Modern Instrumental Methods of Analysis

Prof. J.R.Mudakavi

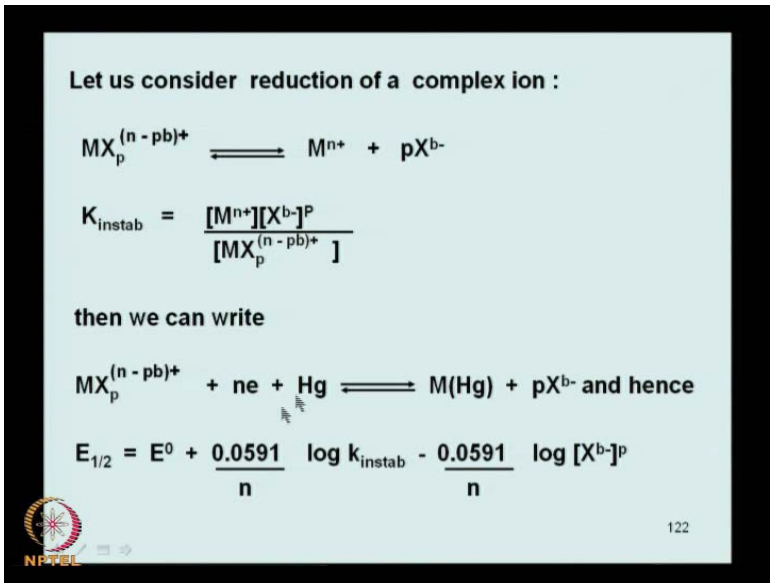
Department of Chemical Engineering

Indian Institute of Science, Bangalore

Lecture No. # 39

Polarography-2-Applications

(Refer Slide Time: 00:22)

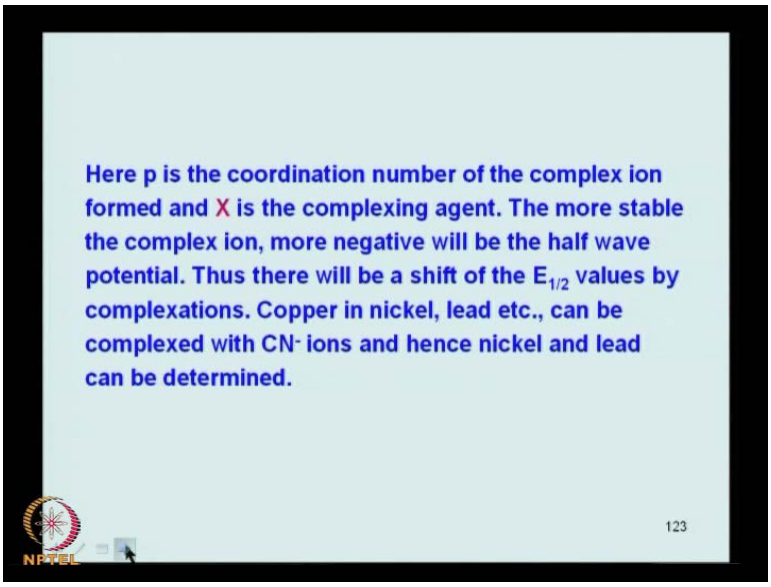


We will continue our discussions, on the polarographic metals of analysis and we are discussing about the complex ion about a metal ion complexing with element X with a with a with p molecules; and its balance is b minus and we can represent the complex like MXp raise n minus pb plus and this compound, if I dissociates to Mn plus and pXb minus we can write the instability constant as shown here that is Mn plus into Xb, X raise to b minus whole raise to p; this p comes as the exponent divided by the and dissociated molecule .

Then we can write the equation like this and MXp raise to n minus pb plus and n electrons plus mercury that is the reduction process and complex will dissociate and get reduced to the metal and form amalgam with the mercury and the elegant we get released. . So, we can write for this equation E half is equal to E not 0.0 591 divided by

n, log of K instability minus 0.0591 divided by n logarithm of X base to b minus whole raise to p.

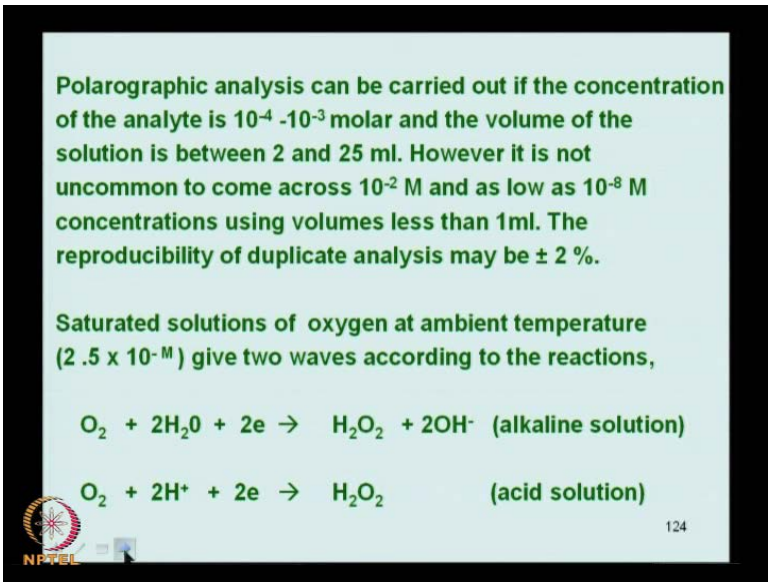
(Refer Slide Time: 02:12)



So, the here p is the coordination number of complex ion formed and X is the complexing agent, the more stable the complex ion more negative will be the half wave potential therefore, there will be a shift in the E half values by complexsation.

So, we can determine copper in nickel, lead etcetera we can we can complex them with cyanide ions and hence nickel and lead can be easily determined, because their instability constants will change.

(Refer Slide Time: 02:49)



Now polarographic analysis apart from this direct reducible metal ions and complex ions can be carried out, if the concentration of the analyte is of the order of about 10 raise to minus 4 to 10 raise to minus 3 moles and the volume of the solution usually what we take in polarographic determination it varies between 2 and 25 ml.

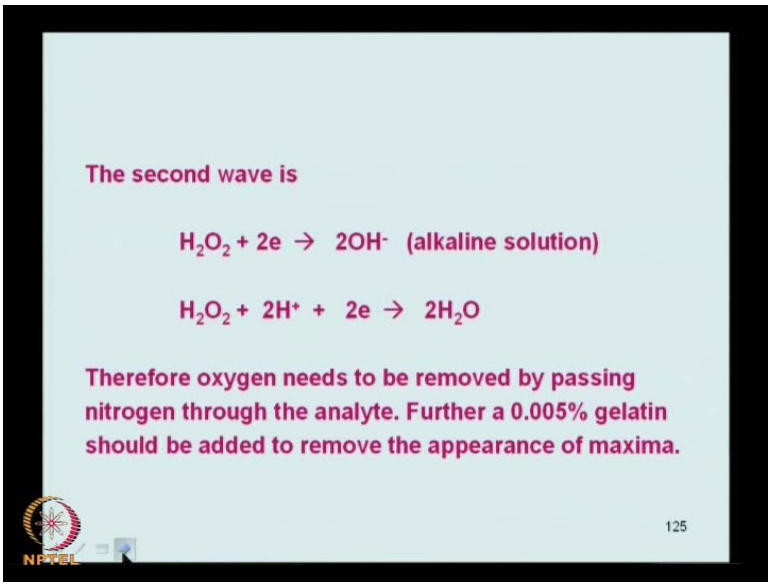
However it is not uncommon to come across concentrations of 10 raise to minus 2 molar also and as low as 10 raise to minus 8 molars concentration using volumes less than 1 ml it is not very uncommon at all therefore, the repeat reproducibility of the duplicate analysis becomes very important when you are operating in this large range. So, it should be preferably within plus or minus 2 percent

Now, we said that the polarographic solutions in which we are trying to reduce the metal ions at the cathode, mercury cathode and we are, if you remember our previous discussion we had pass the nitrogen through the solution to remove the dissolved oxygen why we do that? That is because the saturated solutions of oxygen at n v n temperature that is around room temperature etcetera.

In this concentration that is 2.5 into 10 raise to minus 5 moler they give two waves according to the following reactions that is oxygen can get reduced to 2 H2 O2 in presence of water and this happens in alkaline solution and if it can get reduced by hydrogen ions in acidic solutions forming H2 O2. So, reduced product is always H2 O2

plus 2 OH minus in the aqua solution this is the reason, why we do not want oxygen to be present in the sample solutions?

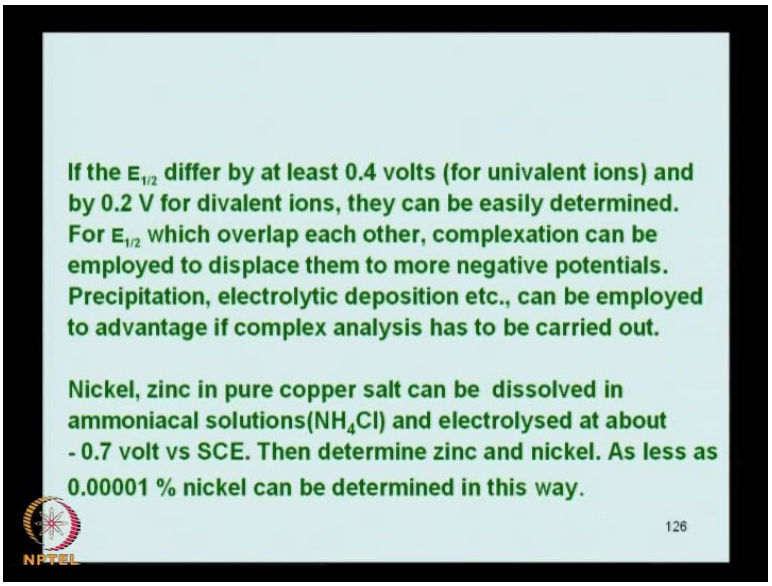
(Refer Slide Time: 05:13)



So, the second wave that is oxygen gives 2 waves; one is for the reduction of oxygen to hydrogen peroxide and hydrogen peroxide also can further react with 2 electrons to give OH minus ions.

So, it has to give second wave and in acidic medium, the reduction product is only water therefore, oxygen must be removed by passing the nitrogen through the analyte, further 0.005 percent gelatin also should be added to remove the appearance of the polarographic maxima that we have discussed in the last class.

(Refer Slide Time: 06:04)



So, if the E half that is the decomposition potentials differ by at least 0.4 volts we will be able to determine, the elements in succession that is if you have two or more elements to be determined then their E half should be different at least by 0.4 volts that is for any invalent ions like sodium, lithium, potassium, thallium, etcetera.

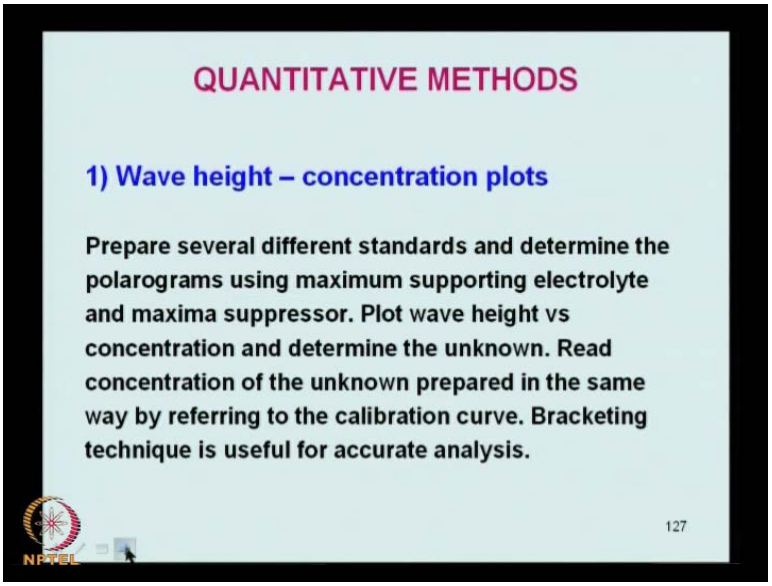
And for divalent ions the E half should be at least for 0.2 volts then they can be easily determined with sufficient clarity, otherwise there is a problem of overlapping of the reduction potentials. So, you will not know where exactly to stop, the where exactly the first element is getting reduced and decomposition potential of the second element starts up.

So, for E half which overlap each other as usual, what we can do is? We can complex them with complexing agents and then we can try to displace them to more negative potential. So, that the decomposition potentials are fairly well separated. We can also use precipitation, electrolytic deposition, etcetera to advantage you take complex analysis have to be carried out and polarographic analysis is part of the total analytical program. For example, nickel and zinc we can determine the pure copper salt by dissolving in ammoniacal solutions and electrolyze at about 0.7 volts, where SCE saturated calamine electrons then what we can do? We can determine the zinc and nickel as separately as complex ammoniacal solution.

So, as less as 0.00001 percent nickel can be determined in this way like this, you can gauss the potential of the method to determine very small quantities even in by polarography. Now, how do we really do the quantitative analysis as usual as expected, but you would like to do is to determine the decomposition potential and the wave height; if the concentration is more wave height should be more and if the concentration is less wave height should be less.

So, you can instead of concentration you can take this standard solutions determine the wave heights and for different concentrations and portly calibrations curve of the wave height verses the concentration then we can do as usual an unknown or wave sample and determine its wave height and refer it to the calibration curve.

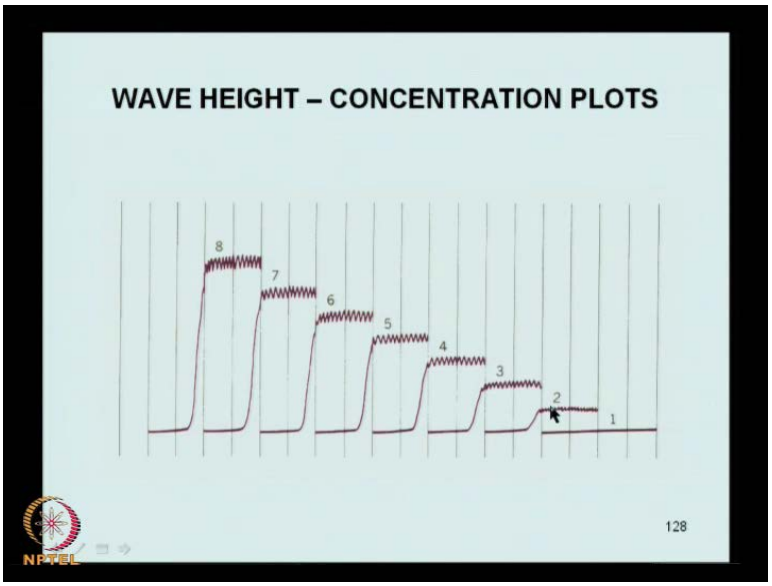
(Refer Slide Time: 09:42)



So, what we do normally in wave height concentration plots we something like this, we prepare several different standards and determine the polarograms using support maximum supporting electrolyte and maxima suppressor.

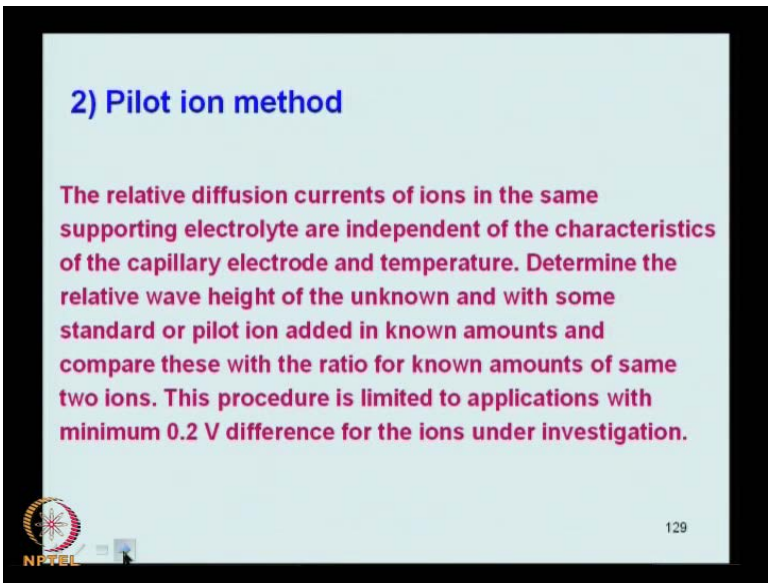
It is important that we suppress the maxima continuously all, in all almost all the samples otherwise there will be an automatic error included in the measurement of the decomposition potential and then what we do is? We plot wave height verses concentration and determine the unknown. You can read the concentration prepared of the unknown in the same way by referring to the calibration curve usually bracketing technique is useful for accurate analysis.

(Refer Slide Time: 10:32)



So, this is a typical plot of the wave height concentration plot. Here, I have a blank and some concentration here. this is a theoretical presentation and 3, 4, 5, 6, 7 etcetera you can see, the wave heights; that is the distance between this and this and this and this and this and this they are all increasing and we can plot the wave height verses concentration curves it is a very simple technique basically.

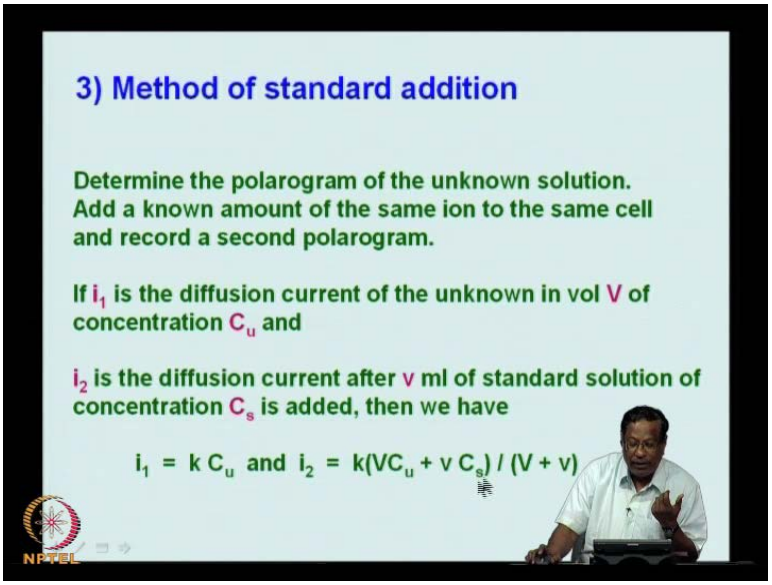
(Refer Slide Time: 11:11)



Then we have, what is known as pilot ion method? In the pilot ion method, what we to do is the relating diffusion currents of the ions, the same supporting electrolyte we have

to recognize that they are independent of the characteristics of the capillary electrode and temperatures. So, we determine the relative wave height of the unknown sample and its some standard and pilot ion added to that in known amounts and compares this with the ratio of the known amounts of the same two ions. This procedure is limited to the applications with minimum of 0.2 volts difference for the ions under investigation. So, this is another second method more details we can read it from this standard text box, but I am only trying to give you different techniques.

(Refer Slide Time: 12:09)



And the third one is the method of standard addition, I have to tell you at this stage the method of standard addition is not necessarily a priority of the technique what we have discussing that is polarography method of standard addition can be applied even means spectro photometric analysis or hydroid generation or atomic absorption or i Cp or reading of the thing. But principal remains the same. So, I am going to explain to you the principal of the method. So, what we do is? In this case as for us, because we are discussing the polarography we have to determine the polarograms of the unknown solution.

That means you determine the wave height of an unknown solution and then what you do? You take the same unknown solution add a more amount of the same ion to the same cell and recall it second polarograms; that means, first you are taking the known sample and determining the polarograms as usual then you are taking the same amount of the

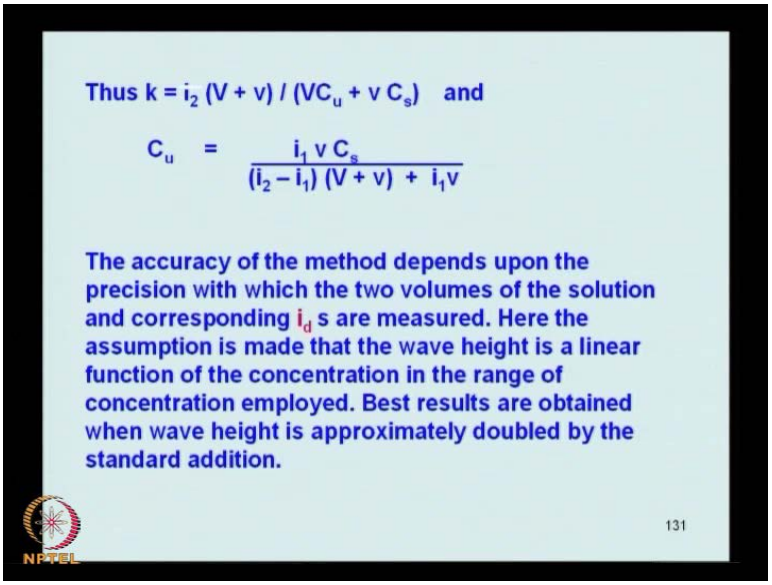
solution again in another another beaker and to that you are adding the known amount of the standard solution.

So, I have two entities now; one entity is ion is the diffusion current of the unknown in volume v; that is the original volume of the concentration, Cu let us say unknown and i 2 is the second measurement, what you have made? It is the diffusion current after a small amount of standard solution that is small v is added corresponding to a concentration of Cs.

That is the second volume we are adding a standard solution and determine the wave height current. So, we can write these two equations; that is i 1 is equal to k into Cu that is unknown, the current is proportional to the concentration of the unknown with a proportionality constant of k and i 2 would be also be proportional to the concentration of the unknown as well as concentration of the standard, but the volumes are different.

So, i 2 would be proportional to the v that is I had I had designated as capital v into Cu plus small v into Cs divided by k multiplied by k divided by capital V plus small v that is the total volume of the solution. So, why we are getting the second solution because it is the volume of the measurement measurement volume is changing by the addition of the small amount of the standard solution. So, we are having two different equations and two unknowns.

(Refer Slide Time: 15:40)



So, k we can solve for k is given by i 2 into V by plus v divided by V into Cu plus v into Cs and unknown concentration of the unknown can be determined by the simple expression i 1 into v Cs divided by i 2 minus i 1 into multiplied by total initial volume plus standard volume added plus i 1 v. The derivation of this equation is very simple. I will not be going to the details of this derivation, but it can be found any of the standard text books. So, the accuracy of the method depends upon the precision with which the two volumes of the solution and the corresponding id that is diffusion currents are measured.

We have to be very accurate, otherwise there is no point to be doing this method especially standard edition method that should be exact and measurement of the diffusion current also should be exact. Here the assumption, what is maid? Is that, the wave height is a linear function of the concentration in the range of concentration employed; that means, assume that it is a bear lambda straight line maker we are by adding the unknown solution to the standard solution by adding the either wise the standard solution to the unknown solution the total concentration would still be in the bear lambda range, if it goes beyond that, we will not be able to determine the unknown with sufficient accuracy.

So, that is when after pre conditions and we will have to be vary of these conditions, we have to make sure that we satisfy this condition all the time. Specially, when we are employing this standard deviation technique standard addition technique. So, best results are obtained when wave height is approximately doubled; that means, you are unknown volume and the known volume should be unknown should be should be double of known volume standard edition. So, concentration should be half.

So, we will stop our discussion about the polarography more details, can be had totally at this stage that polarography has developed into a very well defined beautiful analytical technique. Especially, for the separation and speciation of the elements in different matrices and with respect to speciation, polarography is rather superior to atomic absorption, atomic emission, etcetera, because their speciation is not so easy to define. Because, the changes in this balance itself are not so prominent in the atomic absorption or atomic emission. But, in polarography just by speciation; that is the exact composition of the metal ion as a complex in the known solution it can represent or it can give you a

differentiable diffusion current which can be correlated to the chemical existence of the different species of the same element also.

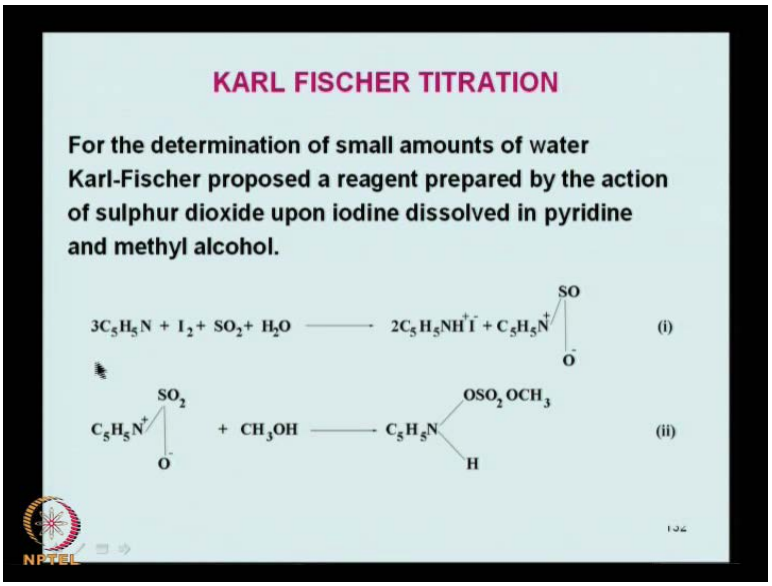
So, that is an advantage, why lot of people especially with variable with variable oxidation states and chemical compositions they prefer voltammetry, polarography and anodic striping voltammetry to exactly identify the different chemical species of the element in a given matrix. Now, I want to turn your attention to the Karl Fischer reaction. So, Karl Fischer reaction is a very beautiful Karl Fischer reaction is a very beautiful way of determining the moisture contained in a given sample.

Suppose, I give you a any salt and you want to determine the moisture contained in that. Normally, one will just heat it to about 100 or 150 degree centigrade and remove the water by evaporation and dry it and put it cool and it take to a weighing machine to determine the difference correlate it to the concentration of the water that is present in the given sample, but situation may arise when you heat it the compound may undergo different transformations it may decompose.

So, in such cases you cannot wait and if the concentration of water or moisture is in parts per million level. If, it is in percentage level? Yes. It can be done by simply weighing and noting the differences, but if it is in part per million levels you want to determine and we do not have a simple method of quantitative determination of water in any of the samples at parts per million levels

The only way, we know of this kind of analysis is Karl Fischer titration and the beauty of this is the Karl Fischer titration can be applied to organic substances as well as inorganic substances, in situations like pharmaceutical preparations, where water should not be there and such situations can be monitored by using Karl Fischer titration. And for the determination of very small quantities of water. So, Karl Fischer is a scientist they are scientist they proposed a reagent prepared by the action of sulphur dioxide upon iodine dissolved in pyridine and methyl alcohol.

(Refer Slide Time: 22:58)

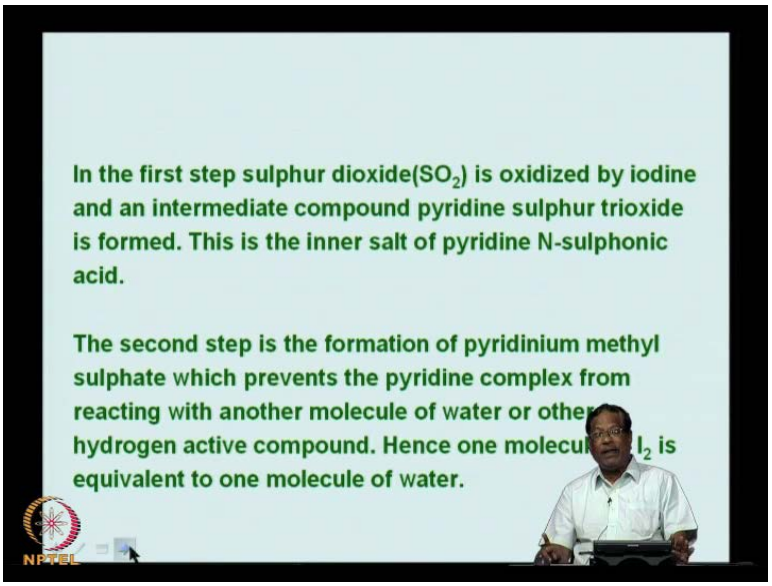


So, the reaction may be able to be represented like this that is pyridine, 3 molecules of the pyridine C5 H5 N, will react with 1 molecule of iodine and sulphur dioxide 1 molecule in presence of water. That is, if water is present then it forms a new new complex like this 2 C5 H5 NH plus i minus this is a salt just like sodium chloride Na plus Cl minus, I have 5 H5 NH plus and i minus and then it forms a complex C5 H5 N plus SO 2 is joined here with nitrogen SO minus.

And if, I add methyl alcohol because the reaction is with methyl alcohol also, the methyl alcohol will react with this charge on the oxygen for me OSO2 OCH 3; that means, we ensure that one only one molecule of hydro water is reacting in this whole system and we prevent the reaction with another molecule of water by having methyl alcohol.

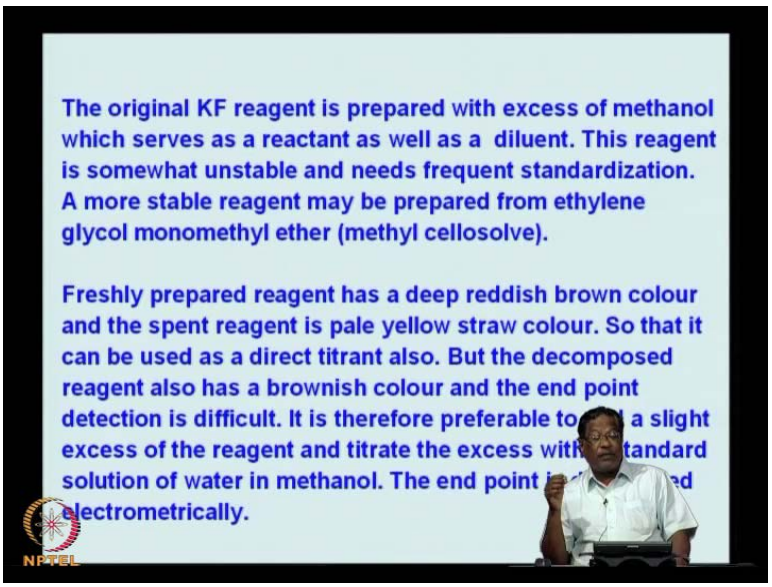
Methyl alcohol binds to this position and then it for produces an ester. So, the first step, in the first step sulphur dioxide is oxidized by iodine and an intermediate compound of pyridine, sulphur trioxide is formed; that is, this is the inner salt of pyridine and n-sulphonic acid that is very easily understood this one C5 H5 N plus. SO, O minus is the inner salt of the pyridine n-sulphonic acid.

(Refer Slide Time: 25:02)



The second step is the formation of pyridinium methyl sulphate which prevents the pyridine complex from reacting with another molecule of water or other hydrogen active compounds. Therefore, we make sure that only one molecule of iodine is equivalent; that means, it is reacting and only one molecule of iodine is equivalent to one molecule of water.

(Refer Slide Time: 25:44)



So, the stoichiometry is established that 1 is to 1 stoichiometry. So, the original Karl Fischer reagent is prepared with excess of methanol which serves as a reactant as well as a diluents, this reagent is somewhat unstable when it is prepared freshly it is good.

But, over a period of time it decomposes and then it is unstable therefore, what we have to do is? We need to standardize the Karl Fischer reagent frequently. Regularly, whenever you want to use any Karl Fischer reagent first step is to standardize the Karl Fischer reagent and then use the new standard value for the calculation of the water in the sample in which you are analyzing.

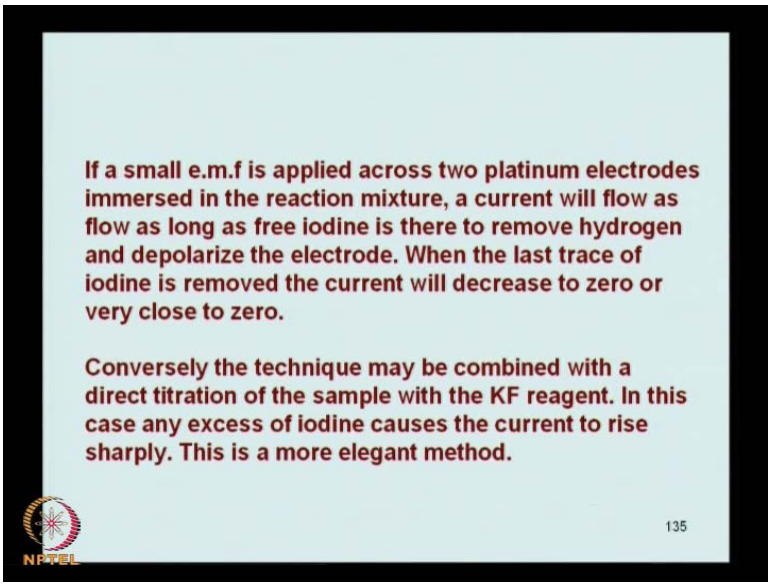
Now, a more stable reagent can be prepared from ethylene glycol mono methyl ester; that is a compound it is popularly known as methyl silozol there are number of mono ethyl ether which are known as methyl silozol, butyl silozols and then carboxi methyl silozols etcetera.

But, methyl silozol is a fine it works for the system others also do work. So, freshly prepared reagent has a deep reddish brown color and this paint reagent has a pale yellow color. So, just by looking at the Karl Fischer reagent you will know whether the reagent is good enough or not.

If it is having deep brown color with less yellow tinge you can say that, the material is useful otherwise one has to prepare fresh reagent. So, that it can be used as we can also use it as a direct titrant that is you take the dark brown Karl Fischer reagent and titrate it in a solution containing your salt. You either dissolve the salt or suspend the salt in methyl alcohol and then titrate it directly using a just like any ordinary college titration from the burette take the Karl Fischer reagent and titrate it until you get a pale yellow color.

So, but the problem is the decomposed reagent also has a slight brownish color and the end point detection is somewhat difficult therefore, what people do is? We add a slight excess of the reagent and titrate the excess with a standard solution of water in methanol. So, we know how much of water we are we are adding and to determine how much of the Karl Fischer reagent is consumed. So, the difference is going to tell you the actual Karl Fischer reagent that has been added extra. So, it is end point is easily determined even otherwise end point can be determined electrometrically; that means, when the reaction is complete you will not see any current.

(Refer Slide Time: 29:39)



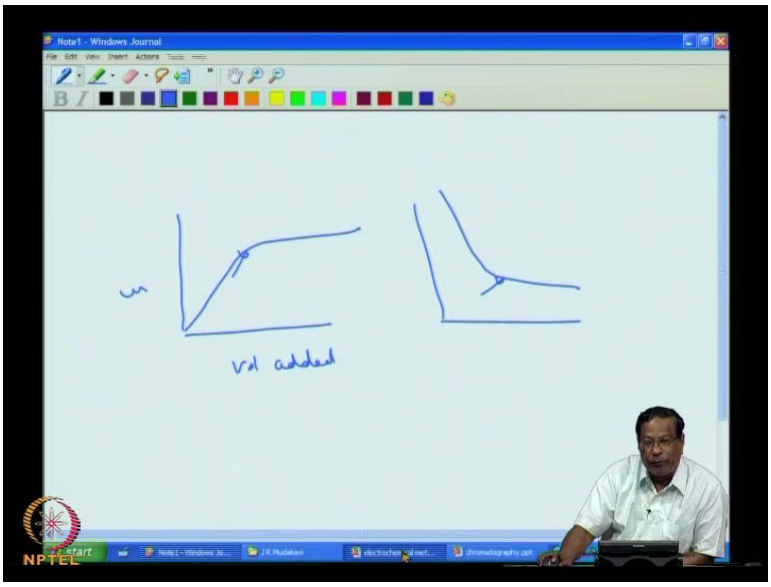
If a small e. m. f is applied across two platinum electrodes immersed in the reaction mixture, a current will flow as long as free iodine is there to remove the hydrogen and depolarize the electrode. When the last trace of iodine is removed by reacting with the water molecule present in the sample. Then what happens to the current? The current will decrease to zero. So, initially you will get a very high current reading and then as you keep on titrating the iodine will get consumed and the current will keep on decreasing and when the last trace of iodine is removed the current will become zero or very close to zero.

So, such reactions are known as dead stop reactions. So, dead stop reactions will exactly be able to pinpoint the end point of the titration irrespective of whether you will be able to they able to identify the color change or not. So, long as you are measuring the electric current when the moment it reaches zero that is the end point. So, the electrometric end point for Karl Fischer reagent is much more reliable and dependent for the determination of water.

So, conversely what we can do is, the technique may be combined with a direct titration of the sample with the Karl Fischer reagent; in this case any excess of iodine causes the current to rise sharply either you can take it in the beaker or in the burette. So, you can do either way add excess or then titrate or you can straight away titrate the substance

with the Karl Fischer reagent and any excess of iodine causes the current to rise shortly, if you are adding Karl Fischer reagent extra.

(Refer Slide Time: 32:00)



So, either way it is a dead stop reaction in one case you would see a figure like this that is volume added and current. So, it keeps on increasing and otherwise in the other case what happens is it will become a dead stop reaction. So, either way you should be able to determine the end point of the reaction, because that will not be that will be either increase or no increase depending upon whether you are able to whether how you are conducting your experiment we are taking it in the burette or in the conical flask whichever way is you are handling.

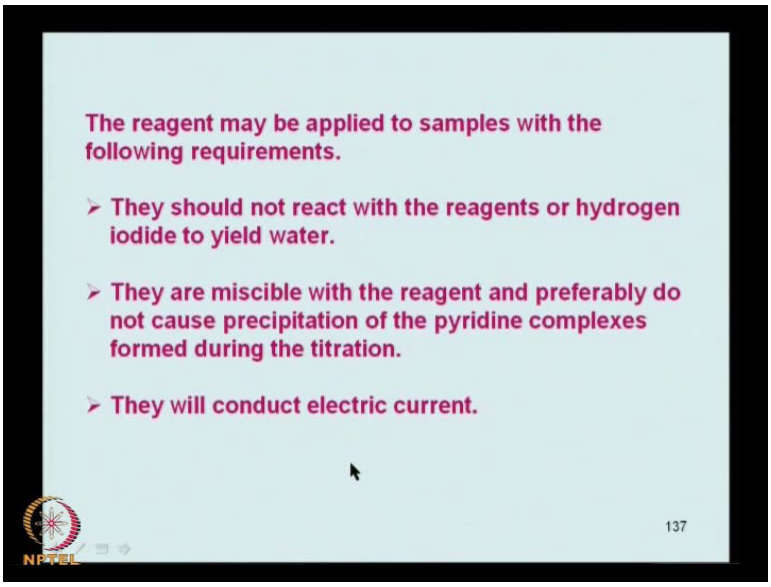
Sometimes what happens is, if the sample is very costly then we do not want to waste it. So, we take a small quantity in the conical flask and take the Karl Fischer reagent from the top that is from the burette. And if it is not so costly then we can do the normal Karl Fischer titration as usual. So, the technique can be used either either way, but the direct titration with the Karl Fischer reagent is a more elegant method.

So, the apparatus is basically vary simple the source is a 3 volt battery it is a torch battery basically, even two simple torch batteries with 1.5 volts output will be sufficient and what you need is a micrometer and you need a resistance with a 500 ohm resistor and 0.5 hat radio potentiometer. The potentiometer is set so, that there is a potential drop of

about 18 mille volts across the electrodes and does not require any adjustment until the whole battery is exhausted.

So, Karl Fischer reagent may be you can standardize the Karl Fischer reagent with 5 to 6 milligram of water dissolved in methanol or you can titrate directly with ah disodium tart rate dehydrate it is a primary standard and water contained is exactly known you do not have to worry about, how much of water is there? And this because this disodium tart rate dehydrates contains 15.66 percent of water. So, they try to standardization of Karl Fischer is very easy and you just have to weigh the disodium tart rate dehydrate and titrate with Karl Fischer reagent. And you are ready for the actual analysis, the application of Karl Fischer reagent is very invariably employed in several kinds of samples all over the world including pharmaceutical industries and other industries where water contained in the salt is very important.

(Refer Slide Time: 35:45)

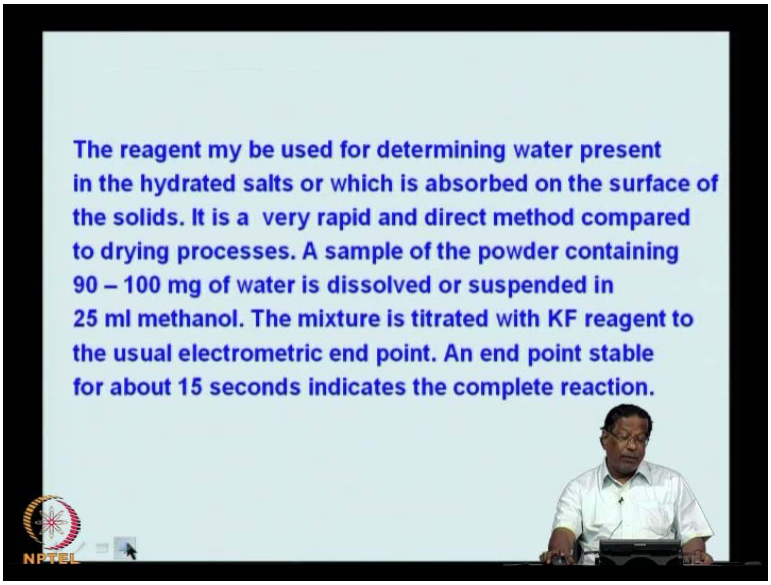


Now, there are certain problems with Karl Fischer reagent, the reagent may be applied to the samples with the following requirements, what are those requirements? They should not react with the reagents or hydrogen iodide itself to yield water. We say, if the one of the reaction product is water you will never see the end point of the titration metal because more and more water keeps on getting produced.

So, the non production of water is one of the most important aspects of this it should not if the one of the reaction products is water you should not be using this method at all

then the products the reactants and the product should be miscible with the reagent that is another requirement. And preferably it should not cause precipitation of the pyridine complexes, because we have already said that the pyridine disulphonic acid salt it is an inner salt and if that salt precipitates there is no further reaction with the methyl alcohol. So, it should not precipitate during the titration and if you are conducting a potentiometric titrations then they will preferentially there should be electric current should be passable; that means, the solution should be fairly dilute and easy reproducibility should be obtained.

(Refer Slide Time: 37:21)



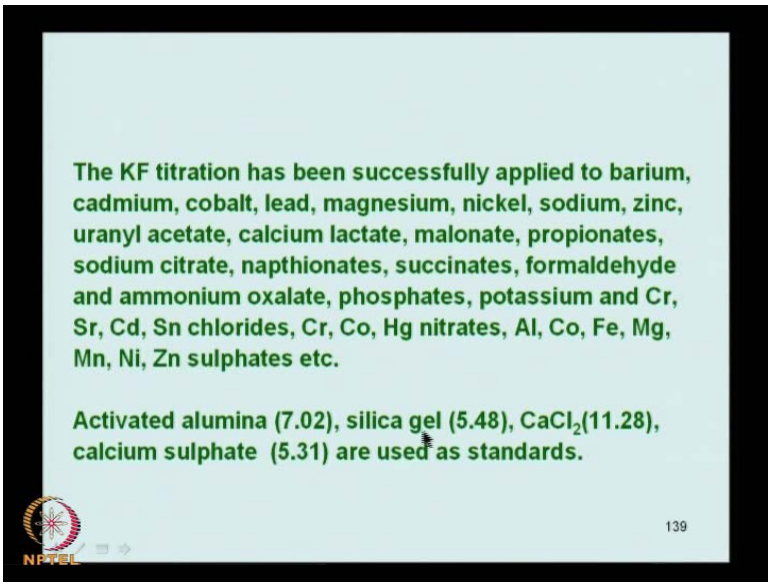
So, the reagent may be used for determining water present in the hydrated salts most of the salts solutions, what your salts? What you buy in your laboratory they all have water of crystallization and the water of crystallization is determined by titration with Karl Fischer reagents and which is this is absorbed on the surface of the solids. So, water may be hydrated hydrated salt or sometimes the water may be just absorbed you keep the salt open it will absorb moisture, absorb on the surface that also can be determined; that means, even if the samples do not have the water of hydration, but they just absorb water from the atmosphere such water also can be determined by the Karl Fischer titration.

It is a very rapid and direct method compared to the drying process. A sample of the powder containing 80-100 milligram of the water is dissolved or suspended in 25 ml of methanol, why 25 means? It is only a question of convenience it is a titration basically a

titration procedure and 25 ml is fairly good amount for titration you can manage with stainless well and good and you will be saving the Karl Fischer reagent and Karl Fischer reagent cost somewhere about 400 to 600 ah rupees for 500 ml.

So, the mixture is basically titrated with the Karl Fischer reagent with the usual electrometric end point an end point stable for about 15 seconds is what you should look for that indicates the complete reaction otherwise you know the end point will come again it will become brownish again you will add end point etcetera add add the Karl Fischer reagent and there will no end. So, the idea is to for about 15 seconds if the end point is color is stable straw color.

(Refer Slide Time: 39:52)



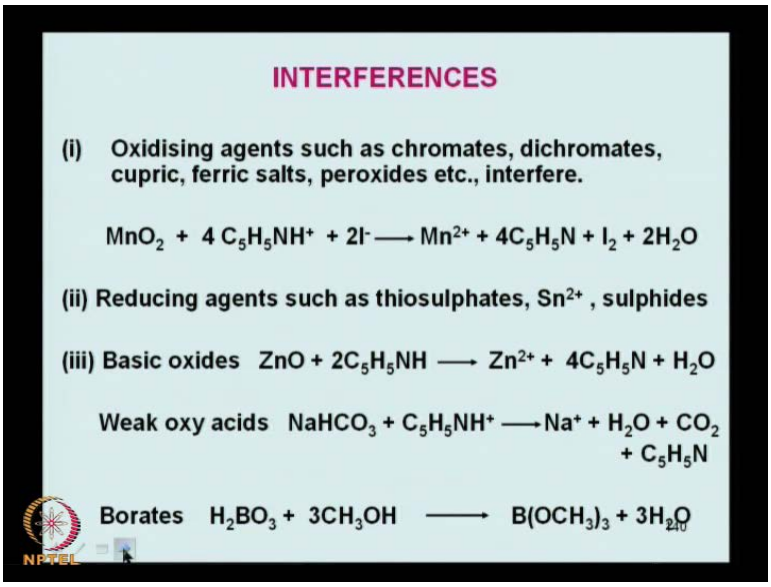
Then that indicates the end of the reaction. So, the Karl Fischer reagent has been successfully applied. I am giving you a small list of the chemicals you will see the applicability of the Karl Fischer reagent, the wild applicability and popularity of the Karl Fischer reagent with respect to the following salts.

We can apply them to barium, cadmium, cobalt, lead, magnesium, nickel, sodium, zinc, uranyl, acetate, calcium, lactate, malonate, propionates etcetera; these are all organic salts of these metals and then sodium citrate then napthionates, succinates, formaldehyde, ammonium oxalate, phosphate salts and potassium salts, chromium salts, strontium, cadmium, tin chlorides in a chloride salts then nitrate salts of chromium, cobalt, mercury etcetera. And then sulphate salts of aluminum, cobalt, iron, magnesium,

manganese and then nickel, zinc sulphates etcetera these things are it just goes to indicate a variety of the salts that are that can be that can be adopted for the determination of water by Karl Fischer reagent.

You can even take activated alumina and find out, how much of the water is absorbed on to that? We can take silica gel approximately 5.48 percent is water is absorbed on to that and then calcium chloride, calcium sulphate these are used as standards for Karl Fischer titration.

(Refer Slide Time: 41:54)



Not just methanol, but the other standards are also quite possible to use in the Karl Fischer titration. Now, if it is, if I am singing so much praise about Karl Fischer reagent is it a panacea of all water analysis problems is unfortunately no. There are problems associated with the Karl Fischer reagent that is sometimes you do come across interferences and interferences you can easily guess if you are a chemist, because you will have that kind of chemical intuition, but otherwise for your reference I have listed them in the next slide.

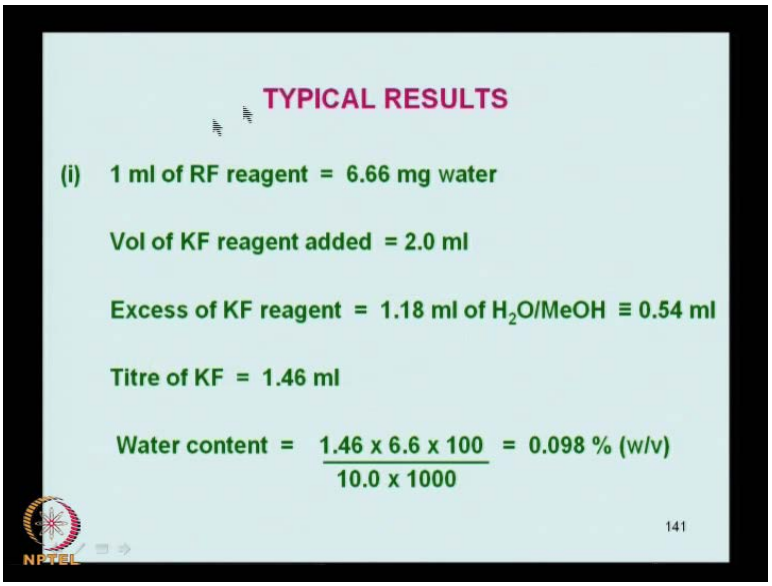
I have the first slide, the next slide what is showing is oxidizing agents such as chromates, dichromates, cupric salts, ferric salts peroxide etcetera. They interfere because they react with iodide they oxidize iodide to the produce iodine. So, a simple reaction representative reaction is MnO 2 and it reacts with iodide to produce manganese 2 plus followed by pyridine and iodine plus 2 water molecules.

So, this is the first kind of reaction where we have water as the product. So, such reactions cannot be employed in Karl Fischer titration, because if we were determining water we cannot be producing water by the reaction and then we have thaiosulphades, stannous chloride, sulphides etcetera these are reducing agents and they reduce iodide to iodine that is not very idle then basic oxides are there zinc oxide they react with pyridine directly and get reduced to zinc and pyridine and again they produce water one of the precondition for not applying the Karl Fischer reagent. And then weak oxy acids like sodium bicarbonate they react with pyridine ion then they produce water again not very good and then borates react with methyl alcohol which is one of the components of the Karl Fischer reagent and they produce B OCH3 thrice that is methyl borate, methaoxy borate and again they produce water not very ideal.

So, any reaction where water is produced we cannot use it for Karl Fischer reagent method to determine the amount of water that is present any salt. Still it does not reduce the importance of Karl Fischer titration, because the applications are much more enormous much more complicated and complicated and applications are more complicated salts for which there are no methods available, but that is what I meant and the Karl Fischer reagent is a fairly good reagent of course, it is a dirty reagent its smells quite rotten.

But, the importance can be realized from the fact that they for the invention of this Karl Fischer reagent noble prize was awarded for to the inventors typical results, what we normally use in Karl Fischer reagent is shown by the next slide.

(Refer Slide Time: 46:36)



That is this if I use 1 ml of the Karl Fischer reagent standard value is 6.66 milligram of water and volume of Karl Fischer reagent added is about 2 ml. So, excess of Karl Fischer reagent is 1.18 ml of water methanol mixture that is 0.54 ml excess is added.

So, the titer value is 1.46 that is from 2 ml excess is 0.54. So, titre value is 1. 46. So, 2 minus 1.46 are 0.54. So, the water content can be very easily calculated using this expression 1.46 into 6.6 into 100 divided by 10 into 1000 that is we directly convert the water into percentage straight away.

So, that is about the Karl Fischer reagent and I will stop here, our discussions on the electrometric methods the reasons, why I have introduced Karl Fischer reagent? In this topic is because it has got an electrometric end point which is exact. So, though it would be nice, if you remember this Karl Fischer reagent for your future references anywhere you want to determine water of hydration water of absorption etcetera and that is the only way we know for quantitative analysis of water.

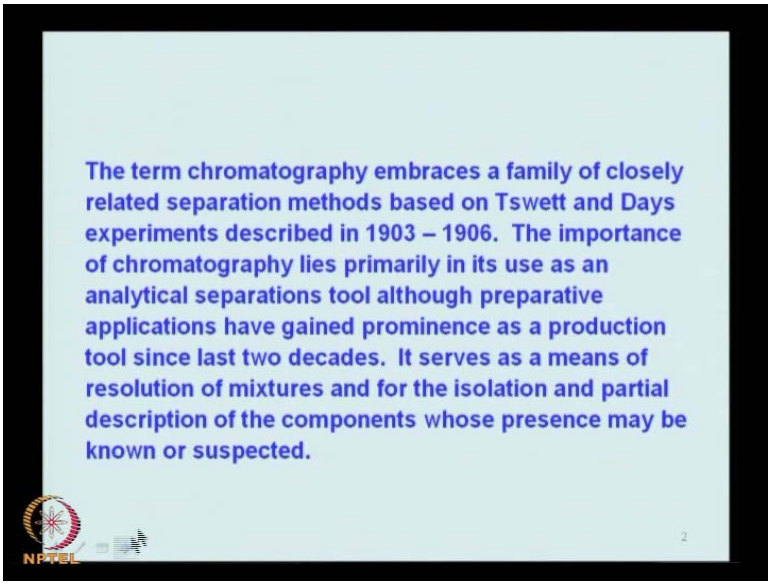
So, what I would like to do now, I will complete our discussions on this electrometric methods of course, there are several other electrometric methods like a anodic striping voltammetry and then conductometry, coulometry and high frequency titration etcetera. But, the inputs what I have given to you so far, with respect to electro chemical techniques will serve as the basic learning material and you will be able to graduate to other methods whenever there is a need for you to look into those other techniques and

you will be able to adopt yourself very easily to other techniques whenever there is a need.

So, I will end our discussion on the electrometric techniques here now. And we will move on to chromatographic techniques. So, this is an entirely different aspect from an electrometric techniques or spectrograph spectroscopic techniques, spectrograph and electrometric techniques etcetera. Chromatography is an entirely different together altogether. So, we need to understand that chromatographic techniques basically serve the purpose of separating the components in a given mixture.

So, with this basic objective defined, I will take you to this another world of chromatography and I would like to start off with the with the title that is again in my next slide.

(Refer Slide Time: 50:35)

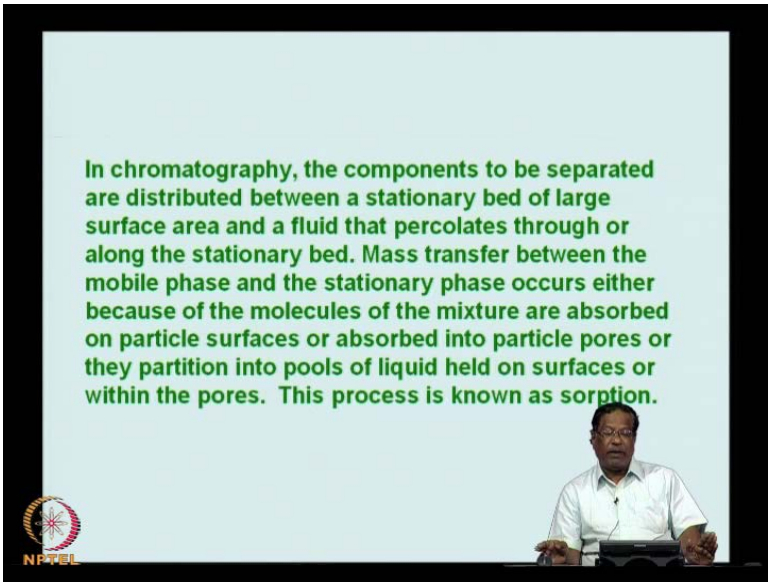


And this is followed by my definition of chromatography that is, what I have told you the basic aim is to separate the components. So, the term chromatography embraces a family of closely related separation techniques based on the observation of doctor Tswett and days they made a number of observation and experiments during 1903-1906. We can imagine that the chromatographic techniques are not really very old you will appreciate that they are only 100 years old now, but the amount of amount of knowledge and the amount of advances that have come through in chromatography techniques is truly enormous and mind boggling.

So, the importance of chromatography lies primarily in its use as analytical separation tool although nowadays since last 15-20 years chromatographic techniques have been used as a production tool. Since, last two decades especially high quality pharmaceutical products they many of them are being produced using preparative chromatography that is you not only produce you separate the components and take out the pure components and start marketing the drugs and pharmaceutical, enzymes etcetera.

It serves as a means of resolutions of mixtures and also for the isolation and for partial description of the components whose presence may be known or suspected. You may be knowing all the components still you would like to separate or you may suspect that it could be contaminated etcetera and still you should be able to separate.

(Refer Slide Time: 52:48)



If the situation so, demands. in chromatography the components to be separated are distributed between a stationery phase and a mobile phase; that is the stationery phase will have large surface area and through which liquid will pass through, it percolates through the stationery phase along the stationery bed and must transfer between the mobile phase and the liquid phase takes place occurs either, because the molecules of the mixture are absorbed or absorbed and the particles surfaces or they are dissolved in particle pores pores between the particles in the liquid they are or they partition into pools of liquid held on the surfaces or within the pores; this process is known as sorption.

When the when the component passing through a stationery bed dissolved in a liquid as it passes through, it may be held on to the particle size on the surface of the particle or in between the particles there will be some space. And through which the liquid will percolate and in this liquid it may be absorbed and there will be distribution between the stationery phases as well as in the mobile phase. So, the process is known as absorption.

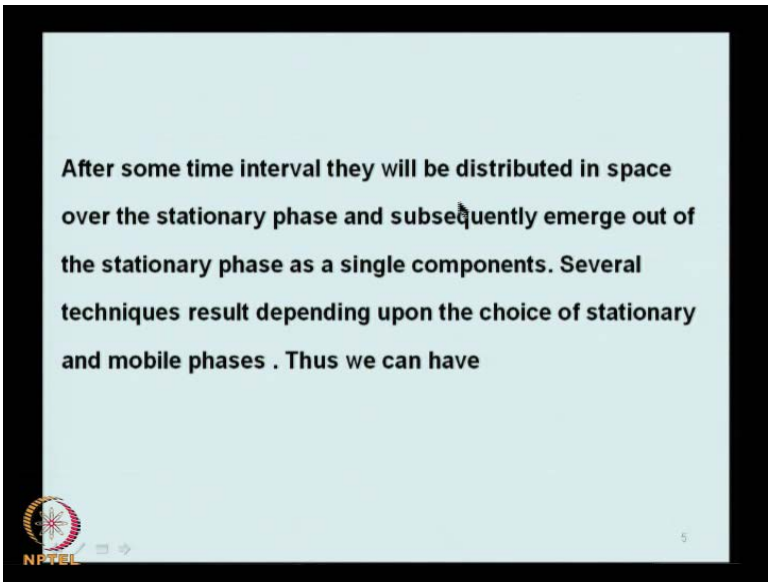
Separation of the components in a sample is based on the fact that the rate of travel of individual solutes solute molecules through a column or a thin layer of absorbent is directly related to the partition that is how the molecule distributes itself between the two; a stationery phase and the mobile phase it is between the molecule and stationery phase the molecules may get distributed and then they run through at different speeds.

The partition coefficient of each component determines how much of it is in a liquid phase? And how much of it is in the stationery phase? So, if selective retardation difference is prevail; that means, if the molecules are held selectively and they travel at different speeds each component can travel through the column or along the stationery phase at a rate dependent upon this option characteristics if this option is very high, the absorption is very high; that means, the travel would be slower why are the stationery phase otherwise if it is not at all absorbed it will just run through. So, after sometime what happens sometime interval they will be distributed in space over this stationery phase?

Suppose I have a long column of about 2 meters then initially they would have all be in a simple mixture after about 1 feet there will be separation and they will be running at a different speeds. So, after 2 feet there will be further separation of the component and at the end of the column they will come out individually separately.

So, you can imagine the whole process like a running race. So, the fastest man who is not absorbed will come out fast and the slowest man who is getting absorbed and released absorbed and released his speed will be reduced and he will come out later, but everyone will be a winner at the end; that means, all the components will be passing through the column and coming out; that means, every participant is a is able to reach the winning goal post.

(Refer Slide Time: 58:01)



So, if you a look at the slide, the after sometime interval they will be distributed in space over the stationery phase and subsequently emerge out of the stationery phase as single components. Several techniques result depending upon the choice of the stationery phase and mobile phase we can have a number of stationery phases and number of mobile phases that is how? We have a number of chromatographic techniques and then the next slide I am going to show you this. We will we will discuss, what are the different types of chromatography and how we can employ them for our separation purposes.

.

.

.