we go about doing the quantitative analysis using a spectrophotometer? That is what we are going to discuss now. In any principle any colours solution can be subjected to a chemical analysis by spectrophotometry. You take any substance which is coloured you can make the measurement. Now they are some sometimes you come across substance which are not coloured, which are colourless. Then what do you do there are numerous reagents that reacts selectively with the analyst to give you an absorbing species.

So, sometimes you may react them with a reagent, but you may not see the colour also. In that case what happens is what is required it may be absorbance in ultra violet region or near IR. So, near IR means off course will see some slight red colour, but in ultra violet reagent we may not see any colour at all. So, there is a vast amount of literature

that exists which detail reactions of this type. So, typical inorganic reagents include thiocynate ion for iron cobalt and manubrium.

All the thiocynate ions react with iron to give a red colour and cobalt red colour and manubrium also a red colour. Now hydrogen peroxide is another reagent very simple chemical reaction reaction, which you can do in your laboratory for titanium vanadium and chromium iodide ion that can be use for forming iodides for bismuth iodide and tellurium. So, there are organic reagent chelating reagents for example, diethyl di tricarbonate for copper dithiozone for lead and one time phenanthroline for iron dimethyl glyoxaline for nickel etcetera.

So, what I want to tell you is there is specific reagent, which you can use to determine the analyte, using a specific reaction. And such reports are available for almost for all the elements expect radioactive elements, all the elements of the periodic table expect may be some radioactive elements. And rare elements off course they may not be there and some elements will which we do not need for chemical analysis, but 90 percent of the periodic tables are determined by spectrophotometry.

There are certain elements like hydrogen and then helium neon organ krypton and several other gases they we do not we are not many spectrophotometry reagents, oxygen, nitrogen. You do not need spectrophotometry for that there are not many other methods for the determination of these gasses, but if it is a metal ion, if it is scummy metabolite, if it is an organic compound, if it is a bio chemical compound, they will find number of magnetically methods for the determination of such analytes.

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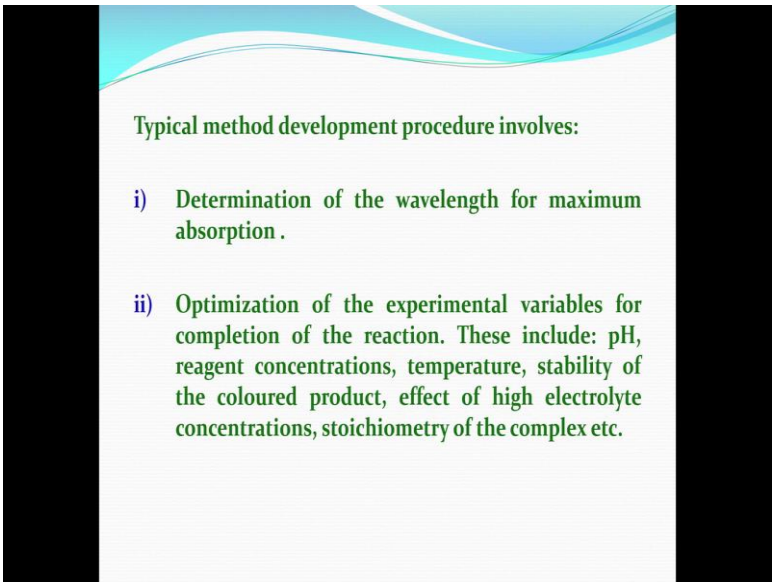

Now typical method developments; so how do I go about developing a method? For an unknown substance suppose you are researcher, you want to develop a method and for a matrix for which it has been not done. Even though I have told you that there are several almost anything can be determined by spectrophotometric, still you will come across challenges for which there are no pre-set method no standard method available. Such things happen most of the time because the matrix is different. May now what be a matrix a matrix is a substance a matrix is a substance associated with your analyte. So, the matrix the element makes and suppose you want to determine the world, there are several requirements of world measurement.

For example, somebody may geologist wants to determine have a how much of gold is there in a giver oar, where is a jeweller may not need gold in an oar, but he wants to know how much of gold is mixed with the copper to reduce it is calorie to reduce it is carat value 18 carat 24 carat like that that is his requirement. Some times in electronic connections, we need very small quantities of gold and excess gold goes into waste they. So, somebody wants to recover all the gold and that requirement is different that is in ppm level, whereas in a chemist a geologist needs the estimate gold in milligram per litre that is ppm. A jeweller wants to determine in percent and fluent man cost conscious in electronic industry wants to recover the waste gold in ppm level. 1 or 2 ppm there may be 5 ppm may be 100 ppm. So, that is the requirement. So, in each of these cases the associated compounds present in the given sample is known as matrix.

So, spectrophotometric one disadvantage is that along with the matrix the method response changes. The reaction may remain. The same quite often the reaction will not proceed because of interferences, but still you need a method which will work in almost all matrix. Sometimes it does not happen. In that case we have to develop your own method, and how do we go about doing it. That is by doing determination of several parameters of given process.

For example, look at the next slide what I have written here is determination of the first thing is suppose there is a chemical reaction giving a colour. Then we want to know at what wave length is the maximum absorption. So, once you determine the wave length for maximum absorption, I want to optimise the conditions for the maximum colour reaction maximum optimum colour completion of the reaction. So, what are the parameters that are influential in optimising a chemical reaction? Obtusely they include pH reagent, concentrations, temperature and then stability many of the substances are not stable for one more than 1 or 2 hours.

Some times what happens is the stability may be there much together for to rehearse together. So, I have to know how long a given colour complex is stable on it is own, and then effect of high electro light concentrations you will never get substances which are having only the analyte element, there may be several other wave elements electro light. For example, you want to determine magnesium in or zinc in sea water, zinc is magnesium zinc may be in ppm parts per million or may be less than that, but it also contains 3.5 percent 35 thousand milligram per litter of sodium chloride. So, high electro light concentration is one effect, which everybody you would like to determine. And then I want sometimes you want know the stoichiometry.

What is the type of complex etcetera, these are the different parameters normally we need to optimise before a method is developed?

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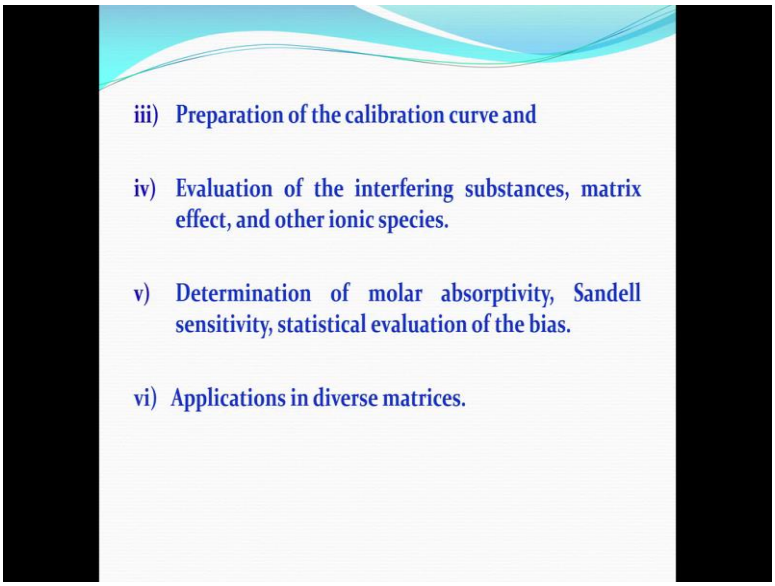

And then you also want to determine the how long is the colour liner with respect to absorbance, because that is the essence of Beer-Lamberts law. So, in Beer-Lamberts law we make a plot of absorbance or transmittance versus concentration. So, that tells us in what range, we should be working because we are know that Beer-Lamberts law. There are several variations from the bear lumbers law at very low concentration and at very high concentrations.

And then sometimes there are other interfering substances and matrix effect and sometimes unknown soppy ionic species and all these things are possible and we have to evaluate the effect of such things. Then determination of molar absorptivity sandell sensitivity statistical evaluation of the bias is more important. For example, molar absorptivity will tell us about sensitivity of the method. And then sensitivity is also defined by sandell sensitivity that is the concentration of the substance that gives you 0.004 absorbance per centimetre square. So, that is the sensitivity for comparison of different methods.

So, statistical evaluation off course is very important especially if you are handling the oars etcetera. Because oars do contain certain amount of variation in composition, in homogeneity in built in composition of oars. So, there are also applications in the diverse matrices, this I have already explained to your number of times.

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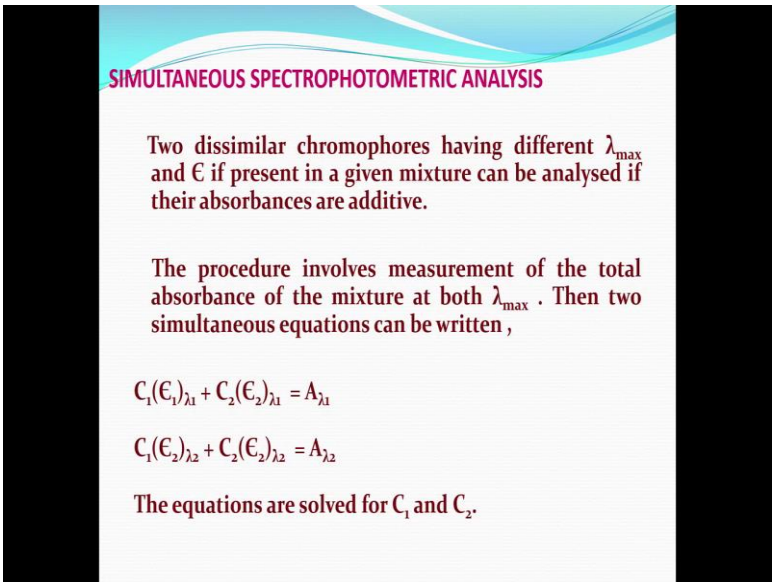

And then sometimes, suppose you have developed a method there is certain amount of requirement of 2 or 3 substances, when which we want to determine simultaneously. So, what is important is there are 2 dissimilar chromospheres, 2 different colours present in the sample having different lambda max and concern epsilon is molar extension coefficient. So, if present in a given mixture, can we determine 2 substances in a given mixture that is the question?

So, basically the answer is always yes. We can do that what we do normally is measurement we make the measurement of the total absorbance of the mixture at the maximum wave length absorbance of each complex. Then we setup 2 simultaneous equations, what we then we write concentration of the first substance molar extension coefficient of the first substance, at lambda 1 that is the peak wave length of the first complex and then second complex second element, molar extension of the second element, but it is absorbance at lambda 1. So, the total measurement there will be certain amount of contribution from both substances at any wave length. So, we write c 1 epsilon 1 at lambda 1 plus c 2 epsilon 2 at lambda 1 is equal to absorbance lambda 1 that is the total absorbance.

Similarly, we write the make the measurement at the at the second lambda max. That is c 1 epsilon 2 remember epsilon also changes, according to wave length. So, c 1 epsilon 2 lambda 2 plus c 2 epsilon 2 lambda 2 at a lambda 2 absorbance lambda 2. So, you can

even call this epsilon 1 into itself. Because obtusely molar extension will change that is we put c 2 epsilon 1 to then the equation this then there are 2 variables and 2 equations. They can be solved for c 1 and c 2. These premises to use 2 different substances in the same sample by use making only 2 measurements.

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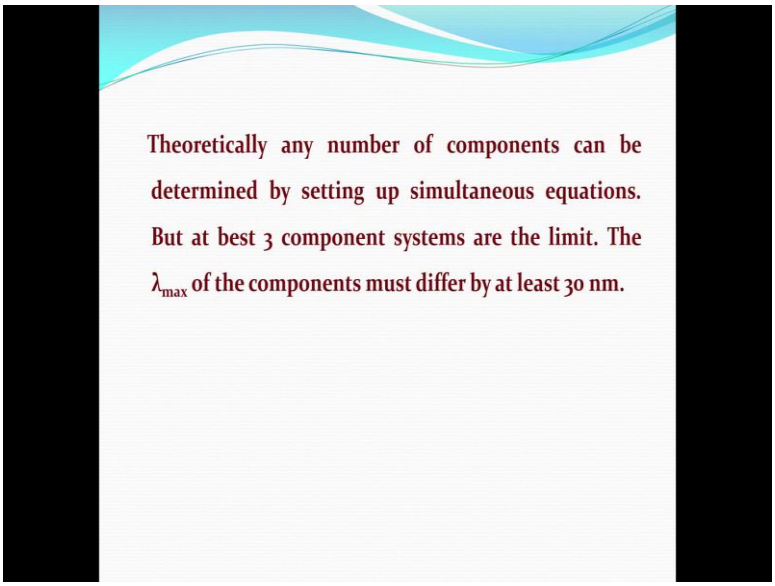

Theoretically we should be if we extend the idea, theoretically any number of components can be determined by using setting up simultaneous equations, but it does not work all the time. At best we can people have tried 3 component systems, but the overriding requirement is each lambda max should differ by at least 30 nanometres. So, if the lambda max is within that the percentage of error for each element increases exponentially depending upon the distance separate lambda max distance separated by the different peaks, but still it has been used in number of applications and it is wonderful technic to do it by separation simultaneous analysis.

Then I will not go into details, but whenever there is a need you can always come back and look up the internet and other things where you can determine 2 or 3 substances simultaneously in a given sample. The idea of this course is to give you an overview of the technics rather than make you expert. An expert you can become on your own after studying all the inputs. So, that is for you to absorb the ideas and then use them for your requirement.

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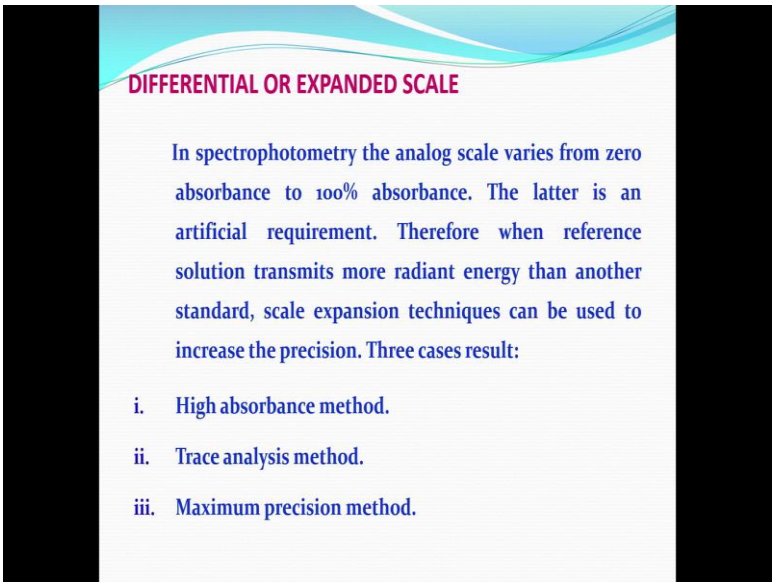

Then there is another technic that is known as differential or expanded scale. And here what happens is this is more applicable to filter photometers. In the filter photometers what happens is there is only analogue scale, and as I have explained you earlier highly concentrated substances are prone to errors. And highly diluted substances dilute coloured substances are prone to errors. So, what we do is here we extend the range in both cases high absorbance or low trace analysis one is high absorbance is for higher concentration, trace analysis is for lower concentration and maximum precision method is for concentration ranges in between that 2, but you need very high accuracy.

For example, in the determination of gold silver and several other valuable materials which require? How we go about doing this is I will explain to you in the next class. So, basically what I have thought you in this class is to the commercial instruments; what are their capabilities, instrumental parts where I also talked you about the sample cell in this absorbance cell etcetera.

Now I have given you how the whole instrument is assembled in each case that is number 2. And the approximate cost and functionalities of single cell double cell and then portable equipment etcetera. Now we are study in the different methods of using spectrophotometry for pollution control analysis. Now what we will like to do is first explore the instrumental capabilities then we will go into the details of individual parameters.

Thank you very much. Have a good day.