Modern Instrumental Methods of Analysis
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Lecture No. # 10
Ultraviolet and Visible Spectrophotometry-6 Applications

We will continue where we had stopped in the last class, that is photometric titrations. Another application that is important in spectrophotometry is the kinetic methods. That means the rates of reactions can be followed by spectrophotometry as a function of time and what you would be measuring is absorbance.

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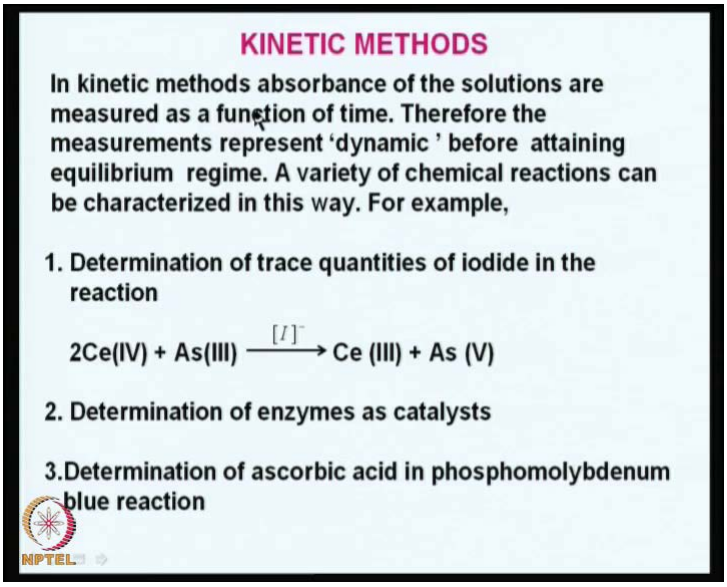

So, in kinetic methods absorbance of the solutions are measured as a function of time. Therefore, the measurements represent dynamic conditions before attaining equilibrium

regime. A variety of chemical reactions can be characterized in this way. For example, determine trace quantities of iodide in a reaction. That is a reaction of cerium with arsenic. So, what happens in the reaction is cerium gets reduced to cerium 3 and arsenic gets reduced to arsenic 3. Arsenic gets oxidized to arsenic 5. So, the reaction can be shown like this 2 cerium plus arsenic goes to cerium and arsenic 5.

So, here the change in the absorbance of iodine is used as a catalyst in this reaction. So, in the change of iodine concentration itself can be monitored by measuring the absorbance of iodine in this reaction.

Another important example, very classic example is determination of enzymes as catalysts and another one is determination of ascorbic acid in phosphomolybdenum blue reaction, that is, ascorbic acid acts as catalysts in the reaction.

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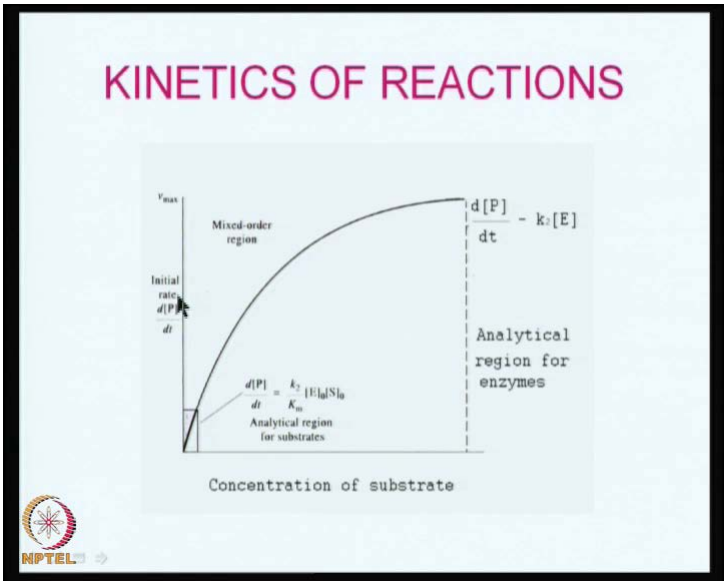

So, you can see that in kinetic methods, you would also be plotting the rate, initial rate d b by d t versus concentration of a substrate. You will see that initially the response is rather linear. That means it is a sort of first order and as the reaction proceeds, we can see that the reaction is no more linear, but it follows a curved path and this is d b by d t minus k t e. These are all Michaelis Menten equations coming from different orders. First order, second order etcetera and these are mixed order regions in this area. Mixed order, that is, neither first, second or second or third some mixture of the reaction will be occurring.

So, analytically the region of interest would be in this range, that is where it responses linear and this is the analytical region for enzymes. One is concentration of the sub-state and then, initial rate, that is d b by d t. This is how they are plotted.

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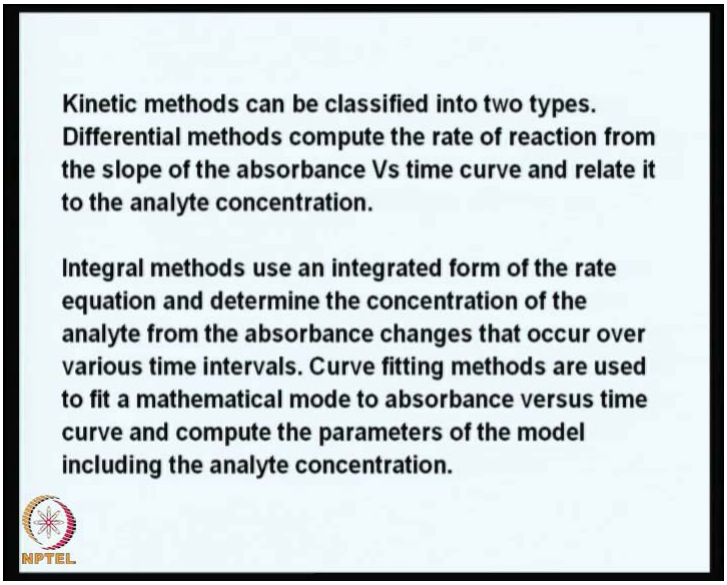

So, in general, kinetic methods can be classified into two types. One is differential measurement. For example, differential methods compute the rate of reaction from the slope of the reaction, where we saw the absorbance to be a linear function of the time curve and related to analyte concentration. There are integral methods that used an integrated form of the rate equation and determine the concentration of analyte from the absorbance changes that occur. That means you measure the absorbance, refer it to the calibration curve to find out what the concentration is.

So, this if you do it over several time intervals, you can compute the concentrations of the substances. Several curve fitting methods are used to fit a mathematical model to the absorbance versus time curve and compute the parameters of the model including the analyte concentration. So, this is how people would like to do, but in general, there is a rider for example, the spectrophotometric follow up of the reactions. What happens is in such methods, the reactions with half lives greater than 10 seconds; they are amenable for spectrophotometric follow up. Otherwise, less than that it is not easy to follow the reactions.

Another problem is the temperature control. As you know most of the kinetic reactions are highly temperature dependent in general. Every time the rate reaction doubles and that is the standard accepted norm. Need not exactly double, but it may vary almost up to double the value of the reaction rate. So, temperature control becomes very important in kinetic follow up reactions. Therefore, you would need at least plus or minus 0.1 degree centigrade of the sample that is to be measured. That means the sample should not vary in temperature more than 0.1 degrees throughout your measurement. So, then only you can get some sort of reliable results in many commercial instructions accessories are available and they are available as modules. You can just fit them. For example, in

chemical kinetics, you can have a temperature controlled sample holder and water will be circulating in these cells. I can show you different kinds of temperature controls.

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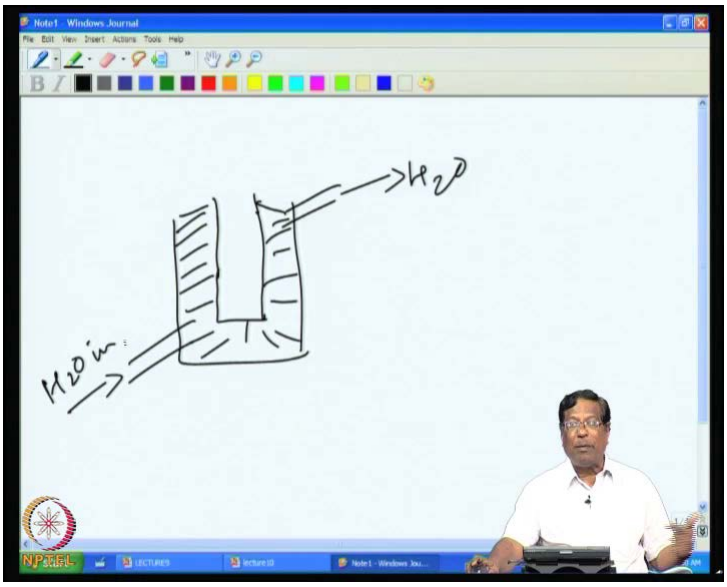

You can draw something like this. This is a spectrophotometric cell and this is encased in a compartment like this and water would be circulating, water in, water out and this cell is going to be inserted into the spectrophotometer. That means the temperature of the sample solution would be controlled by just passing cold water through the sample. So, such modules are readily available across. Several manufactures provide this kind of modules that are available and they can be attached to the spectrophotometers for kinetic measurements.

Sometimes, these reactions involve less than half lives of less than 10 seconds. So, in such cases, what do we do is we go for a stopped flow mixing technique. In this case, what happens is the streams of reagents that is there will be two reagents coming through

two different channels and we allow them to mix and allow a sufficient amount of time for them to react and then stop the flow. So, in such cases, what happens is the reaction will be going on in the stopped flow cell and you can be measuring the absorbance as a function of time and this stop flow mixed solution. You will stop it downstream suddenly and the reaction can be monitored very easily.

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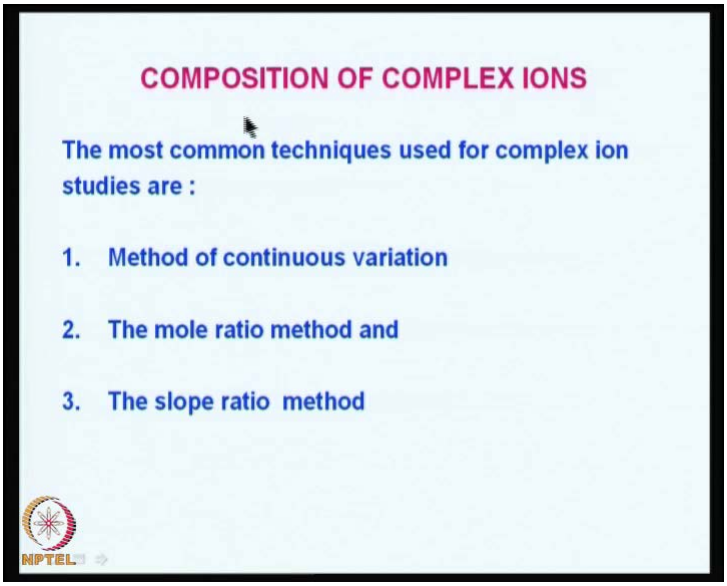

Now, we will go to another aspect of the use of spectrophotometer the most. This involves composition of the complex ions because in normal life, we use lot of complexes in our day to day lives and sometimes, it is important for us to determine the composition of the reactance when the complex forms. So, there are three methods. One is method of continuous variation, one is mole ratio method and another is slope ratio method.

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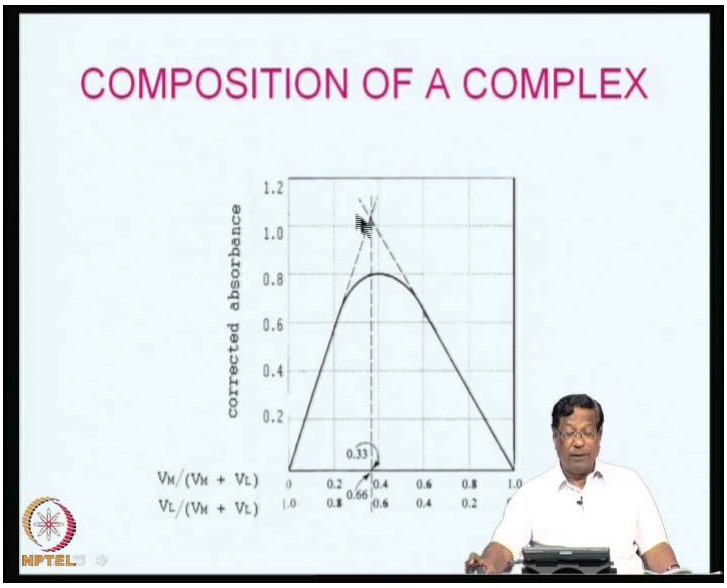

This is mole ratio method. Here what do you do is you can see the concentration is plotted air. We are assuming that there are two reagents. Here one reagent is 0, another is 100 percent, that is 1 here is 0.2, 0.8, 0.4, 0.6. So, this way it increases and the reactor reactant b decreases. So, the whole composition range is represented in this mole fraction area. So, you can correct the absorbance to the volume of the reagents, that is V m and V l. V m represents metal and V l represents ligand and the absorbance can be corrected for the volume changes and then, you plot absorbance versus mole fraction and you would end up with a curve somewhere something like this.

Here in this case, it is approximately 0.5 and 0.33. So, that means the complexes 1 is to 2 ratio 0.33. One is 0.33; another is 0.66 approximately 1 is to 1. Suppose, you get a lambda max somewhere here at 0.5, then the complex has to be 1 is to 1. Like that you will be able to determine the composition of the complex just by following this mole ratio method and this is a very standard technique.

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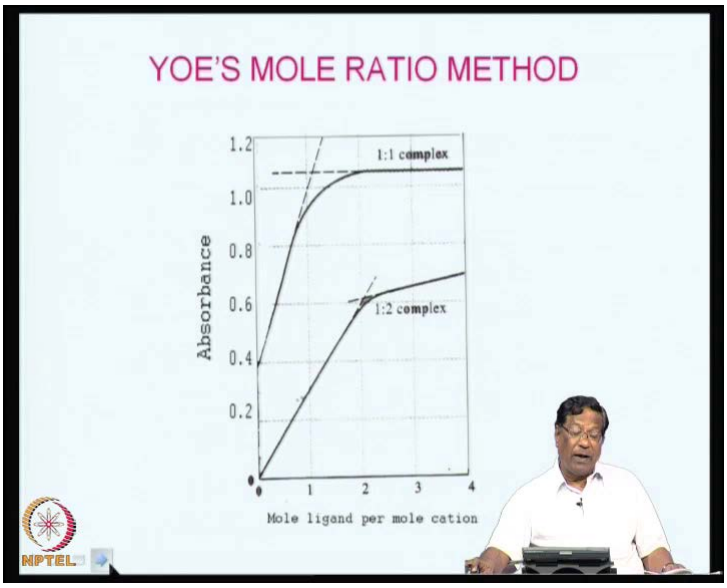

To determine the composition of the color complex, this is the continuous variation method. This is also mole ratio. Only because we are using one mole of the metal and we increase ligands in terms of stoichiometric, that is 1 is to 1, 1 is to 2, 1 is to 3 like that and then, you plot the absorbance versus mole of the ligand per mole of the cation and then, extrapolate the two regions, where the cut is the composition, where intersect each other would be the composition. Here, I have shown you 1 is to 1 complex because one mole of the reactant, one mole of the substance to give you maximum absorbance. After that whatever amount of ligand you add will not give you more absorbance. That means 1 is to 1 complex has been formed. Here, it is 1 is to 2 because the maximum occurs around 2. Like that if the composition is 1 is to 3, 1 is to 4, 1 is to 5, 1 is to 6 hexadentate complexes etcetera, they can be determined using this method.

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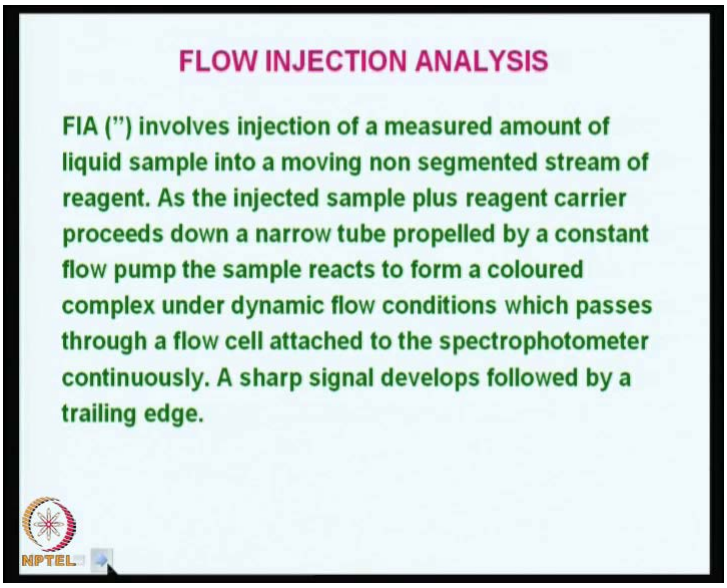

Then, another is the slope ratio method. There what do you do is you plot the absorbance versus composition and then, take the slope of the ligand, take the slope of the metal, take the ratio of the slopes and that to give you the concentration of the substance in the complex. So, another application is flow injection analysis. In flow injection analysis, it involves injection of a measured amount of liquid sample into a moving non-segmented stream of the reagent. So, as the injected sample plus reagent carrier proceeds down a narrow small tube in general or you have two tubes, one segment is coming like this and another segment is coming like this. They will form a Y section.

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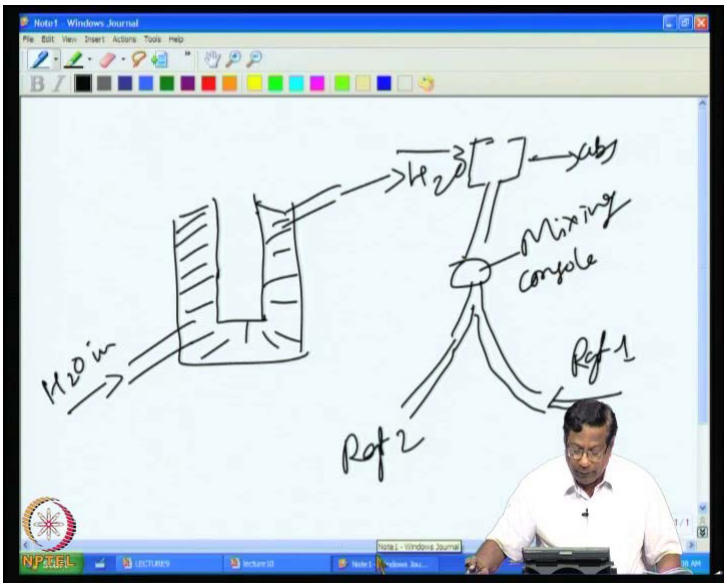

For example, something like this. This is reagent 1, this is reagent 2 and the sample would be, one of them would be a sample. Here, we have a mixing console and then, the reaction will proceed here and then, to produce a colored complex and that will be continuously passing through a reference cell, where the absorbance would be measured. This kind of absorbance can be correlated to the composition of the complex and then, as the sample reacts, you need two pumps to pump the reagent. Then, as they proceed through the narrow propelled by a constant flow pump, the sample reacts to form a colored complex under dynamic flow conditions. What is it meant? It means the both samples are continuously mixing reacting with each other forming a colored complex and then, proceeding to the absorbance cell followed by absorbance measurement and this passes through a flow cell attached to the spectrophotometer and absorbance will be also be measured in a continuous manner.

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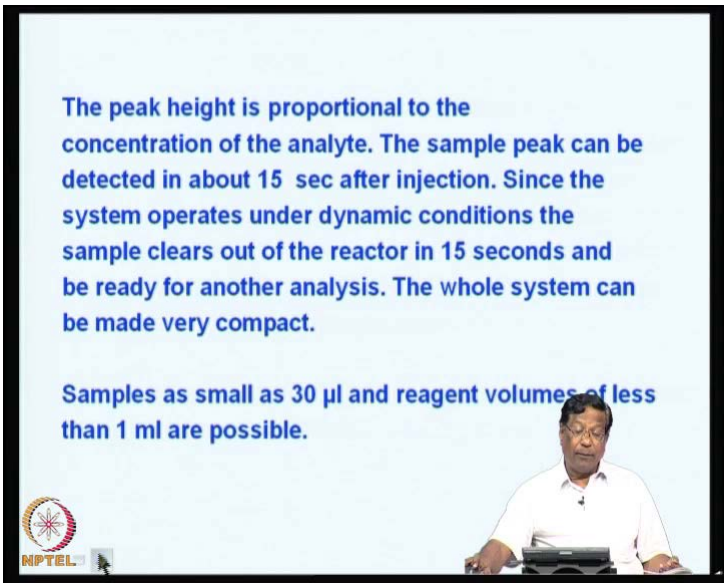

So, a sharp signal develops followed by a trailing edge. The peak height would of course be proportional to the concentration of the analyte. The sample peak can be detected in about 15 seconds after injection. Since, the system operates under dynamic conditions, the sample clears out of the reactor in about 15 seconds and then, the whole you can have a system where the reagents will be passing through without the reactant, one of the important reactant. That means again baseline conditions would be available for the system for you to inject the samples and that sample will again form a colored complex.

So, the advantage of these things, flow injection analysis is that you can handle samples as small as 30 micro liters and reagent volumes are also very less, almost 1 ml or something like that. You can control and you can have measurements made like this.

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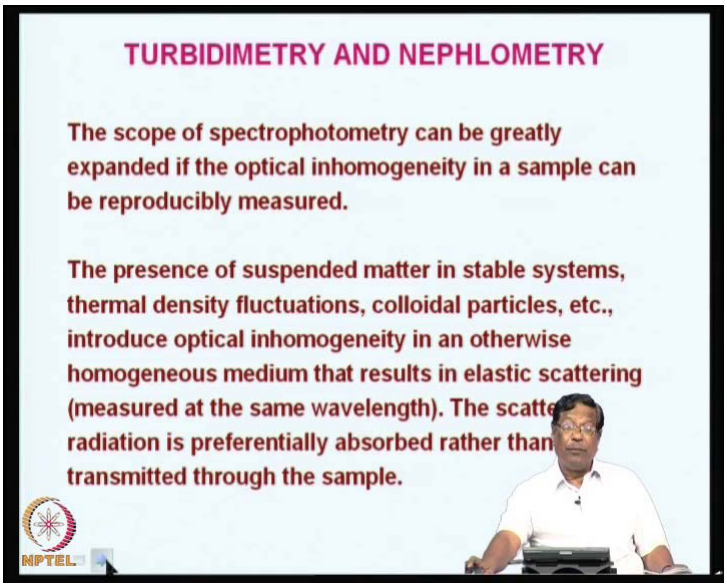

You will be surprised to know that when flow injection analysis was invented somewhere around 1985, within two years there were about 1400 publications based only on flow injection analysis and then, followed by determination using either peak height or peak area etcetera, there were about 58 national and international conferences and then, about 15 books were returned within a span of two years.

You can gaze the importance of flow injection analysis in spectrophotometry. Using spectrophotometry for all these samples, where we could really monitor the atmospheric concentrations, flowing a river water condition (( )) process parameters and so many other parameters that were used to be determined on continuous bases using flow injection analysis.

So, there are separate reviews, books available for flow injection analysis. If you wish to do this flow injection analysis for different parameters, you should refer two more

detailed texts. Now, let us turn to another aspect of spectrophotometry that is known as Turbidimetry and Nephelometry.

So, this is basically where the substance forms the precipitate and it does not dissolve. Now, you would see that every substance has got certain amount of solubility in a given solution. Now, if the solubility exits, there will be precipitate and several reactions are there which will form precipitates preferentially. For example, if you take silver solution, add a little bit of chloride, you get silver chloride precipitate and then, if you take sulfate solution, add barium chloride to that and you will get barium sulfate reaction precipitate.

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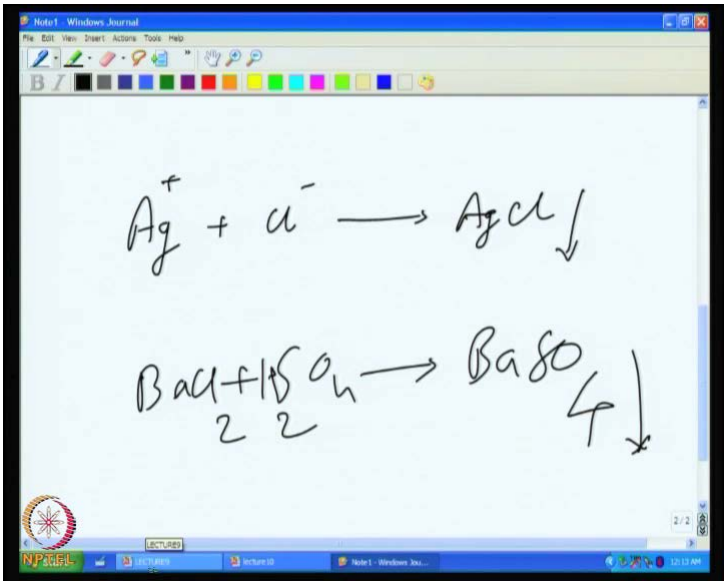

Now, both these precipitates can be written very simply like this silver plus chloride minus this goes to Agcl. This is a precipitate. Now, you can write similarly barium plus chloride sulfate going to barium sulfate. This is also a precipitate. Several thousands of reactions are there like this which will form precipitates and if we can find a way of

converting these precipitates into a reproducible entity, then there should be some amount of possibility for the determination of the absorbance or scattery or reflectance or some other properties related to this, but using a spectrophotometer.

So, the scope of spectrophotometry can be greatly expanded if the optical in homogeneity in a sample can be measured, that is basically a precipitate is in homogeneity in a given sample. So, the presence of suspended matter in stable systems thermal density fluctuations that also could lead to phase transitions and then, presence of colloidal particles, all these systems if they are present in a given solution, they introduce optical in homogeneity, otherwise, homogeneous medium that results in elastic scattering. That means measured at the same wavelength, that is some are elastic, some are inelastic elastic scattering measures. The wavelength at the measures, the absorbance at the same wavelength in elastic would give you absorbance at some other wavelength. So, the scattered radiation is preferentially absorbed rather than transmitted through the sample. This is the basis of spectrophotometer.

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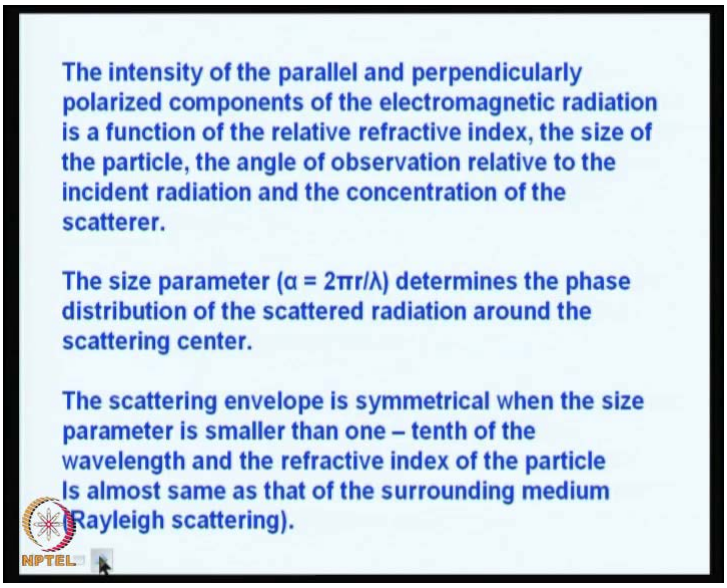

Now, the intensity of the parallel and perpendicularly polarized components of the electromagnetic radiation is a function of the relative refractive index. So, you need not essentially have precipitate alone. Even there are optical in homogeneities, still the relative reflective index would change. So, it also depends upon the size, the particle, the angle of observation. Sometimes you may like to observe the out coming radiation in the same axis as the incoming radiation. That means in the same direction. Sometimes you may like to observe it in the perpendicular direction also.

So, it depends upon the angle of observation relative to the incident radiation and the concentration of the scatterer also. So, the size parameter also is very important in such consideration because larger the size, the diffractions, scattering etcetera will be much more. So, that determines the phase distribution of the scattered radiation around the scattering center.

So, the scattering envelope basically is symmetrical when the size parameter is small. How much small it should be? It should be smaller than about one-tenth of the wavelength, that is very very small particle and the refractive index of the particle should also be almost same as that of the surrounding medium. This is known as Rayleigh scattering. This way of covered earlier in the second class, that is third class interaction of matter with radiation.

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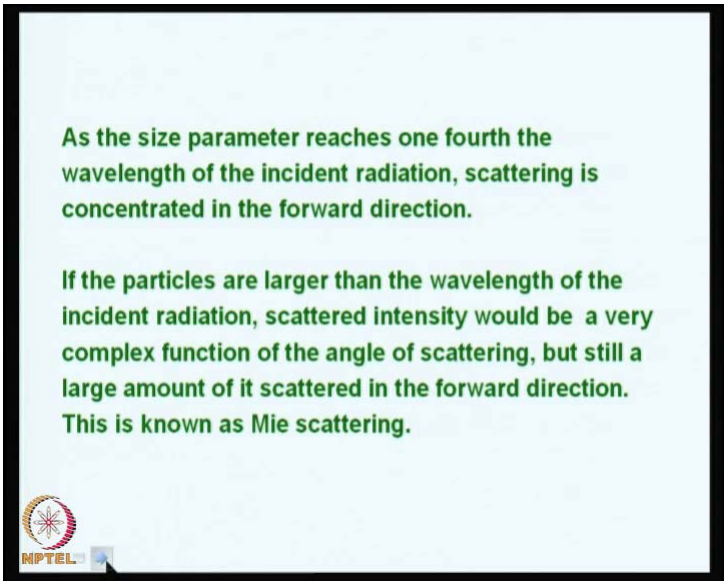

Now, imagine the size of the parameter increases keeps on increasing and follows goes beyond the size of the wavelength, one-fourth the wavelength of the incident radiation, then the scattering is concentrated in the forward direction. That means, you can measure just like spectrophotometry, you can measure the absorbance across in the same direction in which the radiation is passing through.

Suppose, the particles are larger than the wavelength of the incident radiation, then what happens is scattered intensity would be a very complex function of the angle of scattering, but still a large amount of it would be scattered in the forward direction. This is known as Mie scattering. This also we have covered in our third lecture.

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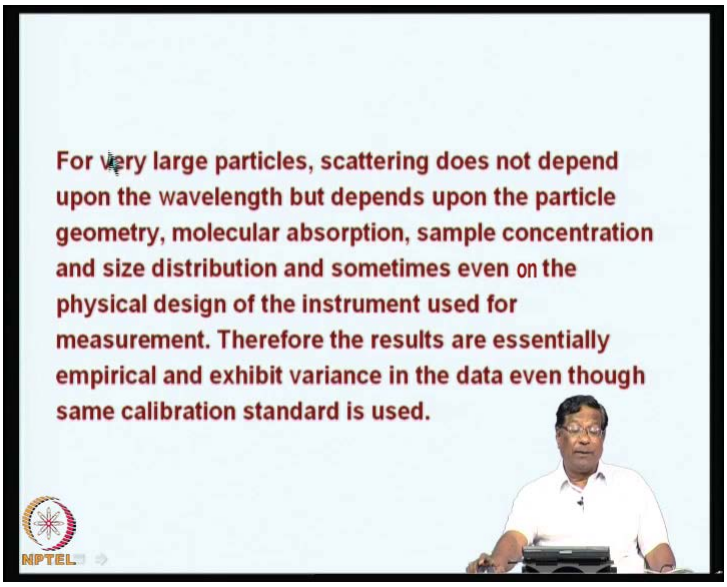

On the interaction of matter with radiation, so suppose you have still larger particles, very large particles, then what happens is scattering does not depend upon the wavelength, but it depends upon the particle geometry and molecular absorption and then, sample concentration and size distribution and sometimes even the physical design of the instrument keeps on changing your measurement. Therefore, the results are mostly empirical in such cases. Now, if the results are empirical, it means that the results are not exactly reproducible. That means every time you do an experiment, the result would slightly vary, but not exactly reproducible.

Therefore, such a condition requires that every time you do a scattering measurement, you need to have a reference and that reference value also will change, but whenever you choose an actual system, it will be applicable only under the conditions of the experiment in which the sample is measured and the reference is measured simultaneous or side by side. If they are measured simultaneous, then the results cannot be taken forward to get the normal conclusions of such an experiment.

Therefore, if the results are empirical, it is mandatory that you must use a standard sample and measure you known also immediately along with the sample. Then only the results will be tenable, but they cannot be extrapolated to other conditions basically because it is semi-empirical. It cannot be reproduced exactly, but for that matter, during those conditions of experiment provided you are able to keep all other variable constant during the whole serious of measurements, then those results would be acceptable. Therefore, this condition usually occurs whenever you have very large particles. So, you need to use, you need to calibrate every time.

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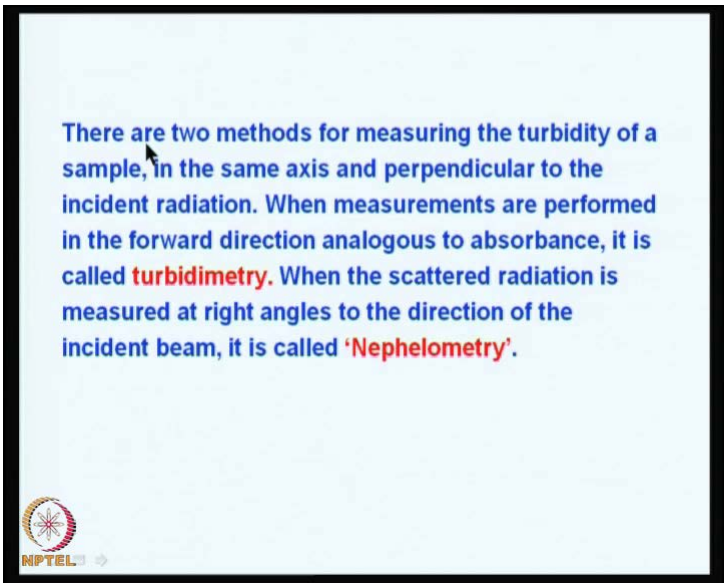

Now, under these conditions, there are two methods for measuring the turbidity of a sample. One is in the same axis and another is perpendicular to the incident radiation. Now, I will show you very simple arrangement.

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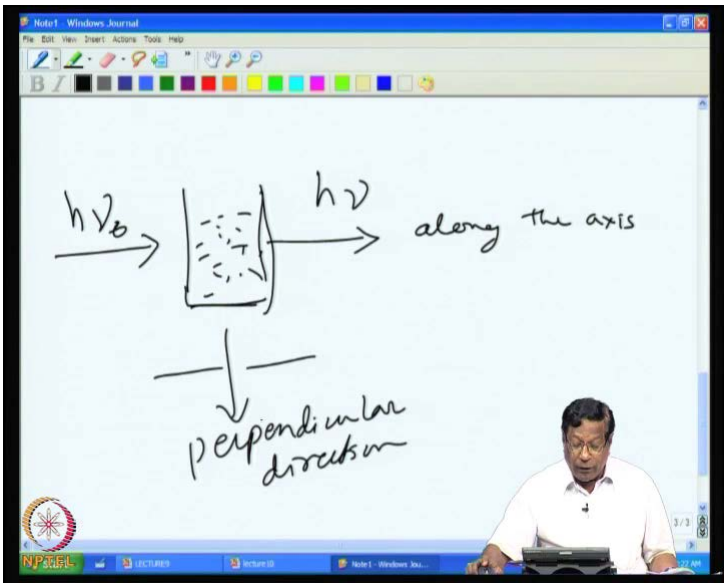

How you can measure the absorbance? Here is your sample, here is your incoming radiation and then, you can choose to measure this is h miu 0, this is h miu, this is 0 and this is your sample holder. This contains the sample and this contains the substance. Now, you can measure along this direction or you can measure along this direction. This is perpendicular, this is perpendicular and this is along the axis. So, the one is called Nephelometry and another is called Turbidimetry.

Now, these two methods, when measurements are performed in the forward direction analogous to the absorbance, then it is called Turbidimetry. When the scattered radiation is measured at right angles like what I showed, the direction of the right angles to the direction of the incident beam, then it is known as Nephelometry and both possibilities exist and the problem is when to choose Nephelometry and when to choose the Turbidimetry.

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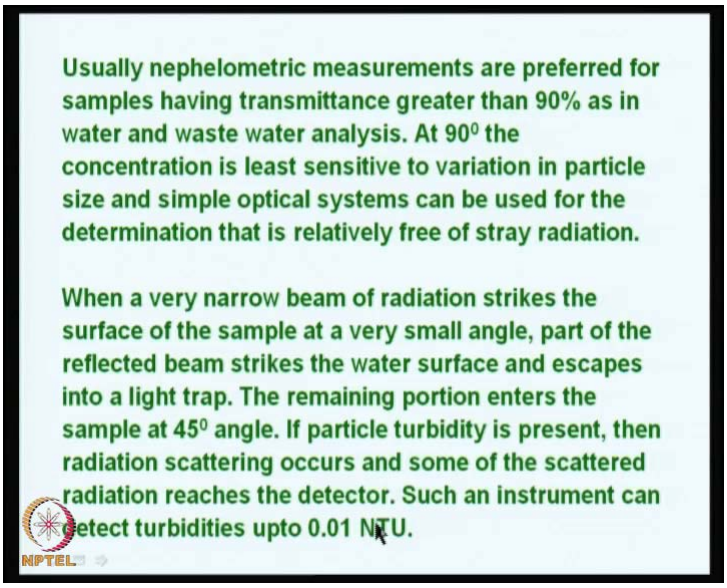

Usually nephelometric measurements are preferred for samples having transmittance greater than 90 percent as in water and waste water analysis. At 90 degrees to the observation to the emission radiation, the concentration is least sensitive to the variation in particle size and simple optical systems like our filter photometers. What you have discussed earlier, they can be used for the determination that is relatively free from stray radiation.

So, when a narrow beam of radiation strikes the surface of the sample at a very small angle, part of the reflected beam strikes the water surface and escapes into a light trap. This is the how we go about doing nephelometric measurement that is a water is flowing. You allow a radiation to fall on the water level and then, strike it. Let it strike the water level and then, go out. Whatever particles are there, they will absorb the radiation and you have to measure the water surface and whatever is reflected, that strikes that gets lost

into a light trap. The remaining portion enters the water at a 45 degree angle and when it comes out, if the turbidity is there, then the radiation scattering normally occurs.

Now, some of these scattered radiation can be made to reach the detector or at 90 degree angles and you can measure the absorbance and such an instrument is known as nephelometer. Nephelometers such as the one what I described, they can detect turbidities up to 0.01 NTU. That is standard units and these water turbidity, the quality of water can be determined using Nephelometry. So, you need a standard, one standard sample which shows around 4000 NTU and that can be diluted sequentially to get 45 etcetera.

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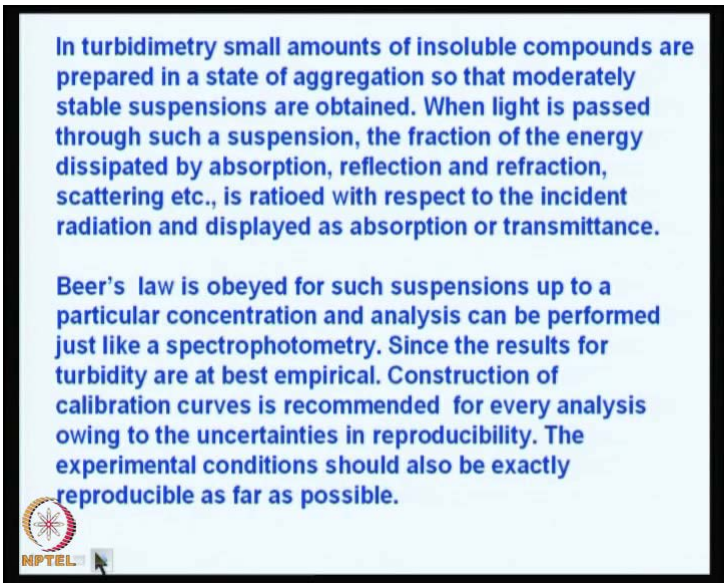

If the drinking water is having about 5 NTU, then it is acceptable as a drinking water. In India, it is very very difficult to get drinking water corresponding to that standard, but the standards are there all the same and this is the way to measure the turbidity of the sample

drinking water or any other sample. For that matter, the turbidity can be measured like this. Now, in Turbidimetry, we are talking about Turbidimetry.

Now, in Turbidimetry, what happens is small amounts of insoluble compounds are prepared in a state of aggregation, so that moderately stable suspensions are obtained. When light is passed through such a suspension fraction, the energy is dissipated by absorption, reflection and refractions, scattering etcetera, whatever is lost in terms of absorption with respect to the incident radiation is ratioed with respect to the incident radiation and displayed as absorption or transmittance.

Now, for any quantitative analysis, Beer's law must be obeyed and if the Beer's law is not obeyed, you cannot have a quantitative determination so far. Such suspensions only up to a very small particular concentration, Beer's law does get obeyed and analysis can be performed just like spectrophotometry. Since, the results for turbidity are at best empirical, construction of calibration curves again is recommended. That means whenever the results are empirical, you had to have a calibration. Every time you do this kind of analysis using turbidimetry for every analysis because of the uncertainties involved in the reproducibility. Therefore, the experimental conditions must be reproducible as far as possible.

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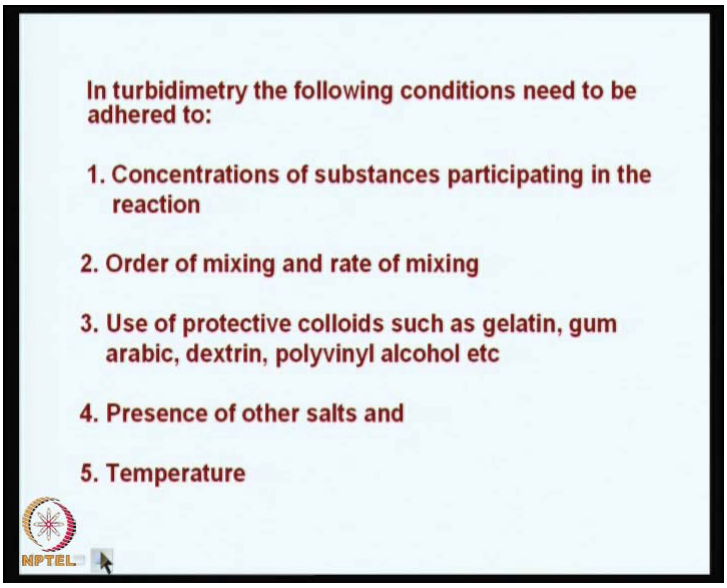

Now, what are the types of experimental conditions you will have to control? These include for example, concentrations of substances that you have to control. Anyways, standards and unknowns participating in the reactions, that is, reagents as well as the standard, what do you want to determine and then, you have to have order of mixing and rate of mixing. You cannot change this.

If you change the order of mixing and rate of mixing, the absorbance are going to change in turbidimetry. Another one is suppose, the substance is there and it got a precipitation reaction. In general, what happens is the precipitates have got a tendency to settle down and if the precipitate settles down slowly, the absorbance what you would be getting would be increasing because the precipitate will go down from the optical path and absorbance will be increasing. That means if you take the sample measure over a period of time, the absorbance what you are going to get are entirely different.

Therefore, you have to find a way of how to keep the suspension stable. For such stable, to make the system stable what you need is increase the viscosity of the sample. Now, when viscosity of the sample increases, the settled velocity of the particles will decrease. Therefore, within a reasonable amount of time, you should be able to get reproducible results. Now, you can use protective colloids such as gelatin, gum, Arabic, dextrin, polyvinyl, alcohol etcetera. All these substances increase the viscosity of the sample. Now, sometimes most of the substances are used as 1 percent solution about 1 ml or 1.5 m l, 2 m l depending upon the standardization of the procedure and you have to add these reagents also either along with the reagents or before, so that viscosity is already more when you want to determine the absorbance.

So, the stability of the reagents depends to a great extent on the use of substances such as gelatin, gum, Arabic, dextrin, polyvinyl, alcohol etcetera. What are the other substances that you have to control the rate of reaction? One thing is there should not be presence of any other salts in the same mixture. If the presence of salt increases, then the particle size of the precipitates, even any reaction where precipitation occurs, presence of salts affects the size of the particle. Therefore, prior to the experiment we should know what the concentrations of the other salts are which do not react with the reagent. So, presence of other salts is very very important.

Suppose, you want to determine the particle size or absorbance in the sea water that will have a different composition compared to river water or compared to lake or compared to a bore well water, all these possibilities are there. If you want to determine some substances in urine, for example, again the concentration of the substances other or salts assume very high importance as far as reproducibility in turbidimetry and nephelometry are concerned. That is why, it is important to know the presence of other salts in the sample what you want to analyze.

Finally, the temperature of course influences the particle size to a very large extent and the temperature of the reaction must be maintained constant which like using an accessory with a flow through cell in which the sample is contained, in a cell where the cold water is circulating, so that you can control the temperature up to 0.1 degree centigrade. In all these things, the rate of mixing I have not explained to you very properly because this is a very very special aspect of turbidimetry. You can use hand mixing, you can use machine mixing etcetera, shake the substance and even if you want to determine the substance given in a turbidimetry, even simple operations of mixing like this or like this or like this. Suppose, you have a machine in which it keeps on checking like this, all these parameters will affect the turbidance readings.

Therefore, rate of mixing is also very very important aspect and we always teach the student to try to reproduce as far as possible the shaking. Especially, if you are doing a turbidimetric reactions theoretically, the turbidity can be turbidance can be determined in number of ways. In theory precipitation reactions, any precipitation reactions can be converted into turbidimetry because most of the substances have got some solubility product about which if that concentration increases, it will precipitate. So, substances which give you reactions, which give you the turbidance, can be converted to spectrophotometric turbidimetric methods and they can be converted into good spectrophotometric procedures.

Now, the determinations can be converted excellent results in subparts per billion level PPM is 10 raise to minus 6 sub PPM levels, that is 10 raise to minus 7, 10 raise to minus 8, 10 raise to minus 9. Sometimes concentrations can be determined using turbidimetry and Nephelometry. Some of the examples include the determination of barium chloride water. I had told you earlier in the reactions using glycerol alcohol mixture, the barium, this is the barium chloride reaction barium plus h2sfo goes to barium sulfate. This

reaction if you carry out in presence of glycerol and alcohol, then it is easier to determine up to 1 PPM of sulfate in the system. This helps in environmental samples. For example, if you want to determine the amount of sulfate in the atmosphere, all you got to do is collect the sample and then, dissolve it, put in water or hydrochloric acid.

So, the sulfate whatever is there in the environment gets converted to sulfuric acid and you just bring it to the laboratory, add barium chloride, add a little bit of glycerol and then, the barium sulfate will precipitate. You can take it to the determination spectrophotometer and calculate what the amount of sulfate in the environment is. Very beautiful reaction can be used for determining up to PPM and sub PPM levels, wonderful reaction.

So, another reaction is the determination of phosphate by using phosphomolybdate reaction. It is a very celebrated reaction. Phosphomolybdate phosphorus reacts with molybdenum reagent and then, in presence of (( )), it gives you blue precipitate and the size of the precipitate can be precisely controlled using turbidimetry as well as Nephelometry. If the concentrations are very low, then you go for Nephelometry, otherwise turbidimetry.

Now, sometimes the determination of ammonia using Nessler's reagent, that is also very important because in most of the environmental samples, we want to know how much of ammonia is there in the air and there is a beautiful reaction known as Nessler's reagent which gives you a yellow precipitate and light yellow color. It contains colloidal particles and this colloidal particles if you are able to trap the ammonia into a solution just by passing it in water, we can add Nessler's reagent, that is potassium iodide and mercuric sulfate and that will give you a colloidal mercuric, colloidal precipitate. Then,

that can be correlated to the concentration of ammonia sub PPM level determinations. No other reaction gives you such low level of detection except Nephelometry.

In general, the nephelometers and turbidimeters, they are available as accessories which spectrophotometers sometimes dedicated nephelometers and turbidimeters are available, but they are low cost something like filter photometers which will cost you a few thousands of rupees, but otherwise it is possible to convert any spectrophotometer into nephelometric and turbidimetric measurements.

Now, nephelometers procedures are very important. They provide a practical way for us to continuously monitor the turbidity in water. For example, if you want to determine, he must have drunk coca cola number of times, but whenever coca cola is produced in a factor, the color of the coca cola is one thing, color and taste etcetera, but the water should not have any precipitates.

So, they monitor the color of coca cola and other beverages, beers which are slightly colored, using nephelometry and turbidimetry. In several process, industries like this, beverages and then, in pharmaceutical preparations product quality control, boiler feed, waters and several contents (( )). In such cases, nephelometry and turbidimetry are routinely employed for the determination of substances using spectrophotometry.

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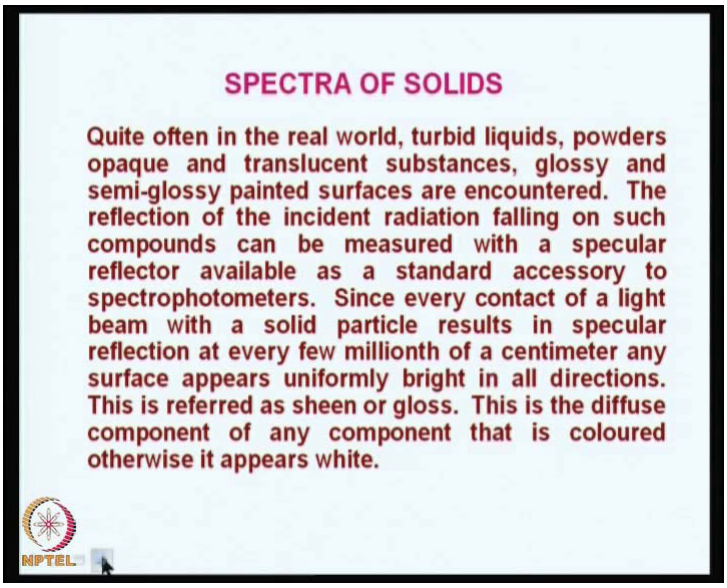

Now, these are some of the things what I have already covered and you can take a look at some of these things whenever time permits and then, another aspect that is required very useful in the determination of substances using spectrophotometry or accessories that is spectra of solids. Quite often we are unable to take the spectrum because it is a solid. If it is liquid, very clear solution, there is no problem. We can always go take it to the spectrophotometer and measure the absorbance. Sometimes if it is precipitate, yes still we can do it by protective colloids and take it to the spectrophotometer and make our measurements. Now, what do you do if you want to determine the quality of the paints or papers, if in the production of paper, one would like to have green colored paper, white colored paper etcetera and how would you control that quality of the papers? That is a point to be considered whether we can do something using same principles that we use in the spectrophotometer.

Sometimes you can imagine a huge machine containing several parts which are painted in green or blue or something, some color and if the parts are painted differently on different days, then the shades must match exactly. Otherwise, the machine will look discolored, disfigured and so many other possibilities are there. Therefore, the color of the solid substances is also very very important for us to determine in a routine manner.

In real world, the turbid liquids, powders, they are all easy and sometimes you would like to know the gloss shine of a material what you want to present. Sometimes in such cases, the reflection falling from the incident radiation reflection of the incident radiation falling on such compounds can be measured with specular reflectors available. They are available as an attachment to spectrophotometers. So, they are also available as standard accessory. Since, every contact of a light beam with a solid particle results in specular reflection, in general you take any surface material. If light falls on them, there is specular reflection, first point of contact reflection. Now, this first point of contact of reflection, it interacts with the next point of contact. So, in general, things will look colored blue, green etcetera, any object that you see.

For example, this color, this is green color, approximately green color. From the blue background, it is looking slightly bluish and this color on every point of contact where the light falls, there will be reflections, specular reflections. Then, within a few millionth of a centimeter, another ray comes from some other sources of light or from the same source of light that also gets reflected.

So, thousands and thousands of beams of light keep on producing specular reflection from the surfaces like this. That is why and they may interact positively and most of the time, they interact in a destructive manner, but the shade will remain and that is how the substance will look blue, green, red color etcetera. Now, it is important. Suppose, you are

producing a bucket of this color, it is important that you would like to know how much of the colored material you want to add in this.

Now, how do you do that after adding? What is the correct color? Master batch is one thing and subsequent batches are another thing. So, you want to know how much of the light it reflects and if the reflection of the light falling on this, if you are able to reproduce continuously, then it is possible for us to have a solid state control for a substance like this. It can be a plastic, it can be a paper, it can be painted machine paper, painted machine part and several other things glosses, all these things. So, this is referred the color. What you see across, coming across a substance coming as a specular reflection. That is first point contact reflection is also known as sheen or gloss and this gloss, you would like to determine, but basically it is something like diffusion. It is a diffused components of the reflected light on any surface.

So, such things if it is colored, it will appear colored, but if it is white substance, again you need to the substance will appear white. Now, you are very well aware that a shirt when it is bought new, when it is fresh, it gives you a very bright white color. You keep on using it after washing sometime, it occur yellow hue. Now, that way to determine the quality of reagent soap powder which washes brightest, you would have seen number of advertisement in TV and other things which wash our TV powder, surf excel, this that etcetera wash white (( )). How do they really control the quality when they use such substances?

So, white substances also need to be monitored regarding their specular reflection, white paper for example. White paper needs to be pure white, otherwise you will not enjoy writing if it appears grayish or darker or brown. So, whiteness of white substances can also be measured using specular substances and here, I have listed a few substances, but

specular reflectors are available as a standard accessory. Since, every contact is this thing, every contact represents a color, you will be able to determine its sheen or gloss and this is the diffuse component as I have already explained to you.

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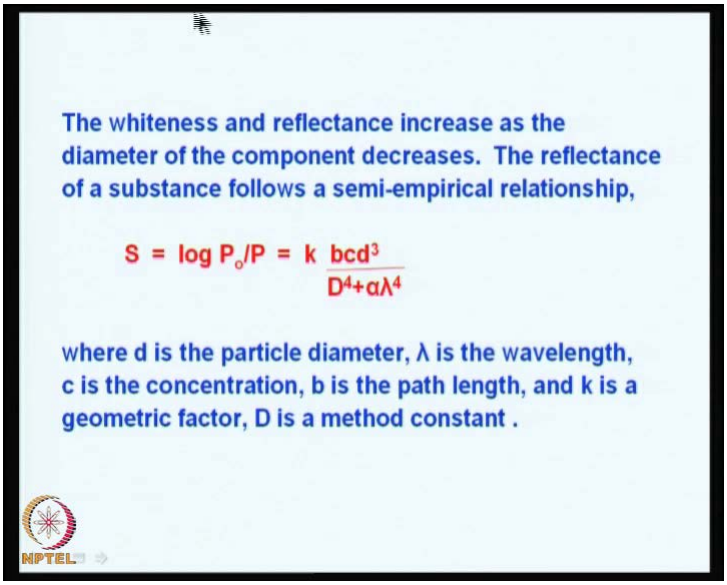

The whiteness and reflectance increase as the diameter of the component decreases. That means the smaller the particle, the better is the reflectance. So, the reflectance of a substance again it follows a semi-empirical relationship, that is S is equal to log of P naught by P. That is incident radiation and the reflected radiation. Now, you should not confuse this with transmitted radiation. That is why we are calling this parameter as S and then, specular reflection S for specular and follow approximately this log of P naught by P. If you consider all the aspects, you can arrive at a general semi-empirical relation like this case k into bcd cube divided by D raise to 4 plus alpha lambda raise to 4.

Now, generally consider all these parameters. Now, you would know for example, d. This d represents the particle diameter. The smaller the particle, higher is the reflectance. Lambda is the wavelength of measurement and c is the concentration that should be proportional and b is the path length. That also should be proportional to the particle size or particle diameter etcetera and k is a geometric factor and d is a method constant. That means whatever you are using for the spectrophotometer as a standard and that standard has got a constant. This you will have to incorporate. The manufactures of standard white reflecting material will give you the value of d.

Now, if you use this value, you can standardize your substance like paper, etcetera into a particular standard value. Then, it is using the reflectance in the spectrophotometry. It is possible for us to obtain that value, keep on controlling the process parameters until you get the correct reflectance. Then, the batch can go for production. So, reflection using reflectance what you need is a good standard. So, the standard is a magnesium oxide standard as a reference and all other measurements also must be made with this reference. Sometimes even titanium dioxide is a very good standard and available as a standard accessory from the reflectance instrument, measurement manufactures reflectance measurements and these are routinely performed in paints textiles and paper industries etcetera.

So, this completes our studies on the spectrophotometer. In the next class, we will study what is fluorescence and phosphorescence in the instrumentation and several aspects are common. So, we will be just describing the basic features of the instrument and wherever there is a requirement, we will go more into details.