Atomic and Molecular Absorption Spectrometry for Pollution Monitoring

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Lecture – 17
Quantitative analysis – I

We meet once again, greetings to you. And yesterday I had started explaining the quantitative analysis by spectrophotometry.

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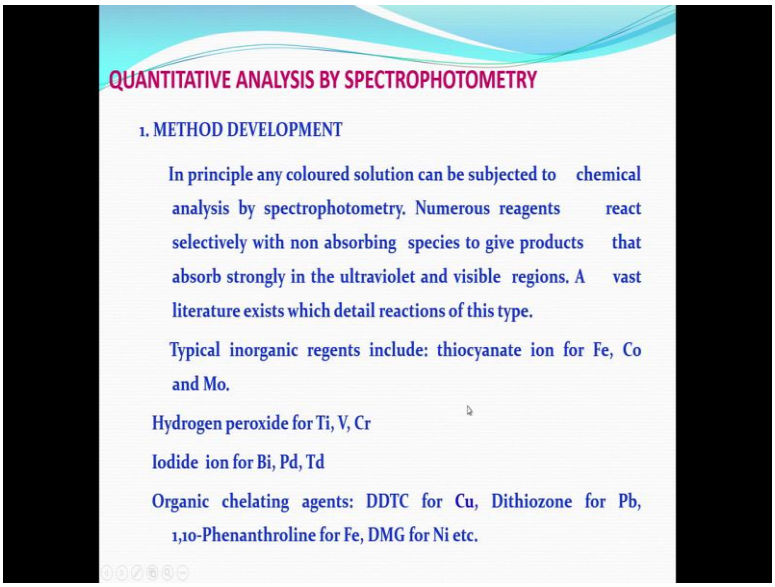

We had completed our analysis, and I mean our studies on instrumentation, and then I had as discussed with you about how we are going to develop a quantitative analysis method by spectrophotometry. I had shown you this technique this slide also, quantitative analysis by spectrophotometry. And how we develop a color system using different organic reagents or in organic reagents etcetera.

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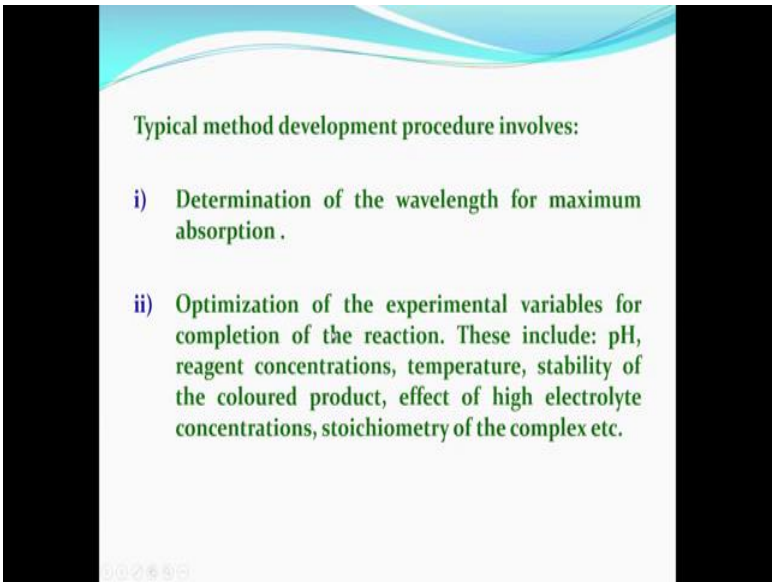

And then I had explained to you a typical method development procedure, that is to determine the lambda max and then to determine the other experimental variables for the optimum color development of the reaction.

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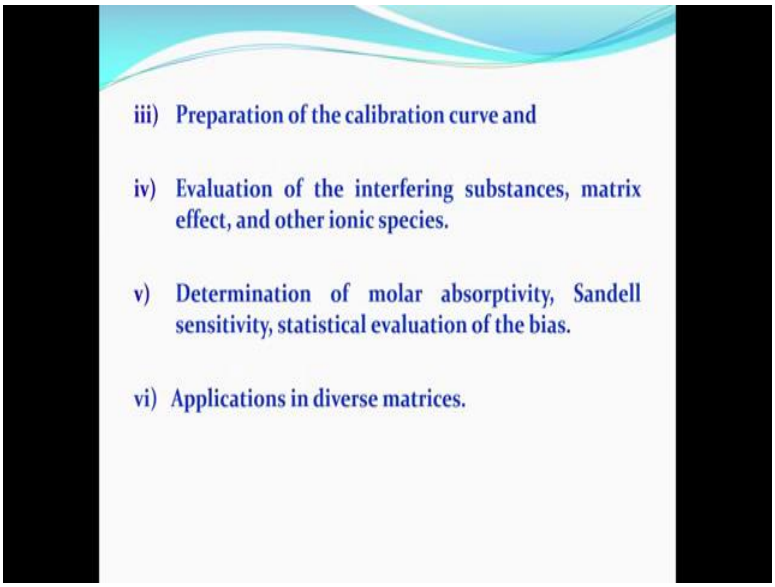

So, these things included pH reagent concentration and a temperature stability of the colored product and effect of high electric electrolyte concentration stoichiometry of the complex etcetera, you know that is typical method development.

And then we had discussed about the preparation how to prepare the calibration curve, evaluation of the interfering substances matrix effect and other things this I had already explain to you earlier. And we sometimes it is important to determine the molar absorptivity or sandell sensitivity, that is and statistical evaluation of the bias. And then applications in diverse matrix also are important because spectrophotometry is basically a matrix dependent technique. So, any change in the matrix will always lead to slight differences in the absorption and absorbencies. And then the calibration curve may go little here and there therefore, it is important to test the method every time we have a change in the matrix.

So, this slide also, I had shown you and then we are discussing about the simultaneous spectrophotometric analysis.

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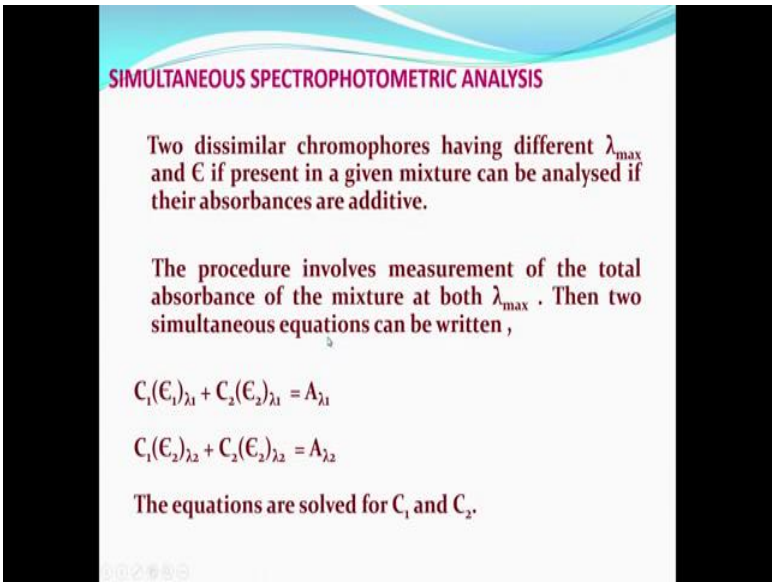

So, here what I said they the means there are there is only one component to be determined in a given analysis. We do not have a problem in the because we can develop a method or a use a development based on the previous slides, but suppose there are 2 substances, which will react with the same reagent. Because basically spectrophotometry is the where the science of measurement has color. So, 2 if there are 2 substances which you colored a complexes then the absorbance will become additive it.

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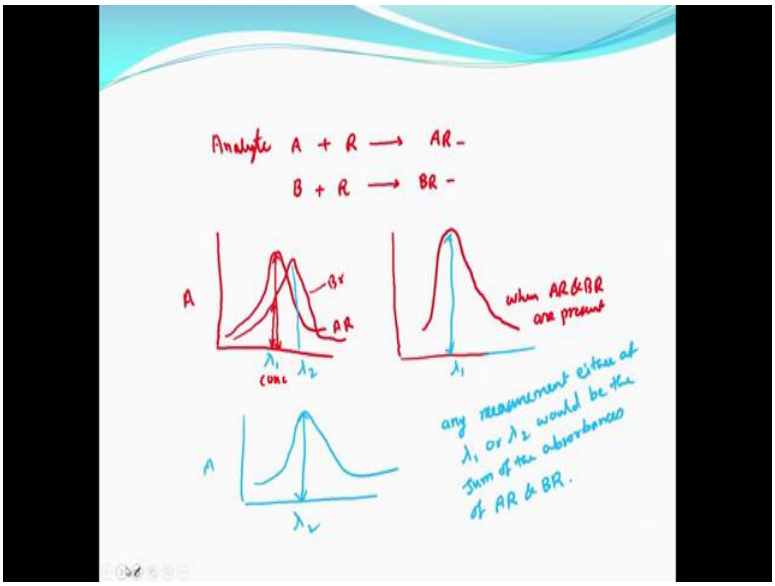

So, I was assuming that, let us assume that there is a some analyte A which reacts with a reagent, to give you a complex AR, and with a lambda max. It is specific lambda max and suppose I have another component B and then the it reacts with the same reagent R to give you a complex BR and this maybe these 2 colors. These 2 colors are different, but if both of them are present in the same system.

Then we will be seeing both the colors. Now if you represent the other observances in a given a system, that is absorbance versus concentration. The complex of AR maybe having a lambda max like this. Because a spectrum like this and complex of BR, I mean this is for AR and complex BR may have a peak something like this. This is BR. So, any measurement of the sample containing both AR and BR would be the sum of this as well as the other one the absorbance of this plus this.

So, the actual absorbance curve may look something like this and go like this. When both when both AR and BR are present. This is for the lambda max of AR. Same thing is true with respect to lambda max of BR. If this is at lambda 1 and this is at lambda 2. Lambda 2 if I measure absorbance this is a lambda 1, lambda 1 this is at lambda 1. Now there is similar thing will happen and I get a curve something like this. And this one will be at lambda 2. So, any measurement either at lambda 1 or lambda 2 would be the sum of the absorbance, would be the sum of absorbance of AR and BR. This is the problem you

may be making measurements, but you will be measuring the sum of the absorbance of both AR and BR at any wavelength.

Now the question is can we solve out it, this is the problem and determining both AR and BR. Otherwise what I have to do is remove them from the matrix by some other means like solvent extraction, precipitation, filtration and things like that, I have AR determining A and similarly make a procedure for removing the B removing the A and make the determination of BR. Then you can have the analysis, but you can a make use of a little mathematics here, and that is by using the simultaneous determination. So, in simultaneous spectrophotometric analysis, what we do is we recognize that there are 2 dissimilar chromophones having different lambda max. And if they are having different lambda max having different colors then the molar extension coefficient also will be different.

So, we set up 2 equations, that is at lambda 1 C 1 A 1 C 1 A 1 plus C 2 A 2 would be the sum of A at absorbance at A lambda 1. C 1 epsilon 2 actually it should be epsilon 1 there is an error and C 2 epsilon 2 C 1 epsilon 2 actually lambda 2 would be a lambda 10. So, the equations are solved for C 1 and C 2. So, if you we have we know they have we have C 1 and C 2 as 2 concentrations and absorbance, we have 2 equations 2 equations and 2 unknowns should be you should be able to sort out the concentrations using a little bit of algebra.

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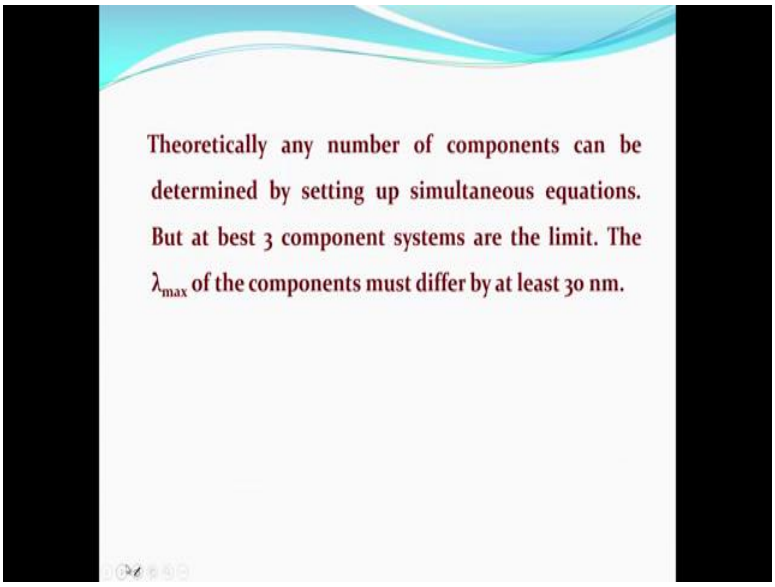

So, theoretically if the given matrix contains, if theoretical if the given matrix contains more than 2 substances, which will give AR BR n and CR, then can I setup up a 3 3 equation system, where similar thing can be sorted bias can be sorted out and then I can determine 3 unknowns also like, this theoretically any number of things can be done, but there is a practical problem with the this approach, what is a practical approach the absorbance lambda max of the 2 substances should be separated by at least 30 nano meters if I had to apply this method to a fair degree of accuracy.

So, this is what I was telling you normally 3 component system people do and people have tried, but more than 3 becomes a little trickier, then it may not be worthy time and effort and many to do such a such as an analysis.

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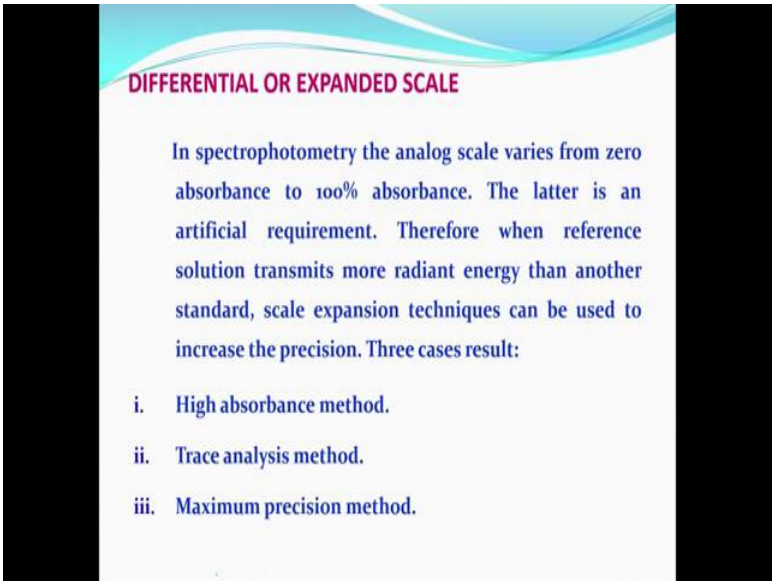

So, now we go to another aspect of how to determine the substances at very high or very low concentrations. This also I had explained to yesterday I am going to do it in a slightly more detail now, because quite often we come across situations where we do not want to dilute a sample or 2 within 0.2 to 0.8 or absorbance. Sometimes we do we do the concentration of the substance is so, less that it may not be worth make concentrating it between 2 and 0.8 absorbance which is the normal absorbance level for all spectrophotometric methods.

So, I can sight you an example of gold. Yesterday I did that the in gold analysis people do not want to dilute and waste. So, if the color is a very high you know that according to

relative concentration error system error principle the error also will be more. So, what can I do if I want to determine a highly colored substance? And the same thing is true in case of body fluids. In body fluids what happens, we normally have a system where the sample maybe very less. Suppose somebody wants to analyze sodium in brain fluid or in the lead in kidneys, it becomes very difficult because you cannot have too much of the sample, a very small quantity of the sample will be there, and if I take that do some pretreatment and then do the analysis.

The absorbance value will be very low. So, the again the error will be very high at low concentration also. This we have studied when we studied the beer lamberts law. So, I am to attend to such situations, a simple calorimeter which does not have the presence creating said hi and computer etcetera, I have still not required you can still make the analysis in a better way. So, what do we do the analogue scale in the spectrophotometer? You know we know that if we choose absorbance, it varies from 0 to 2. That is the minimum is 0 maximum is 2. If I do the transmittance the same scale should be calibrated from 100 to 0. So, 0 transmittance corresponds to maximum absorbance. So, it will be important for us.

So, an analogue scale varies from 0 to 100 percent absorbance that is two absorbance in absolute scale. So, the letter is an artificial requirement. So, when solution reference solution transmits is more radiant energy, and then another standard then scale expansion technique can be used. We had stopped here. In the last class there is there are 3 ways of dealing with this. One is high absorbance method another is trace analysis method and third is maximum precision method.

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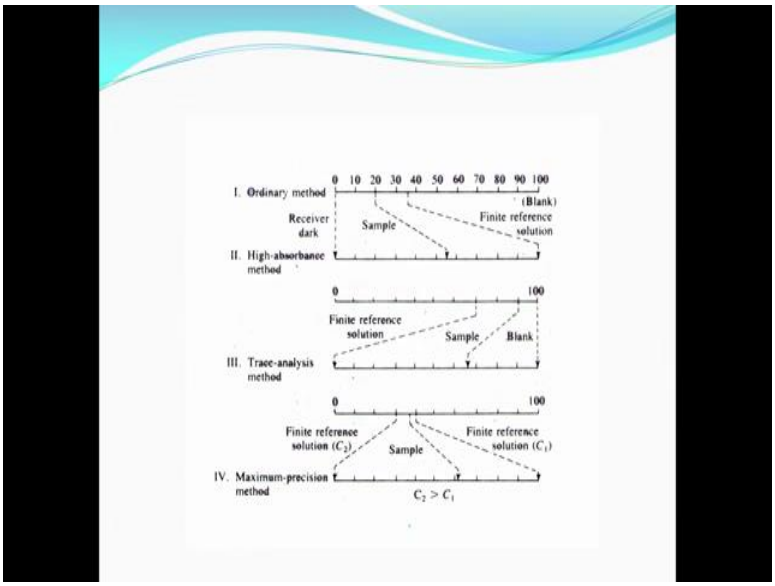

So, let us see what it entails. Look at it now. I have drawn a simple scale analogue scale of a colorimeter. You can see here that the scale in and I have put 4 cases here. 3 cases and the tau 1 2 3 and 4 are there. So, what is happening in this case is the ordinary method has a scale of 0 to 100 on the top. So, I need to analyze a sample which is giving me and absorbance between 20 to 30 percent. So, blind case always around 100 percent reference. And the sample is somewhere between 20 and 40 percent of transmittance.

So, what I do here, so here if the sample is having very low. What I do is I take I want to limit my range to 20 to 40 percent or maybe 35 percent, because my sample maximum contains 35 percent transmittance. So, what I will do is I increase the decrease the light. So, that absorbance is maximum around 2 35 percent is adjusted mechanically to a value of 2. And that comes the remaining 20 percent will be also be expanded by the same method, but it will be somewhere between 40 50 to 60. And any sample in between these 2 would be between 20 and 35 would be measuring between 0.2 and 0.8 absorbance. And it will be showing me a better accuracy. So, this is known as high absorbance method.

Another method is supposing my sample is very dilute sample. So, what I will do is suppose it is about 80 percent transmittance and my sample is somewhere here blank is somewhere here I had just a 100 percent and this 20 percent and 30 percent I have just it know 0 level. So, any sample in between these 2 standard will the scale is expanded from

here to here. That is 0 to I will say about 35 percent. So, this is known as stress analysis method, because the sample is highly transparent here and the error if I measure without any adjustment it will be very high.

So, I am expanding the scale from here to here between let say between 90 80 70 75 percent to 0 percent transmittance. So, this is another way of dealing with substances which are very valuable very small quantity of pure substance available. And transmittance is very low. Now there may be some substances, where I have to adjust it is neither be neither at high absorbance nor at low absorbance. And then what I, but I need the very high accuracy. So, what I will do is, I select this 30 percent as 0 percent and by mechanical means by allowing more light and 40 I adjust it to 100. Anywhere in between can be more accurately described where the concentration can be increased you know and analyzed properly. So, this is known as maximum precision method.

So, there are 3 systems even the put for they have the luxury system is you can choose either this or this or this depending upon the maximum precision required for the analysis.

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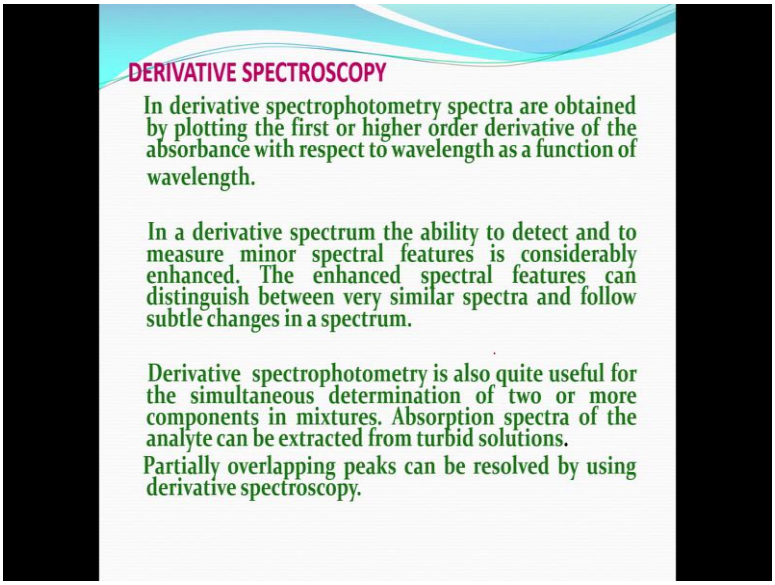

So, now I go to another utility of a spectrophotometer. Now what I need is suppose I have an absorbance spectrum something like this. This is absorbance this is concentration.

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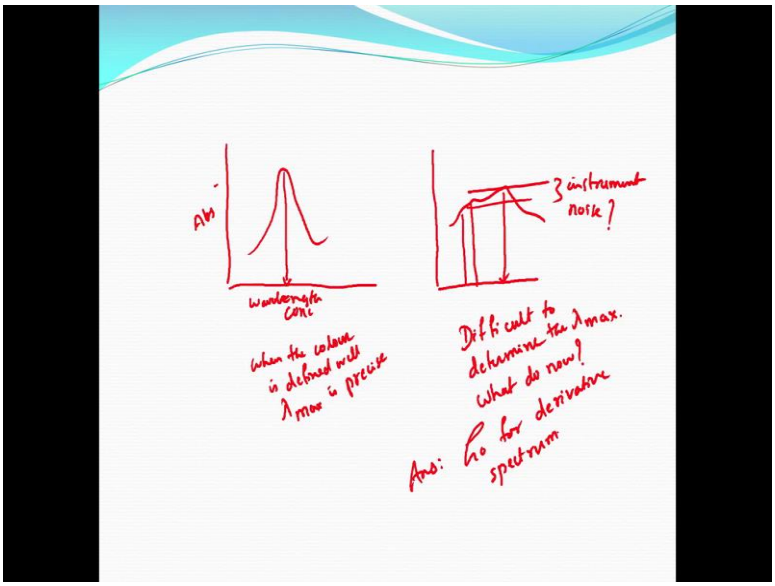

So, if I plot this normally, we expect a peak and peak with lambda max something like this. Then it is very easy for us to find out this is the length wavelength. So, I know where to take the measurement where very absorbance response is optimum.

Now, suppose sometime the color is not very well defined. This is when the color is defined well; that means lambda max is precise. Now suppose the figure is something like this. We do not know whether to choose this peak or this peak or this peak. And whether the difference between this and this could it be an instrument noise, we do not know. In such cases the spectrum so vague that it is very difficult for us to decide where the lambda max difficult to determine the lambda max.

So, what to do now? Answer is go for derivative spectrum. So, what happens in derivative spectroscopy? We required the spectrophotometric delta and scan it and then by plot the spectrum first. Then we can plot the first order or higher order derivative of the absorbance with respect to wavelength as a function, of the length; that means, d a by d absorbance da by d lambda.

So, if you plot d a by d lambda versus d lambda let us see what happens. In a derivative spectrum the ability to detect and measured minor spectral features is considerably enhanced. See the enhanced spectral features can distinguish between very similar spectrum and follow a subtle change in a spectrum. And it is also useful for the simultaneous determination of 2 or more components. If the lambda max is very near.

Now absorption spectra of the analyte can be extracted also from turbid solutions. This is also another very important advantage when we want to do a derivative spectroscopy. So, partially overlapping peaks also can be resolved very well by using derivative spectroscopy.

I am going to show you a derivative spectrum, shortly the, but before that let us see some examples.

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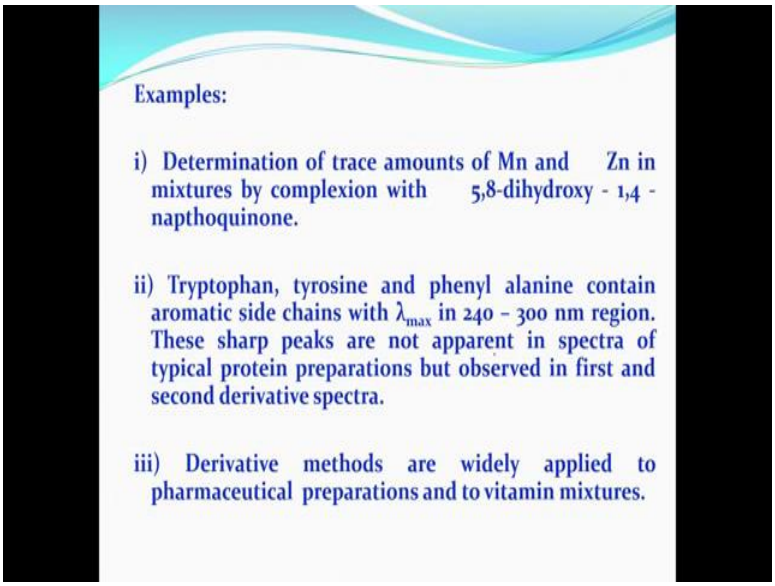

What is happening we want to determine trace amounts of manganese and zinc in mixtures by a complexion with 5 8 dihydroxy 1 4 naphthoquinone. This is an organic recollecting reagent it reacts with manganese and zinc to give you colored substances.

Second example is tryptophan tyrosine and phenyl alanine these are the amino acids they contain aromatic side chains with lambda max between 240 and 300 nanometers. I have explained to you earlier whenever you are doing this chemical UV spectrophotometry, that in benzene ring aromatic systems the peaks are not very sharp. So, the sharp peaks are not apparent in the spectra of typical protein preparations, but they are also observed in first time, but observed very clearly in first and second derivatives spectra. Then for in pharmaceutical applications definitely there will be a very good utility upset substances. So, here I am showing you the absorbance spectrum the first one is what bottom one.

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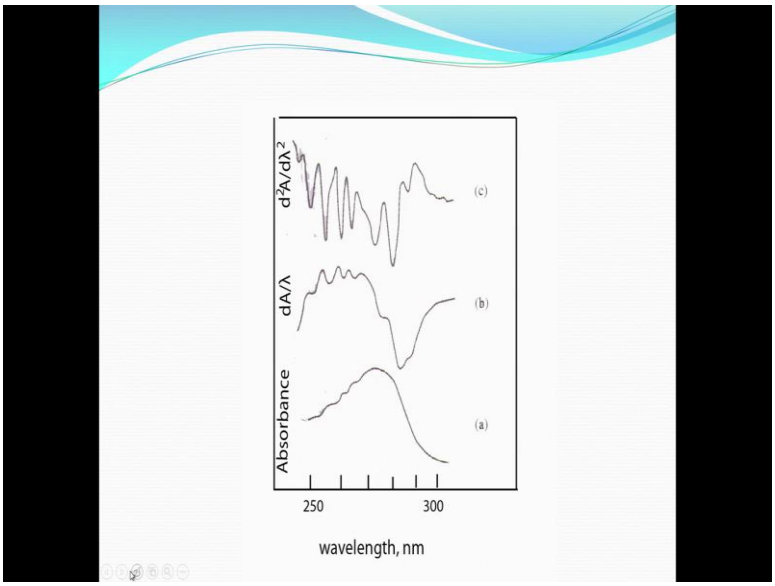

Look here, I have a peak which is very vague. And the it looks as if it has only one lambda max, and the curve is not very well characterized, there is A. Now look at dA by lambda absorbance, first derivative the same peak how it is showing 1 2 3 4 5 and then one trough here and then lambda max extra. So, suppose I do second time d A square by d lambda square. Then what happens I see the lambda max should be negative, the same peaks get converted into negative peaks, but much sharper 1 2 3 4 5 6, so many other things.

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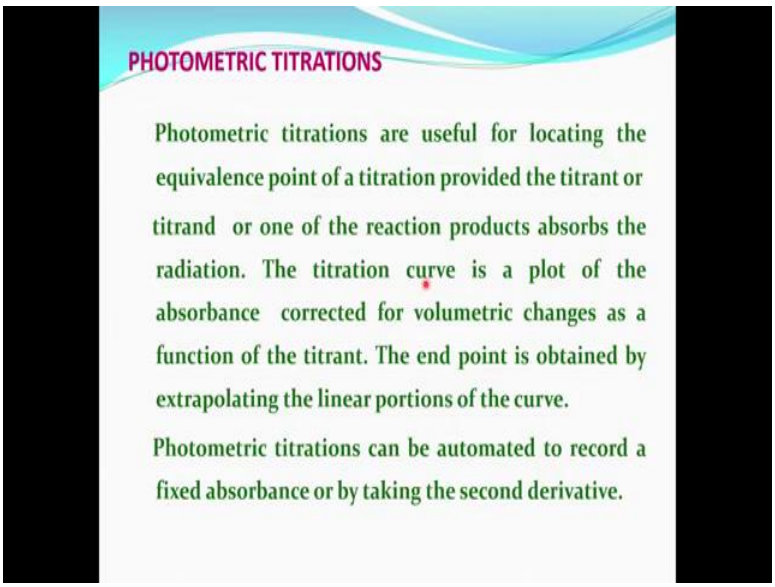

So, this is how the utility of derivatives spectra can be employed to exactly assess; where is the lambda max for different colored system. So, we will also discuss about photometric titrations.