Atomic and Molecular Absorption Spectrometry for Pollution Monitoring

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Lecture – 32
Phenols

While discussing fluoride, I forget to tell you that fluoride determination is done more than 3 to 5 million times a day all over the world because of its importance in drinking water. Now another parameter that is of very crucial for drinking water is phenols. Now you will come across phenols in your day to day life in several occasions the many of the chemicals what you use for floor washing, phenyl and other things they all they all contains phenols and then the in hospitals we use Dettol, Dettol is another phenol and phenols are contained in several natural organic substances like fruits and other food products.

So, all these phenols can enter into water ways and then many of the phenols are toxic. So, there is no need to decide which phenol is toxic, which phenol is not toxic because under given circumstances; under any given circumstance the phenols can aggregate and form a concentrated solution of phenols which can become toxic a group of phenols what I mean is different type of phenols present in water can give can be quite toxic.

Therefore the government bureau of Indian standards prescribes a limit of 0.001 ppm of a phenol in drinking water that is it should not be there basically, but even if it is there it should be less than this and provided one can drink solution containing drinking bore well water or something containing phenols up to 0.001 ppm it is relaxable by another 0.001 ppm maximum 0.002 ppm. So, except almost all phenols.

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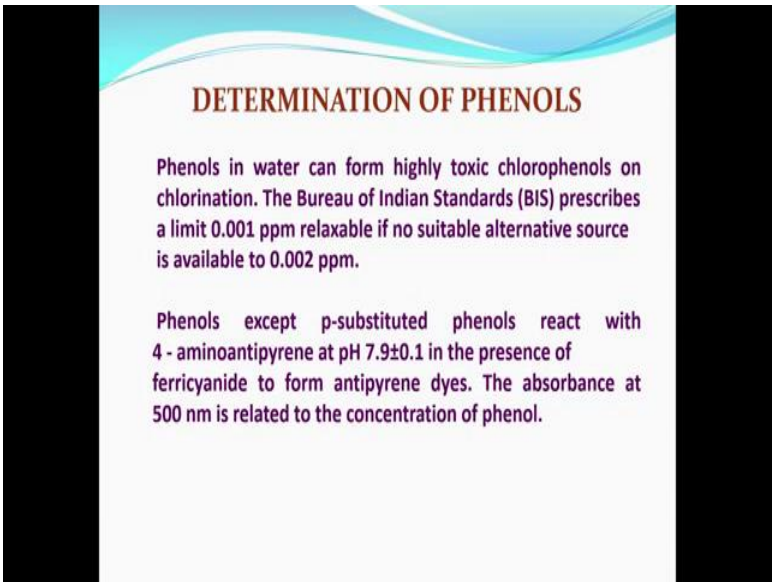

So, to determine the phenols at this concentration we will have to find out a reagent which reacts with phenol in this range. Now one happy situation is phenols except Para substituted phenols react with 4 aminoantipyrene that is the chemical reagent at pH 7.9 plus or minus 0.1; that means, around pH 8 in presence of ferricyanide to form antipyrene dyes.

So obviously, dyes means it will have a colour and the colour is monitored by the absorbance at 500 nanometers and then related to the concentration of the phenol, now it is a little difficult to write the structure of antipyrine complex.

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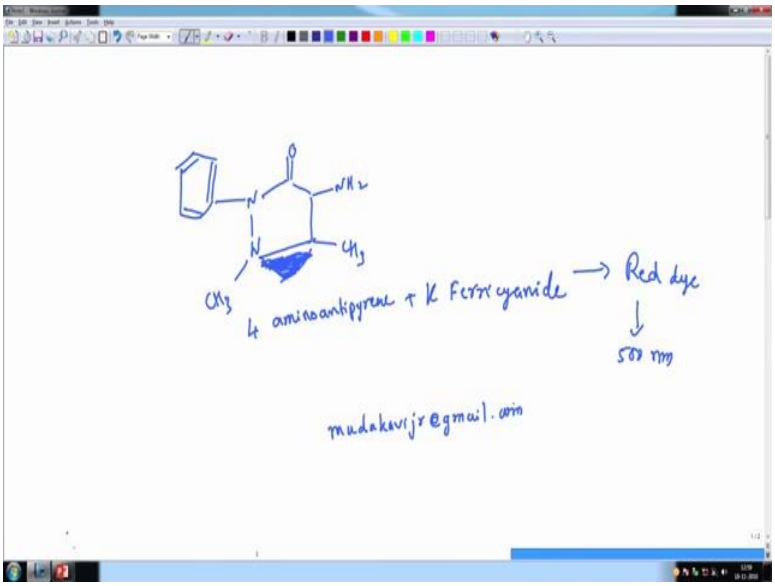

But antipyrine itself structure is fairly simple that is a benzene ring here and then a nitrogen then this is a ketonic group then this is an amino group. There is not this, this is not there. So, assume that it is not there it is a single 5 member ring 1 2 3 4 5 and then there is a Ch 3 group here and this is Ch 3 this is 4 aminoantipyrene. So, this will react with ammonium, potassium ferricyanide that is the basic chemical reaction to give you a red dye which will absorb at 500 nanometers. So, we go here the typical pH is around 8 that I have already explained to you.

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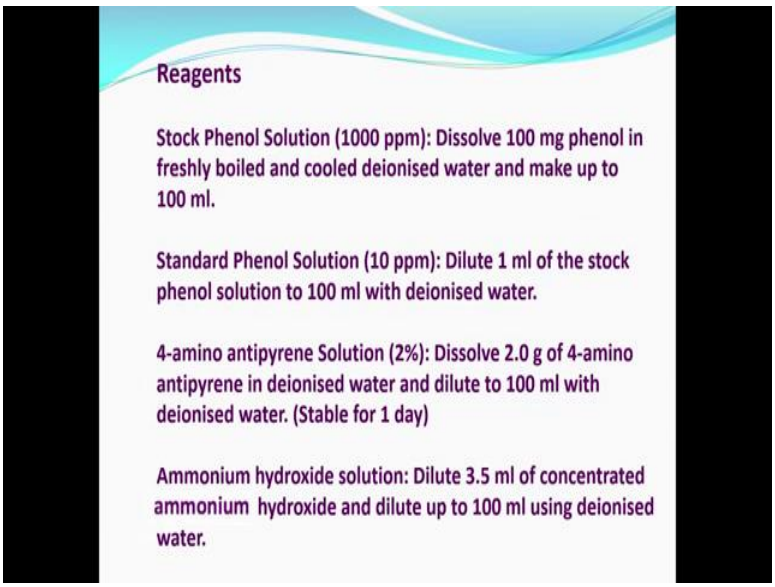

Now, how do we go about doing preparing the reagent that is very simple and you take the stock phenol solutions stock phenol is nothing, but hydoxybenzene that is dissolve 100 milligram of phenol in boiled and cooled deionised water etcetera all these details are available as well as I am giving you. So, you do not have to worry about how to prepare a standard solution etcetera this is all very simplified as far as users are concerned, as far as the users of my course are concerned there you just have to stick to this otherwise one can calculate and do that will take some more time I am trying to simplify the whole system because this is going to be a cook book for you, a ready reference for you to analyze whatever you would like to in drinking water.

So, standard phenol solution you just have to dilute it to 10 ppm appropriate dilution factors you can calculate yourself and that is 1,000 to 10 means 100 times dilution. So, 100 times dilution means I can take 1 ml and dilute it to 100 ml with deionized water and 4 amino antipyrine solution again it is a chemical available across the shelf from chemical suppliers we have to dissolve 2 gram of this and deionized water, but we have to remember that this solution is stable only for one day. That means, instead of preparing 100 ml or 50 ml and discarding it at the end of it you can as well reduce the use the make very small quantities for the amount required for the day.

So, we also need ammonium hydroxide solution that is 3.5 ml of ammonium concentrated ammonium hydroxide and diluted up to 100 ml you can get the solution and you have to prepare a citrate buffer solution. So, this is a little tricky because citrate buffer is a complexing agent also. So, I have given you the recipe here that is you have to dissolve 1.92 gram of citric acid in deionised water and make up to 100 ml etcetera you, but along with citric acid you also have to dissolve tri sodium citrate as a salt.

So, both of them will give you a buffer of about 6.8 and then the property of the buffer is it will not change the pH upon dilution or addition of any acid or alkali the pH will not change now you also have to prepare potassium ferricyanide. So, this is available across the shelf that is from chemical suppliers and potassium ferricyanide you just have to buy it and dissolve 80 gram of that, but this you can store it for in a brown bottle, but it is stable for about 1 week.

See it is important for us to remember. So, many chemical facts whenever you are doing environmental monitoring for example, the stability data what I am giving some of the

reagents like this potassium ferricyanide, it is stable for one day ammonium antipyrine that is stable for only one day, but potassium ferricyanide is stable for one week all these things you should remember and then act accordingly whenever there is a requirement of chemical analysis or monitoring for these parameters. So, that is why I feel that it is important for me to mention the stability aspects as and when required, but if I do not mention it does not mean that the substances are stable for indefinite length of time one needs to look at it from a chemical point of view and arrive at appropriate shelf life storage etcetera, some reagents we have to store in a at 25 degrees or 20 degrees some you have to store in water for example, sample water itself must be stored in around 4 degree temperature you know otherwise you cannot do the chemical analysis because the results will change.

So, going back to procedure what you would like to do know is we have to prepare the standards that is 5 ppm up to 5 ml 10 ppm that is 50 ppm, total I can spread it 1, 2, 3, 4, 5.

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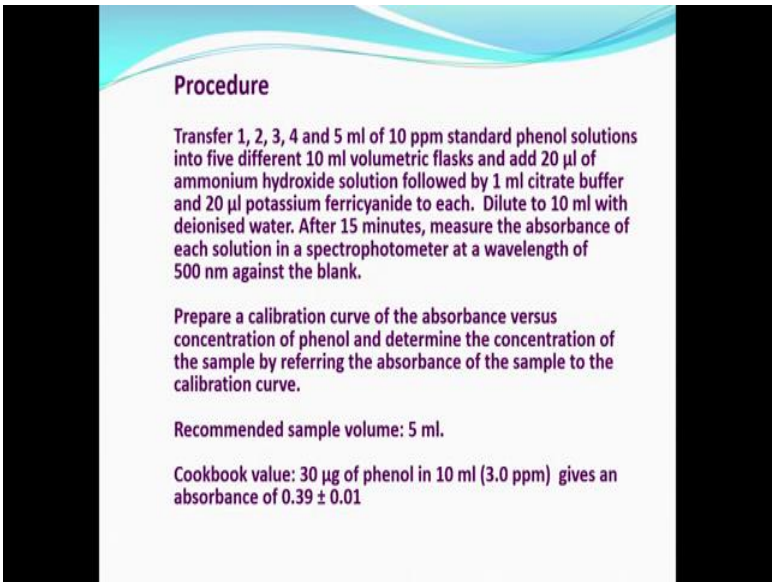

That is as usual we prepare the standard phenol solutions and then at 20 micro liter see I am coming back to that theme of mine where I always say that 10 ml of the standard of the measurement solution is more than enough for any experiment. Especially in analytical sciences we keep on talking about micro liters and you add 20 micro liter of ammonium hydroxide solution followed by 1 ml of citrate buffer and then 20 micro liter

of potassium ferricyanide to each. So, you can dilute all of them to 10 ml. See the calculation is something like this if I take 5 ml of 10, 5 ml of the standard phenol solution I am going to add about 20 micro liter of ammonium hydroxide and 1 ml citrate buffer that makes it 6.2; 6.02 and then another 20 micro liters of potassium ferricyanide that makes it 6.04. So, the remaining volume is hardly 4 ml of dm water.

So, whenever you want to prepare only 10 ml it is essential that you shift to micro pipettes. So, micro pipettes have got a property of a you know the giving you delivering accurate volume, but you need a little bit of experience while handling micro pipettes and the micro pipette tips you can throw them once you use them or if it you are going to regularly routinely use it you can use the same pipette also, but in biological experiments it is better not to contaminate contamination becomes a very important aspect of the success of any experiment. So, in biological experiment one prefers not to use the same micro pipette tips, but in inorganic experiments you can use the same micro pipette tips as long as you feel comfortable with that. So, all the reagents you are adding is going to add up to about 46.4 or 6.5 milliliter remaining is water you dilute it to 10 ml allow it to stay for about fifteen minutes for the colour development and you can measure the absorbance in a spectrophotometer at a wavelength of 500 nanometer against the blank.

So, again I want to emphasize a point at this juncture that you are expected to use not only micro pipettes, but also a spectrophotometer it is not the colorimeter where the size of the sample is a round tube test tube or something like that it should be square with one centimeter minimum and variably if it is necessary required. So, this point I wanted to emphasize. So, any water monitoring body should have minimum equipments and those equipments now I can define what you need is a typical spectrophotometer and micro pipettes and then glass ware, plastic glass ware and heating arrangement if necessary like that once you have this basic minimum this thing which involves an expenditure of about 1-2 about 2 lakhs or something like that you can monitor any of the water quality parameters in your hometown or something like that with the help of my course.

So, now coming back to this determination we have to prepare a calibration curve and we have already said we can prepare 1 to 5 ml that is 1-250 ppm of phenol and you have to just plot it the typical volume recommended volume is 5 ml; that means, maximum volume of your sample should be 5 ml to allow you some allowance for a dilution. And then cook book value is again if this here you should check on and off before you start

any experiment that corresponds to 30 microgram of phenol in 10 ml that is 3 ppm which should give you an absorbance of about 0.39 plus or minus 0.01; that means, you can get 0.38 to 0.40 absorbance, if you get that your reagents are good you are good and the experiment will be good it will be successful. So, that is the value of the cook book which I have emphasized again and again.

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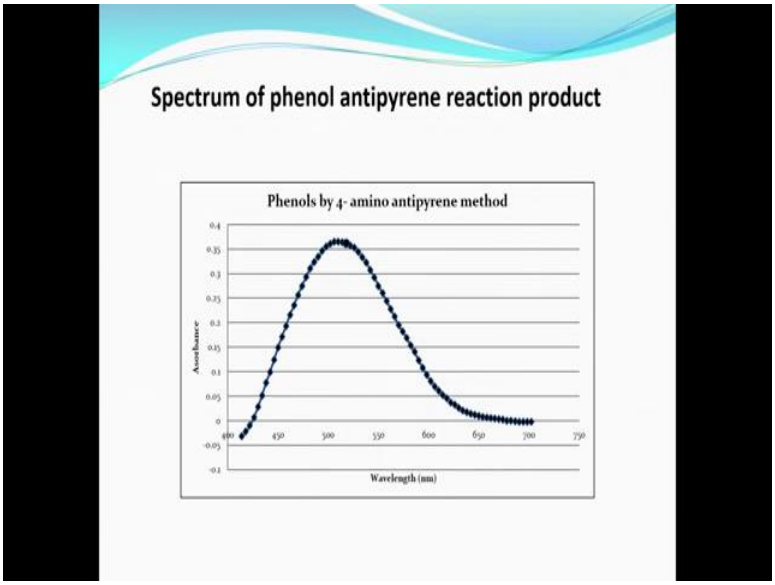

So, this is the typical curve the for the phenol antipyrine reaction product you can see that the product the molecular absorption curve shows a very single peak. So, you do not have confusion like boron carminic acid or something like that. So, here it is the a peak very clearly defined somewhere around 500 there is not much change in the absorbance because between 0.35 and 0.3, we normally stick to 2 absorbance units that is a 2 decimals especially whenever we are working with spectrophotometers we stick to 2 decimals. Third decimal you can round it off to the nearest second decimal and if you do that the approximate wavelength range you can fix very easily without any confusion here. For example, you see that in this figure the lambda max is somewhere around 510 or 520, but the absorbance value as such is not much different. So, it does not matter if you are off by 1 or 2 nanometers wavelength.

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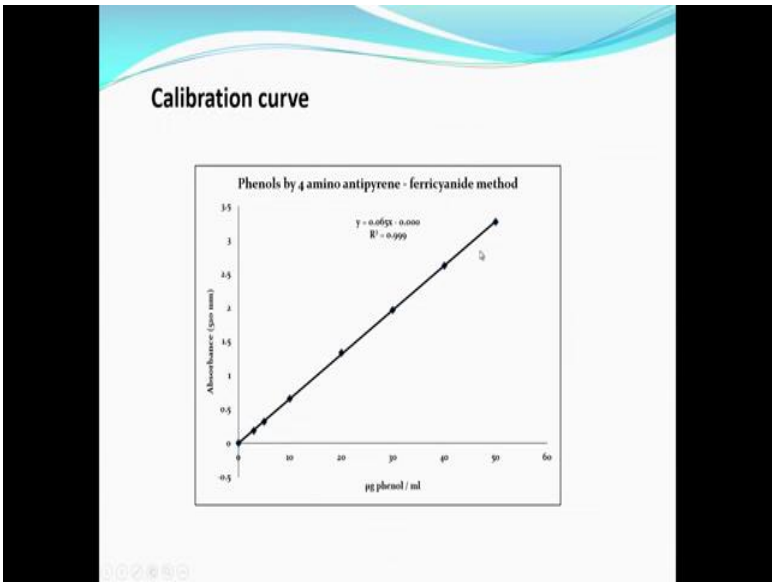

Now, if we go to calibration curve, we can see how beautifully they fit into the calibration curve and you can see that the calibration curve is linear up to 50 ppm and analysis can be completed using very simple chemical means as regards the interference again we should not forget the interference.

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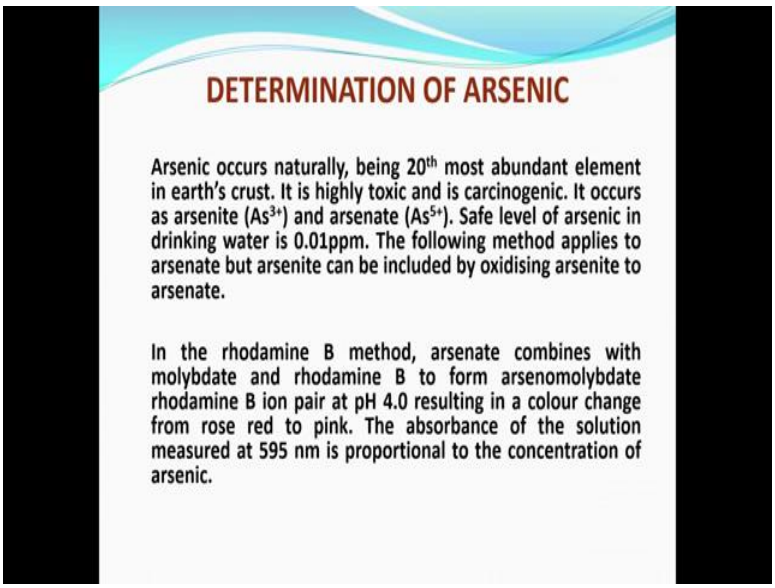

I do not have the slides for interference, but what I would like to tell you is take about talk about minimum interferences that is no interference from iodide bromide cobalt manganese magnesium etcetera into 100 times level that is 100 microgram per ml level

there will not be any interference. And the list you can get this additional list from the text book as well as from me I will give you some of the references, but the interferences listed here are result of our own research work.

So, if you can write down these numbers it would be fine, but it is a little tricky to show you the actual interference data because it will extend the period of this course beyond 20 hours. So, it may not be ideal for me to give the interference data in the text, but you can always contact me and I can I will be happy to provide you the interference data in any other assistance when you need for water, water chemical, water analysis, I hope you know my address I am from Indian Institute of Science retired, now I have a analytical service laboratory you can contact me by Gmail mudakavijr at gmail dot com m u d a k a v i, you can contact me at this address mudakavijr at gmail dot com and coming back to the interference data which I wanted to share with you some of them the copper 100 ppm it does not interfere and then cadmium does not interfere vanadium, does not interfere more than that more than the noninterference I should tell you about the interference actually. So, molybdenum for example, molybdenum definitely interferes and it can tolerate only up to 10 ppm now you can imagine that if you are determining phenol around 1 ppm or 1 ppm or molybdenum is more then there will be definitely certain amount of interference. So, it is always like that and up to 10 ppm molybdenum can be tolerated and phosphate and other things aluminum can be tolerated up to 20 ppm not more because aluminum definitely will form complexes with phenol. So, that brings us to end of our discussion with respect to phenol in the drinking water.

Now, let us discuss about arsenic is again a fantastic element very fascinating element widely distributed in nature, but it is not having any role in the physiology of our human beings or any living beings; that means, as of now we do not we have not come across any arsenic being a an essential element or desirable element in the elemental spectrum, but we know that arsenic is a very very industrial, very very important chemical and arsenic is used in several industrial processes, paints, etcetera, it is very widely distributed. So, the whenever you dig a bore well it is there are chances that arsenic may be in that. In fact, in South Indian including Bangalore, Kolar, Tamil Nadu, Andra Pradesh, etcetera and in Bengal, Dhanbad, etcetera, arsenic is one of the main pollutant in drinking water till recently not many people knew about, but nowadays we know that

the arsenic concentration can exceed more than 2 microgram per milliliter that is 2 ppm in drinking water which is a very very dangerous chemical in the environment.

Now, what does arsenic do arsenic is a very toxic element basically arsenic antimony bismuth tellurium etcetera they are all very toxic elements and they property of concern is the their effect on the sperm count in the mammals, basically what happens is if you know a little bit of biology you will know that the male sperms and female sperms are there in every mammals and the ration of male to female sperms is fixed and if this ratio is disturbed then there will be physiological changes and that physiological changes will be will be reflected in the quality of the quality of the progenies and off springs etcetera.

So, arsenic is also implicated as a carcinogen it causes cancer and therefore, there is a huge requirement for arsenic monitoring and the normal arsenic monitoring is approximately the standard is 0.01 ppm not even 1 ppm 0.01 ppm; that means, if you want to determine arsenic that is 0.01 means 10 nano grams that is the detection limit; obviously, spectrophotometer alone on its own will not be able to detect arsenic. But I have using special analysis technique of either converting it into a concentrate into a gas arsenic hydride and absorbing and converting it into a complex you can give a spectrophotometric finish to the determination of arsenic and complete the determination or one can take a larger quantity of the sample and do the analysis of bring down the concentration bring down the volume so, that the concentration of arsenic increases.

So, this these aspects we have discussed a just day before yesterday in the prior 2 classes before where there are techniques for increasing the concentration or decreasing the concentration of the substance by extraction evaporation and exchange etcetera and many of those techniques are applicable for the determination of arsenic. In fact, there are more than above more than 100-250 chemical techniques for the analysis of arsenic, but what I have chosen here is again it is not a standard method as described by American association of water examination Apha text book, but it is as procedure developed in our laboratory and worked on by colleague Doctor Vijay Kumar.

I have adopted his method with this method we have worked in Indian Institute of Technology, Chennai and it is a very sensitive technique. So, the basic procedure is that the arsenic is reacted with rhodamine B which is a dye and it combines with molybdate and rhodamine B to form arsenomolybdate, rhodamine B ion pair, it is a try ware in

complex. So, the optimum complexation occurs around pH 4 they are resulting in a colour change from rose red to pink and the rhodamine B, I have shown you in one of my earlier samples that is a very good triphenyl methane dye this one I had shown you here the during the my explanation during fluoresceins and this is a rhodamine D; rhodamine B available across the shelf and you can purchase it and use it to form the arsenomolybdate complex.

So, it forms a pink complex with lambda max around 595 nanometers and the absorbance is proportional to the concentration of the arsenic. So, sometimes you will come across not arsenic element, but quite often you will come across arsenic as arsenate as an anion, but also arsenite also. So, if you there is possibility that arsenate will react, but arsenite will not be able to react, but you will have to oxidize it to arsenate and then carry out the reaction as usual. So, it requires an additional step in the application of molecular spectroscope to the determination of arsenic.

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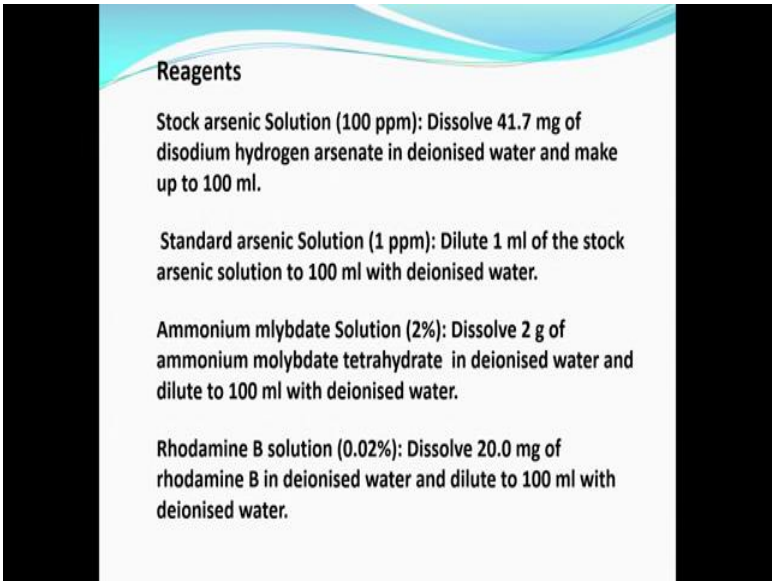

So, what we have to prepare the reagents are arsenic solution hundred ppm that is our stock solution and the you have to dissolve approximately 41.7 milligram I am going to give you only like this because my interest is environmental should not be polluted by the laboratory chemicals also. So, use minimum quantity and minimum volume. So, you have to dissolve (Refer Time: 29:34) arsenate disodium hydrogen arsenate available across the shelf and makeup to 100 ml standard is always one ppm you can prepare you

can dilute 1 ml of the stock solution to 100 ml and ammonium molybdate we have prepare again, I have given you the procedure here by dissolving 2 gram of ammonium, molybdate, tetrahydrate in deionized water rhodamine B solution is available across the shelf it is a very prominent organic dye used in nail polish and several other components cosmetic chemical also and it is also used in river water monitoring which I had explained to you during fluoresceins method and rhodamine B structure it is a triphenyl methane dye.

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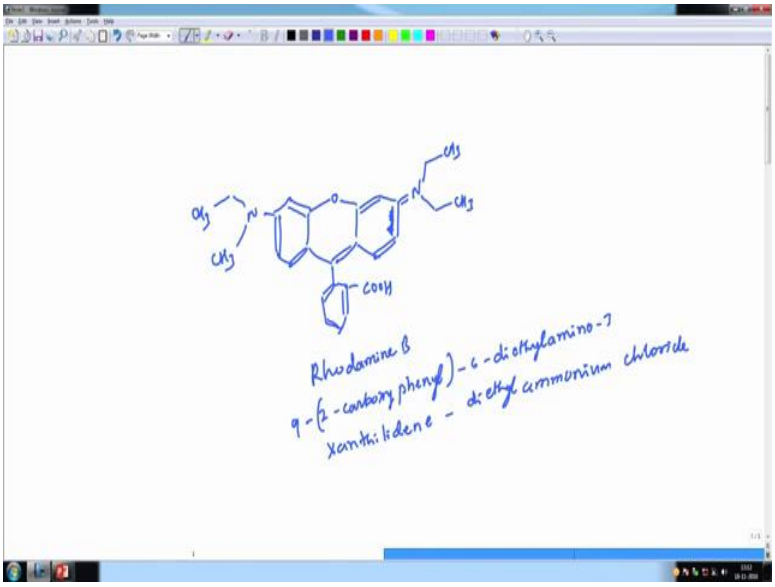

I can give you the structure of rhodamine B typical structure is like this these are all aromatic rings not here, but here this are not there then this is nitrogen group Ch 3 Ch 3 there is one more benzene group these are known as tri phenyl methane dye. There are 3 benzene rings again there is nitrogen Ch 3 this is rhodamine B the structure is 9 2 carboxy phenyl that is the chemical name 6 diethylamino 3 xanthine redene diethyl ammonium chloride diethyl ammonium.

This is a very famous dye especially used in textiles also many of the pink dresses whatever you are if you are wearing chances are that it will contain rhodamines B dye and it is also a good chemical reagent and one can use this as an analytical reagent also. So, these are the typical reagents we have to prepare.

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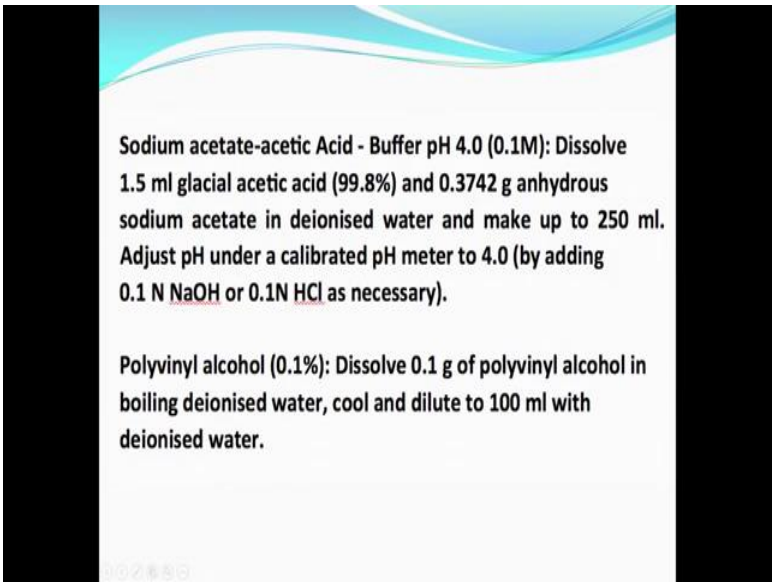

And we also have to carry out the reaction around pH 4. So, the best way to carry out the pH 4 reaction is to use sodium acetate acetic acid buffer. So, for this recipes are available across all text books and I have also given you the numbers here and we have to adjust because normal sodium acetate acetic acid buffer has a pH of about 4.76. So, you have to adjust the pH to 4 because the reaction is most best carried out around pH 4. So, you if you prepare a sodium acetate acetic acid buffer you have to add acid hydrochloric acid until you bring it to pH 4.

So, this is phenyl polyvinyl alcohol the this is a reagent which is used as a stabilizing agent for the triphenyl methane dye complex to remain in solution in the original method developed by my colleague Doctor Vijay Kumar. He had extracted the complex into benzene and then completed the analysis we have worked further on this on this method to show that the addition of polyvinyl alcohol stabilizes the colour in aqueous medium itself, one of the endevours of ours to bring you this course is to keep the procedure simple and then less quantity; quantity should be less and it should be very easy to procure the chemicals for you from the suppliers and it should be very it should not be time consuming for the chemical analysis.

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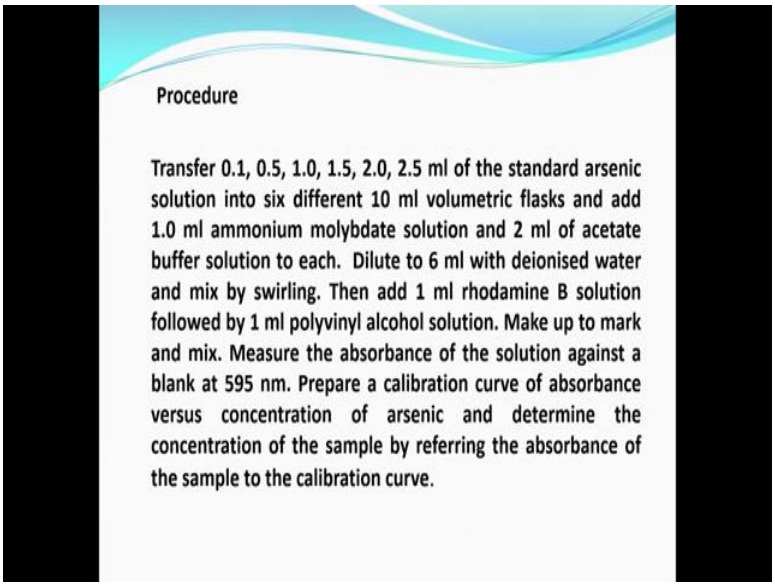

So, proceeding to the procedure what we would like to tell you is you have to take 0.1 to 2.5 ml of the standard arsenic solution that corresponds to how much the working ppm is 1 ppm. So, if you take 2.5 ml, you will be you will be taking 2.5 microgram of the standard arsenic solution. So, that is the highest limit; high end limit. So, in the remaining things are all very simple add ammonium molybdate 2 ml of acetate buffer 6 ml of deionized water and mix by swirling add 1 ml of rhodamine B followed by 1 ml of polyvinyl alcohol solution this polyvinyl alcohol is also available across the chemical suppliers across the shelf you can buy it and you just have to prepare it fresh daily.

So, then you have to make up to the mark and measure the absorbance at 595 nanometer, but you can also prepare a calibration curve of absorbance versus concentration by referring to the sample you can determine the actual concentrations.

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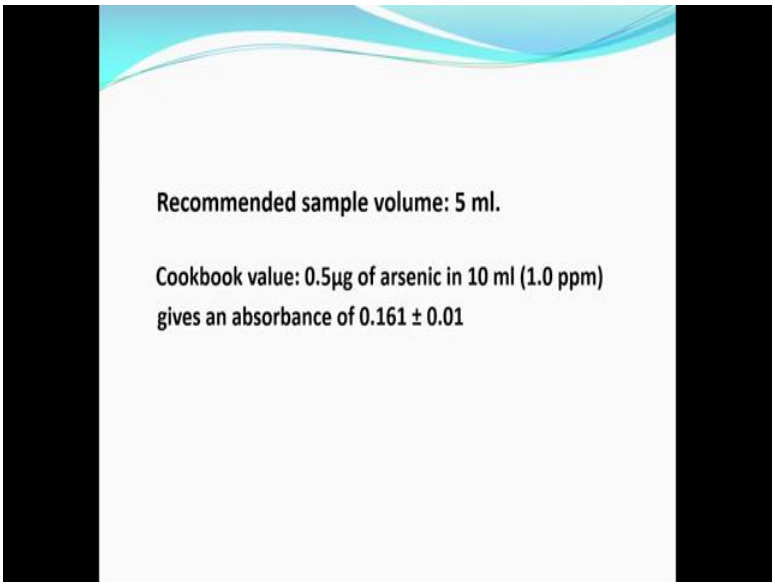

So, the recommended sample volume is 5 ml that is the maximum that is 5 ppm and cook book value if you take 0.5 microgram that is of arsenic in 10 ml that is 0.5 ppm, it should give an absorbance of about 0.161.

So, going back to this, the recommended drinking water concentration is 0.01 ppm. So, for 0.01 ppm, you should get 0.016; 0.03 absorbance. So, your sample volume if it contains 5 ml; 5 into 0.1 is 0.5 ppm. So, 0.5 microgram should give you 0.161 that correspond to the drinking water analysis as prescribed by the pollution control board state as well as central pollution control boards.

So, this is a modified of arsenic determination for you and we are very happy to bring you this method.

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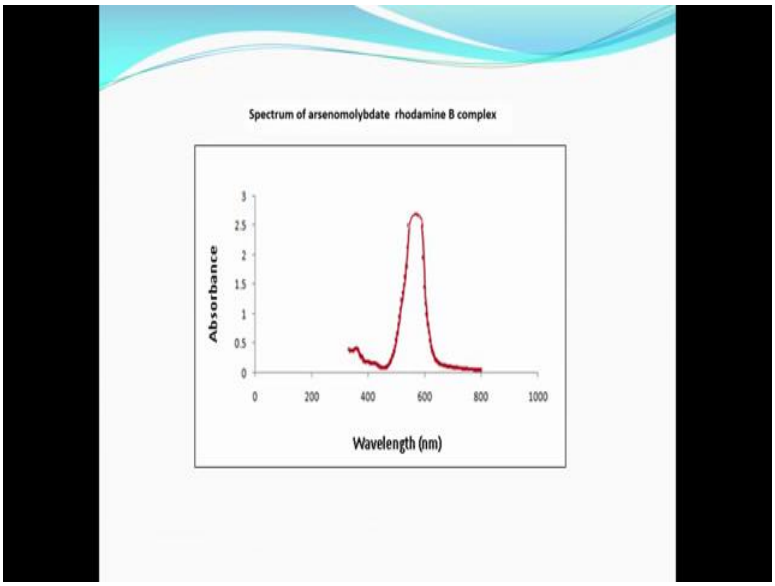

The actual spectrum I am showing you here a, the lambda max you can see there is only one single peak you do not have much choice and it is a very simple method that permits you to determine arsenic in different concentrations at this level. So, this is the calibration curve we can see that whenever we are working at very near detection limits the spread of the spread of the points is a little more. But as I have already explained to you earlier plus or minus 10 percent should be acceptable in spectrophotometry and that is what has happened here, I can I have drawn a linear curve here the linearity curve is given by y is equal to 2.85 x plus or minus 0.018 there is a certain amount of non zero origination, but r square value is approximately very good that is the reliability is 0.974.

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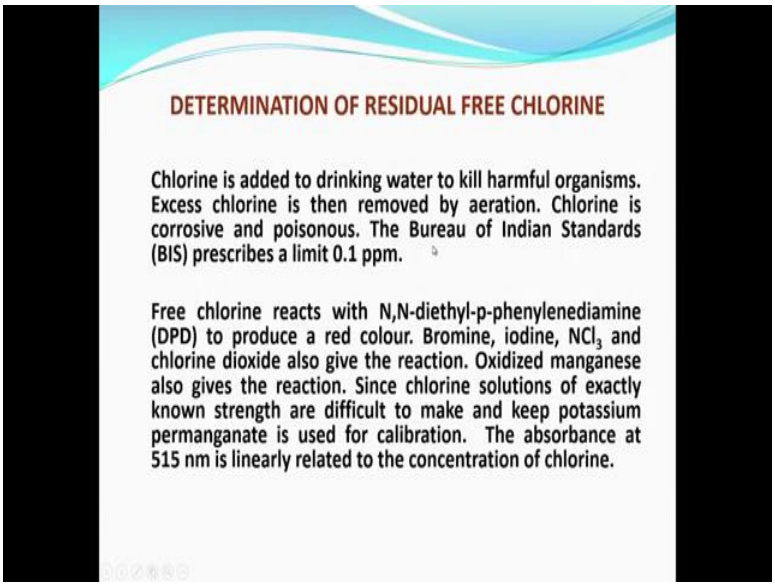

So, we will stop our discussion on this and continue our discussion on other parameters in the next class.

Thank you very much.