Course on Analytical Chemistry
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Module No 01
Lecture 05: Methods (Contd.)

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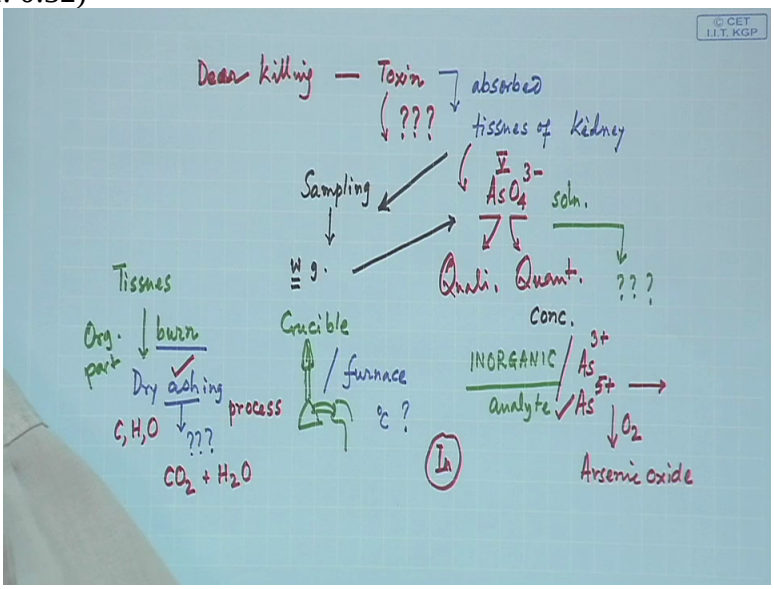

Good evening, welcome to this 5th class of analytical chemistry where we are discussing about some real example where we have seen that deer killing by some toxin and analytical chemist has the liability or responsibility to identify this toxin and whether this particular toxin is responsible to kill those deers in a natural forest or in some other places. So is basically this particular type of toxin which comes under the toxicology research, can be lethal to the human being also.

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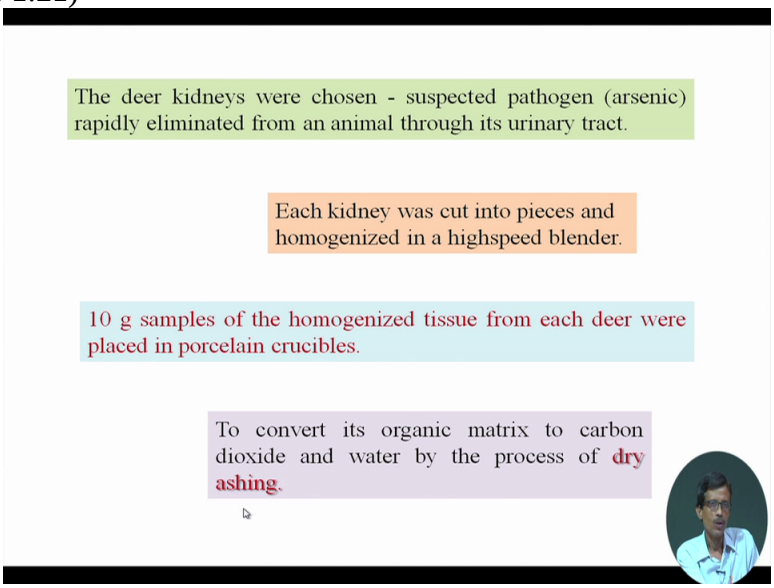

And where we are discussing about the sampling where we have seen that the deer kidneys were chosen wherefrom we take the sample basically because we are suspecting the possible pathogen which can rapidly eliminate from any animals through its urinary tract. So if you are also poisoned with something which is toxic to your body and that can also be eliminated from your body through the urinary tract via kidney. So these particular toxin will be there and it will be absorbed within the tissues of kidney.

So tissues of kidney will be therefore handled to find out the possible poisoning effect of ASO43 -. That we were discussing last time that how to identify, that means we all know that there are now 2 aspects of analytical chemistry, what an analytical chemist can perform to identify whether the species is typically ASO43 - or some other species like that of our ASO33 - or anything else that is responsible for the killing of all these deers.

So initial thing is the identification, that means the qualitative aspect and then the amount. So these 2 are pretty important and these each of them are related to each other because once we find out the amount of arsenic is there and the amount of arsenic poisoning, so we will be talking something related to the concentration of arsenic in all these living tissues in the body as well as in the kidney. So we should have this particular thing. Today we will also discuss probably in this particular class or the next class that how to get these samples.

That means the sampling process which is a very important step in analytical chemistry, how to get these samples? Because we have seen that how we can handle the Martian rock, how we can handle any ore of iron ore now we are seeing something where the biological samples for the biological tissues are there from where we can find out the amount of arsenic or as ASO43 - which is responsible for the killing of those deers.

So to how to get that? So if we have two kidneys, we all know that we have two kidneys, was cut into pieces and homogenised in a high-speed blender. So we know that like (())(4:42) we use some blending process. So similarly these chopped kidneys, so chopped kidneys of these deers will be cut into pieces and then high-speed blender is utilized. That means we are taking out of these species from the absorbed kidney.

So how much we should take? So 10 grams never sample of the homogenised tissues from each deer were placed in the porcelain crucibles then. So we go for blending process and fulfil mom and then we get the sample. So some amount of sample. So the W is important. That means W gram of this because the technique will have some more and the former limitation that how much sample we should take, what type of analysis it is.

So this W gram of sample will be required to get this particular ASO43 -. So how to get that? So that is the real challenge, that is the real question to everybody and even to the analytical chemist and how you get these samples from this particular kidney sample in the solution form. Suppose we think that ASO43 - is in the solution form because as we all know that this is very easy to handle.

Even if you are given for sodium arsenatesample in some unknown solution, you can very easily find out the identification of this particular species as sodium arsenateas well as the amount of that particular species present as the required concentration what we are going to determine. So in the solution state, how we can bring the species which is originally absorbed within the kidneys. So these homogenised kidney samples, we take in a crucible.

So why we take these crucibles? Because the crucibles can withstand a very high temperature and our idea is therefore for the procedure for the methodology is that we get these tissues. So you have the tissues. So tissues has to be burnt. So tissues are there. If we burn them so what is

that? What sort of thing we can do by burning this process? We take this in crucible and if you just simply heat it, so if you can take that, so you all know that if we take this in the on the open burner flame, so it is in the crucible.

So you take this burner and we heat it, so what will happen? So this organic part, these biological tissues, they are the organic part. So organic part will be destroyed 1st because we are looking for something which is in inorganic part. So that is why we should all be careful what we are going to determine. So inorganic analyte we are going to determine from this particular experiment as a real-life example also.

So when we burn this, so a type of burning process we will get and that is known as a corresponding thing which is known as dry ashing. So what is that dry ashing? So this particular burning process where samples are taken in the Crucible and heated by a burner or sometimes it can be heated by furnace at a very high temperature, so the temperature in degrees centigrade is also should be known to us that what sort of temperature, what magnitude of temperature we should use to burn this such that we will get something in the usable form of this arsenic in some outside or in some anionic form.

So this particular thing is known as dry ashing. So is ashing. So we will go for ash formation. So if we keep on burning everything, so what we will find that we try to convert the organic matrix because the matrix we all know, that is the complex form, everything is there because blood vessels will be there, the tissues will be there and all other things are there and we try to burn these. So these kidney tissues, the kidney tissues are known as the typical organic matrix and we just heat them directly in air.

So in presence of air, this particular heating process will take out all the carbonious matter present in this organic matrix along with the hydrogen and oxygen, those are also present to convert them all to carbon dioxide and water. The way we burn the sugar, the way we burn the glucose and all these things, we all know that everything can be converted to carbon dioxide and water. So this particular process is known as dry ashing.

That means everything we are converting to a dry ash. So what is that ash? So what is leaving behind to us is that particular dry ash. So anything over there, that means we are looking for this

arsenateion. So arsenateion which was trapped within the kidneys, so we were trying to go for the dry ashing process. So the dry ashing process would be beneficial to take out this particular species in a Crucible because everything we have taken within the Crucible and we are heating at a particular temperature.

So what we are looking for? We are looking for the ash. So what is that ash? So if something is there, that means the inorganic species or inorganic ions are there. So we can have arsenic as arsenic 3 + or arsenic as arsenic 5 +, so these are the 2 common oxidation states of arsenic we all know. So in this particular case, this is arsenic 5 which is arsenateion and the other one is arsineite ion.

So these can be converted initially due to this particular process because whatever carbon we have, whatever hydrogen we have and whatever oxygen we have in the biological tissues, those will all be converted to CO2 + H2O. So any inorganic species so if we just tell them as that inorganic species is not Indium, is inorganic ? So inorganic species like that of your metalloid arsenic, any metal ion or any other nonmetal species present will be converted.

So this particular species in presence of plenty of oxygen burning. So this particular arsenic so in any form must be that particular area has been sprayed with this herbicide as I told you that some herbicide has been sprayed which is arsenic-based and that herbicide is directly converting to some oxides then. So this will give you that arsenic oxide. Since then presence of plenty of air, that means air is an oxidising agent we all know.

So among these 2, if we consider that if A arsenic 3 + and arsenic 5 + both are present, so in presence of these oxygen, only the higher oxidation state, that means the arsenic 5 will be stabilised. So we will get the corresponding oxide of AS5 +. So due to this dry ashing process, what we get?

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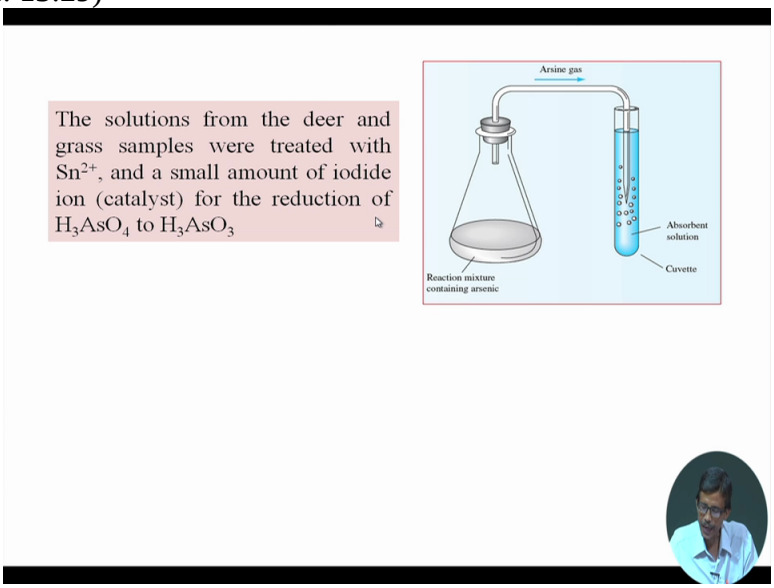

We get the corresponding conversion of the reaction which is converted to AS2O5. So this particular thing will give rise to something where we go for a solution.

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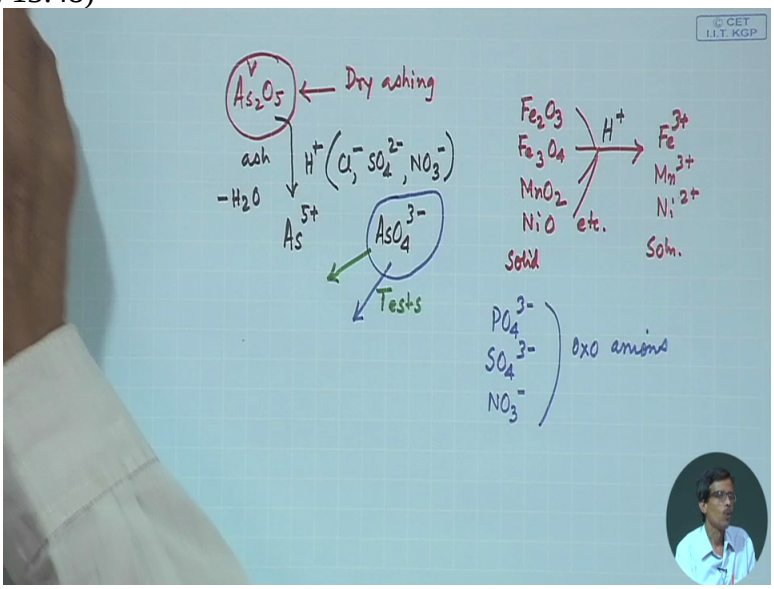

So next step what we will do is that if we get this as AS2O5, so this is the oxide what we get after dry ashing. So after this dry ashing, we get this arsenic oxide. So arsenic in + 5 oxidation state, arsenic 5 oxide, so once we get, so that is a typical example where we can analyse the different oxides as we all know that we can have species like FE3O4, MNO2, NIO, et cetera.

All these metal iron oxides or metal oxides we call, so all these metal iron oxides can be nicely converted to its corresponding solution where iron is present as FE2 + or FE3 +, manganese is present as MN3 + and NI is present as NI2 +. So these are all in solution and this is in solid state. So when we get this, so after dry ashing, what ash we get? This is the corresponding oxide of arsenic.

So how we get this particular species? That means we all know that whenever we have some oxides, hydroxides, carbonates and all, they can be attacked by typical mineral acid. That means, hydrochloric acid or sulphuric acid and so on. So in this particular case also, the treatment of these as the corresponding one, that means if we treat this directly by H + of any mineral acid source, that means if we take hydrochloric acid, we will have CL -, if we take sulphuric acid, we will have SO4 2 -.

If we take nitric acid, we will have NO3 -. So this oxide that is or oxide structure will be attacked by this producing what will go out, this oxygen will be attacked by H + and water will be removed. So arsenic this particular one if the environment is not reducing or anything else, that arsenic is there as AS5 + in solution. But remember that this is not in a isolated form. It will be not present in this particular form because what we are looking for?

We are looking for ASO43 -. So this ASO43 - was the species because this particular species has some stability, is not that when we treat this with acid like these that means the all the ions are forming as the corresponding anion. No such oxo species is forming but these are the typical anion like the there the metalloid arsenic will form as the metalloid like that of our phosphate PO43 - or SO43 - or sometimes NO3 -.

So like this that we are not getting nitrogen as N5 + or sulphur as 6 + or phosphorus as P5 + but these are typically all we all know that these are the oxo anions. So this particular technique from the laboratory techniques what we can see that typical qualitative analysis as well as the quantitative analysis can identify this oxo anions. So there are certain available tests which can detect the presence of arsenateanion in solution.

Suppose you are supplied a sodium arsenateand you are asked to detect that whether your solution has arsenateion or not, then what you can do? You can have some test where some

reagent can be used which can give rise to colour reaction or anything else where we get these as the corresponding test or sometimes if we can determine in total, the corresponding anion, that means the arsenic anion in the medium then we can detect or find the corresponding amount present in the solution.

But this particular case, we will do something else where we see that this can be reduced back. So what we will see?

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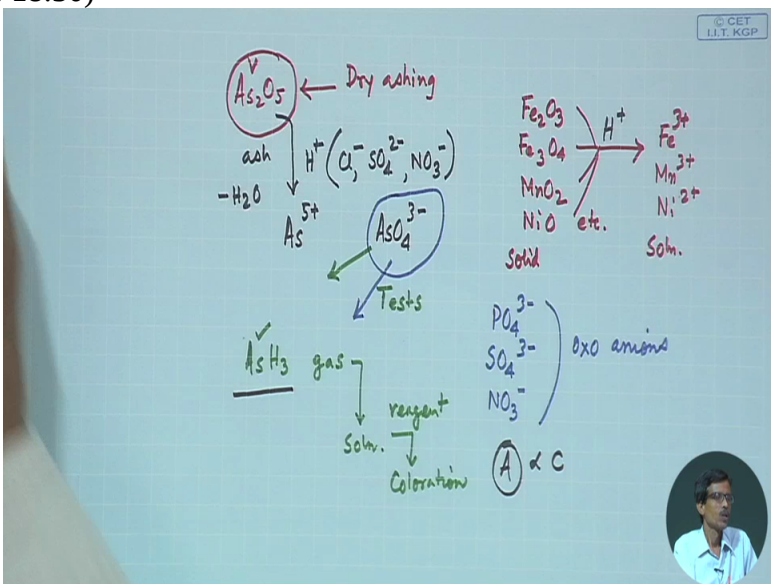

That the solution from the deer and grass samples, both were taken because the kidney samples are giving from there and the grass samples also can be burned in a similar fashion to get AS2O5, that means arsenic pentoxide. So arsenic pentoxide if it is treated with SN2 +, so initially what we get that this is the dissolution of arsenic pentoxide in mineral acid giving H3ASO4. Now what we can do?

We just reduce the corresponding thing by stanus ion. That means stanus chloride is a very simple reducing agent and that stanus chloride can be reduced this particular thing from arsenateto arsineite. So what is that particular reaction is that H3ASO4 + SNCL2, that means the stanus chloride in solution we all know that stanus chloride can give rise to the solution in hydrochloric acid.

So we can from H3ASO3 that means arsineite ion, then Stenic chloride + water or stock so the initial reduction state basically we are lowering the corresponding oxidation state of the oxides of are running from + 5 to + 3 because we are looking for something where we can avoid this arsenic to ASH3 which is the arsine gas and how to take this arsine gas that this is also a poisonous gas and is also sometimes lethal if we inhale.

So this particular technique is giving rise to some idea also that how we handle arsenateion, how we can handle the arsineite ion. If it is there in the solution or originally, there is no need to reduce this from arsenateto arsineite. Then the next step is that if we can have this particular type of conical flask, we know that this (())(20:38) flask and a reaction mixture is taken. That means the solution is taken, that means all these things are there.

And H3ASO3 is present in the solution. From there, if we do something in the 2nd step, what we do? We just get the corresponding arsine gas and this arsine gas is passed into a test tube, a smaller test-tube type of thing we all know, that Cuvette because these Cuvettes, smaller Cuvettes are very useful of definite shape and size and structure which can be directly used as the corresponding sample compartment for spectrophotometric estimation.

So these will give rise to some idea about our estimation through the use of instruments. So not only we are trying to do that this particular arsine gas, is not that in a colour test will give you arsine but we are looking for something which is present in a very low concentration and that low concentration is lethal to those deers. So deers are being killed by a very low concentration of arsenic-based herbicide.

So this absorbent is present, so that is giving rise to this particular solution. Suppose we know that if we have a alkali solution, so alkaline solution is there and if we pass carbon dioxide mother will be converted to carbonate solution in the hike was medium. So that carbonate can be analysed. Similarly what we are passing is the arsine in arsine gas. Arsine gas is nothing but our ASH3.

So this is gas and this particular gas we are buying to get in a Cuvette such that this is there in a stable form and quantitative transfer of this arsenic, whatever amount of arsenic is present, that will be present in the solution. And if some reagent is added, that should give a colouration and

we try to detect this colouration because that colour solution should give some absorbance in the spectrophotometric estimation which is proportional to concentration following Lambert ambience law. So that we will also slowly see but there is in this particular class what we see that how a real-life example can give rise to the corresponding identification as well as estimation of arsenic through the formation of this arsine gas.

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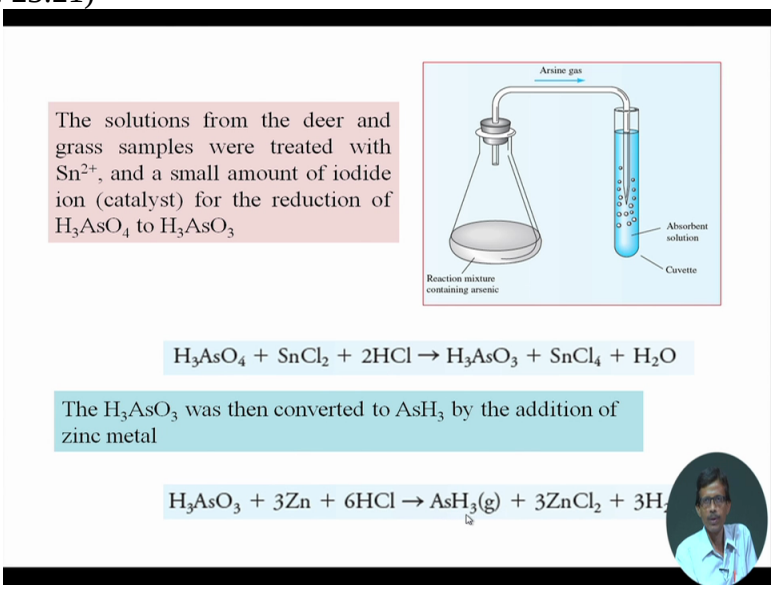

So next step what we will do is that you take this particular one, is then converted to that how this particular arsineite ion because the stanus chloride reduction is very simple and very straightforward reaction to convert the arsenateion to arsineite ion. Then this arsineite ion is then converted to ASH3. So this is your arsine gas and now a strong reducing agent, that means zinc metal, the metallic zinc because stanus chloride will not do.

So zinc metal will be utilised to convert this to give you something which is hydride. So direct conversion of this oxo anion. So this is basically ASO33 - so this oxo anion in as a dignity can be directly converted to the corresponding gas as the arsine gas ASH3. So here also the stoichometric reaction is also simple, so we should also on the corresponding stoichometric reaction for these as this with the reaction of zinc and hydrochloric acid, we all know that the zinc in presence of this hydrochloric acid forming hydrogen gas.

So that hydrogen gas is basically reacting with this H3ASO3. How? Because this is directly reacting and this oxygen is totally in totality converting to your water sample. And zinc is oxidised to zinc as zinc chloride. So this arsenic then in the + 3 oxidation state because why we are converting this this state, we are using this state is that we are trying to reduce this and we did it by using stanus chloride from + 5 oxidation to + 3 state because this hydride is the stable entity what is available as the corresponding tri hydride.

So ASH3 is your prior hydride cause it is not possible to get the corresponding hydride of the arsenic in the + 5 oxidation state which can be ASH5 . Because that is not physically possible to get so that we are not getting. So we will be getting ASH3.

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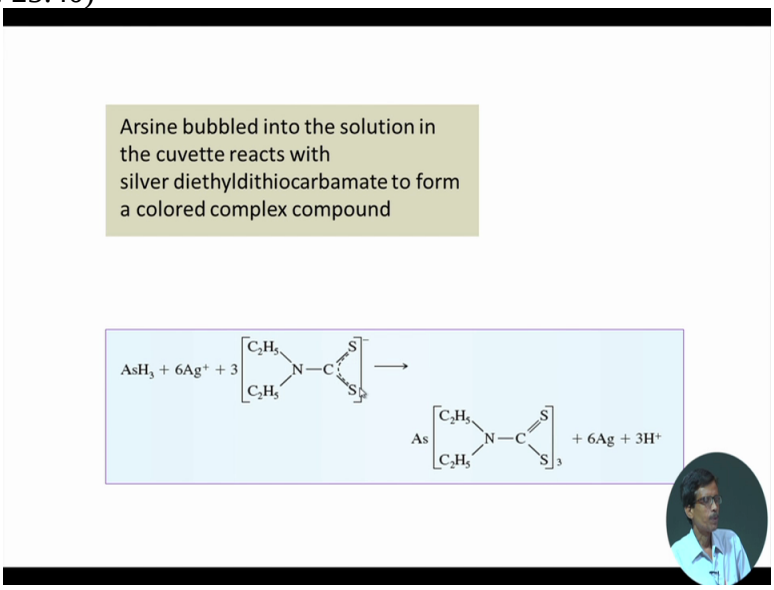

So in the next step what we can do that this particular gas, when gas is there, is bubbled into the solution the way I showed you the test-tube so that particular test-tube is there and this test-tube, we bubbled it and that can be your Cuvette and then we allow it to react with a typical reagent which is silver diethyldithiocarbamate. So all we know that diethyl carbamate is the chelating ligand.

So this is a sulphur sulphur bidented chelating ligand which is a silver salt and the silver salt of that particular salt is is directly reacting with arsine. So what we have? Now arsine was bubbled into the solution that means the Cuvette and that particular Cuvette is basically giving rise to the

corresponding formation of a coloured species. So what we get is that that ASH3 and this particular salt so this particular salt what we get is as the corresponding silver salt.

So the silver salt of this diethyldithiocarbamate so silver is present, so it is stoichometrically 3 units of this, the ligand because this particular part this particular part is binding to this particular arsenic centre.

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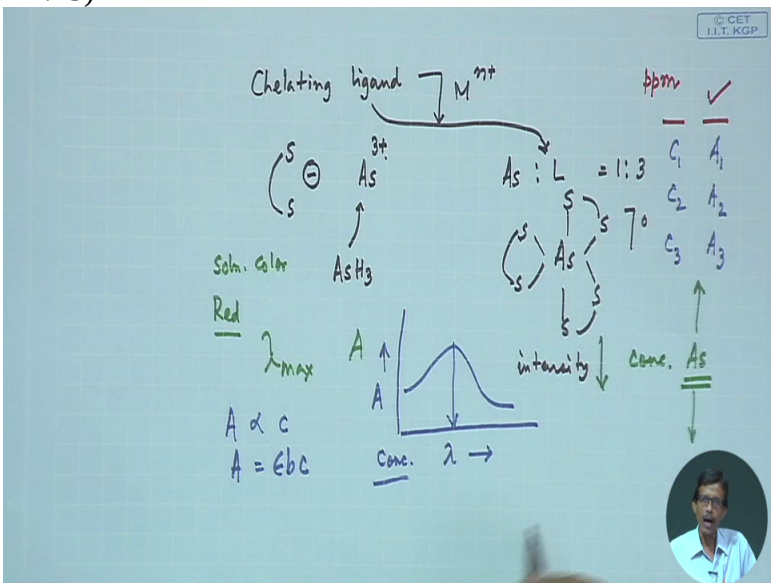

As we all know that this is a ligand system, so we all know that how a chelating ligand is used for binding any metal ion like MN +. In this particular case we are using SS as the sulphur sulphur (())(27:34) diethyldithiocarbamate anion.

So having one negative charge and that will be binding AS3 + which we are getting from ASH3. So this arsenic 3 + will be binding with this SS bidented ligand. So the what should be the stoichometry? That means if your L is the ligand or the reagent, that means the genetic ligand so this is your L, so what should be the stoichometry? Because this stoichometry once we know, that will be useful to determine the unknown arsenic concentration.

So here, this is 1 is to 3. So one arsenic centre will be there and forming chelate with 3 SS unit. So this is the thing. And it seems one charge is coming from here, so you have arsenic in the trivalent state. So overall it is a neutral species. So this particular neutral species is there and

discoloured. So this particular colour and how it is forming giving rise to corresponding intensity.

So the intensity of this particular colouration can be quantitatively measured for the concentration of arsenic in the system. I am not asking to know or remember it as what particular species because so many species we have handled so far. Arsenic was originally present in herbicide in some form, and then we formed in the different oxides, then hydrides and then ultimately as the chelating reagent.

So this is the typical methodology we follow. Otherwise it is not possible to give you a typical procedure for the identification of these arsenic species in the kidneys of all those deers. So this is the target species what we can determine which is a coloured complex. So this is a complex species and that will give rise to the corresponding colouration. And that colour species can be quantified by knowing the absorbances.

As I told you just now, the absorbance at a particular wavelength, so if the colour is red, so if the solution colour is red, so for that solution colour what we get? That the corresponding colouration colour we had a typical lambda max value. And with that particular lambda max, we can also have the corresponding absorbance. And a spectrophotometer is there and we are we get a corresponding absorbance for that particular wavelength.

So this is the lambda axis and this is the absorbance axis but what we do? That for a red colouration, we have the fixed lambda max value and these lambda max values, what we can change now? We bring the other parameters, that means the concentration. So what we will have happen there? That A, the absorbance will be proportional to concentration as we all know that following the Lambert Beers Law that A is equal to Epsilon BC.

This is the mall or absorbivity, this is the thickness that the depth of the solution through which the radiation is passing and C is the concentration. So depending upon all these concentrations, so in different concentration of this arsenic, so if we have a different concentrations of arsenic like C1, like C2 and C3, which will get the corresponding absorbances, A1, A2 and A3. So these concentrations now we fix in such a way that we get these because we are handling a very low concentration. That means, we are handling a ppm range of concentration. So how the

absorbance because there are some limitations for the spectrophotometer that certain point we find that say 0 to 4 or 0 to 3, we can go for the absorbance measurements.

Otherwise, this band is very small or this band is above this particular range of measurement. So in this particular range, that means where we have the ppm concentration, so this ppm concentration will tell us about how much absorbance we can have.

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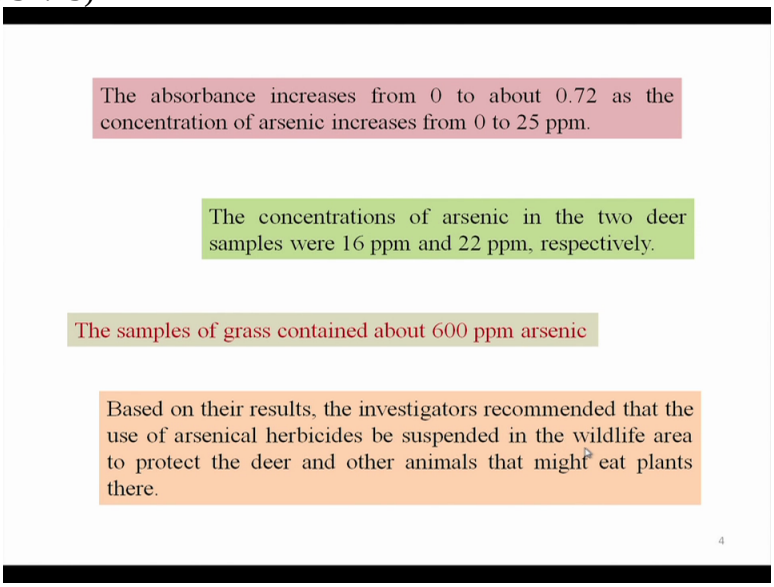

So in actual practice, what we see over here is that the absorbance increases from 0 to above 0.72, you say that is not crossing a value of one as the concentration of the arsenic, the typical concentration of arsenic within this particular chelate is increasing from 0 to 25 ppm. So taking into consideration of all these molecular weights or different species like that of the different oxides, then the arsine what is forming and then ultimately the complex species, we can report these values as the corresponding concentration of arsenic because we are looking for the corresponding arsenic poisoning of those deer is coming from the herbicide.

That means the corresponding acid what is forming from those arsenic as the herbicide. So if we get this, so the whole range we can cover from 0 to 0.72 as the corresponding absorbance range for 0 to 20 5 PM. So in actual practice when we determine the corresponding arsenic concentration for 2 deer samples, what means that 2 different samples were taken from the deer kidneys and the average values are will be different.

So one case it is 16 ppm and in another case, it is 22 ppm respectively. So you see that these are in the range of parts per million concentration. So parts per million concentration is very less. But this particular technique, this particular analytical technique or analytical process is so useful that we can determine this particular low concentration of 16 ppm or 22 ppm poisoning from arsenic and that is comparable to that what is present in the grass samples.

So in case of these grass samples where the concentration of these is very high, so this particular technique is also useful for determining the concentration of 16 ppm or 22 ppm from the kidneys of the deers but at the same time, it is also useful for determination of the corresponding concentration of the grass is contaminated by the herbicide as 600 ppm. So this 600 ppm arsenic concentration is also very useful to understand that how you can determine because what I told you just now that we can determine from 0 to 25 ppm as absorbance range of 0 to 0.72.

So if we try to handle 600 ppm, you see that this is going beyond. So is about 20 times of this value which we cannot measure in the spectrophotometer. So what we should do? We should do the corresponding proper dilution. For these grass samples, we have to dilute them and proper diluted sample we should insert into the spectrophotometer Cuvette and measure the corresponding absorbance.

So all these results basically what is the outcome of this particular analytical investigation and how analytical chemistry is also helpful to us is that the investigators then recommended that the use of arsenical herbicide which is a typical or poison for this deer, so definitely these arsenic herbicides will be poisonous for any wildlife and all these wildlife area should not use for this killing the grass in that particular area to protect the deer and other elements that might eat the grass or the plant available over there.

So is basically gives rise to a typical example of analytical chemistry which can be used for determination of the arsenic poisoning in a forest. Thank you very much.