Experimental Biotechnology
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Module-I
Electrophoresis (Part-1)

Lecture-05
Laboratory Instruments Operation (Part-3)

(Video Starts: 00:24) (Video Ends: 00:59) Hello everybody this is Dr. Vishal Trivedi from department of biosciences and bioengineering IIT Guwahati. Now in today's lecture, we are going to discuss about the different types of centrifuges.

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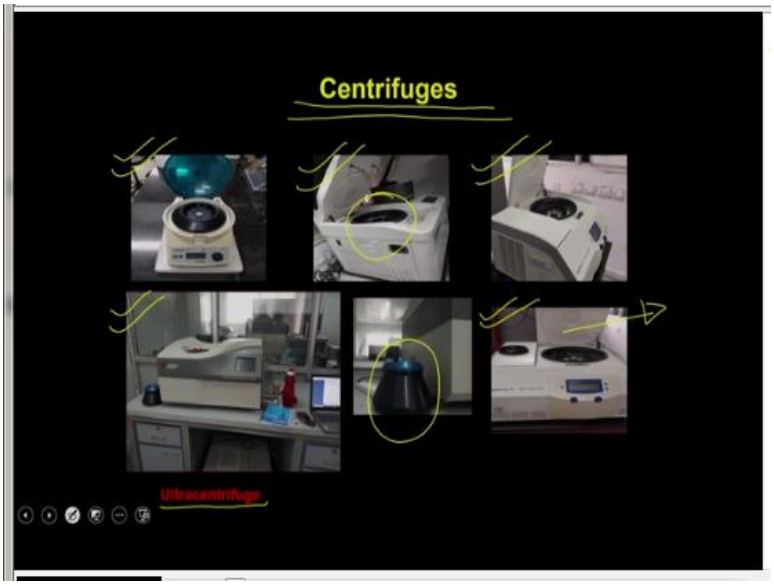

So, when you go into the lab you are going to observe the different types of centrifuges like you have the microfuge, you have the centrifuge like which is going to take up the larger volumes. Then you have the high speed centrifuge and then this is called as the ultra centrifuge which actually can go up to the 1 lakhs G speed or you can have the cold centrifuge or the cell culture centrifuge.

This is the rotor what you use in a typical ultra centrifuge. So, these all these centrifuges are working on a basic principle that you are rotating the object keeping the material into a rotor and that is how it actually separates a material through the process of sedimentations.

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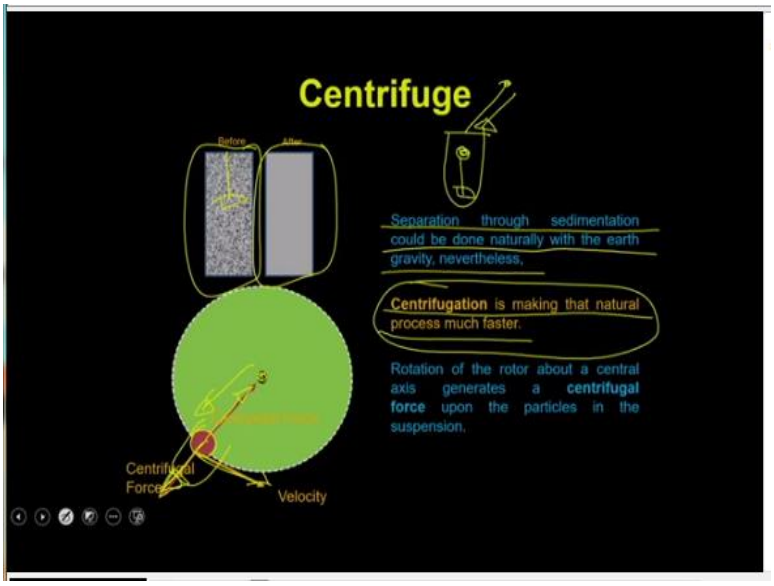

So, you can imagine that sedimentation is nothing but the settling of the material. So, if I suppose take the small mud into a water and mix it and then allow it to settle down the mud will settle down and the water is going to be cleared. So, what this process of settling of the higher particle into the liquid is called as the sedimentation. So, that separation through sedimentation could be done naturally with the earth gravity.

So, it happens because the earth gravity is you know pulling all the particles at the bottom and this process is going to be very slow if you allow it to be done on it is own. But what happened is when you doing the centrifugation you are actually making the same process much faster. How you are making it much faster because as you can see that this is the actually centrifuge where this is the excel of centrifuge and then you are actually spinning the objects.

So, this is suppose your object and you are spinning it, then what happen is the object is going to experience 2 forces. It is going to experience a centrifugal force which is going into the outside of the axis and then you can have the centrifugal force which is going towards the axis and if you are running it into this particular speed of the velocity. So, because of the centrifugal force the object will run away from this axis.

And because you are keeping this object into a test you know into a tube like. So, you can imagine that if I am keeping a material like this into a object and this is connected to axis. Then, this because of the centrifugal force, the object will run towards the away from the central axis.

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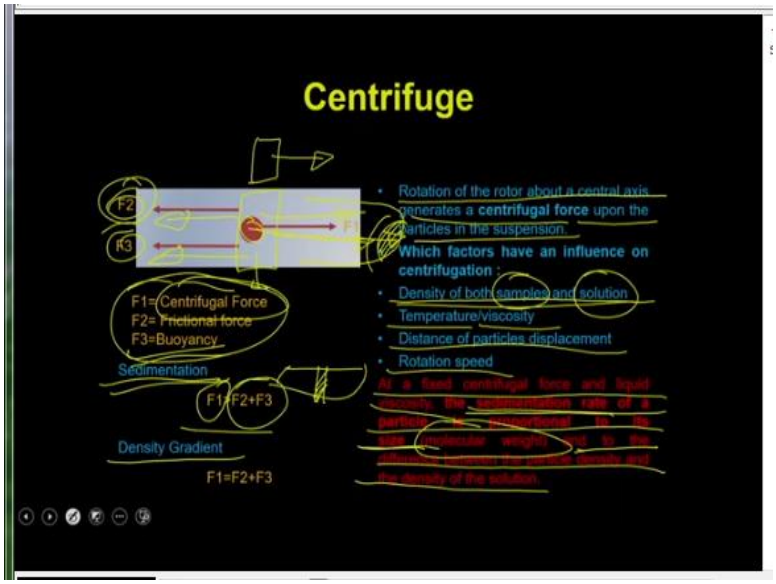

And in this process, what will happen is, when we runs towards away from the axis it actually going to experience the centrifugal force onto this side. Whereas it is going to experience the frictional forces into the opposite side as well as the beyond forces. Beyond forces means the forces which are been dependent onto the density of this liquid in which the material is been suspended.

And where this object is going to stop, this object is going to stop where these 2 forces are all these forces are going to be equalized which means if you will see sedimentation of this particle. If the centrifugal force is going to be bigger than the F2 + F3, then in that case, these forces are not going to stop this movement of this object and then eventually it is going to be sedimented, you can imagine that you have a tube like this.

So, ultimately it is going to reach to the bottom of the tube and it is going to be pelleted, whereas in the density gradient centrifugations you density gradient means you are actually running the material into a high density liquids. So, because of that your buoyancy forces as well as the frictional forces will go up. And because of that the material will not reach to the bottom of the

tube, instead it is actually going to be localized at a place where the F1 is going to be equalized to the F2 + F3.

So, that is the place it is going to be localize which means if I am running into a tube and it is actually the density gradient fluid it is actually going to stop in the someplace where the buoyancy forces plus frictional forces are going to be equalized by the centrifugal forces. So, the rotation of a rotor about a central axis generate a centrifugal force upon the particle in the suspension and the density of both the sample and the solution.

So, what are the forces which are going to influence the centrifugation, the first factor is the density of both the sample as well as the solutions. Then you have the temperature or the viscosity, the distance of the particle displaced and as well as the rotation speed. At a fixed centrifugal force and the liquid viscosity, the sedimentation rate of a particle is proportional to it is size, which means if you are considering the buoyancy identical and then the centrifugal force is going to pellet down this particular material.

And the sedimentation rate is going to be proportional to the size of that particular particle and to the difference between the particle density as well as the density of the solutions. So, the sedimentation of a material is going to be dependent on to the size of the particle, the density of the particle as well as the density of that particular solutions.

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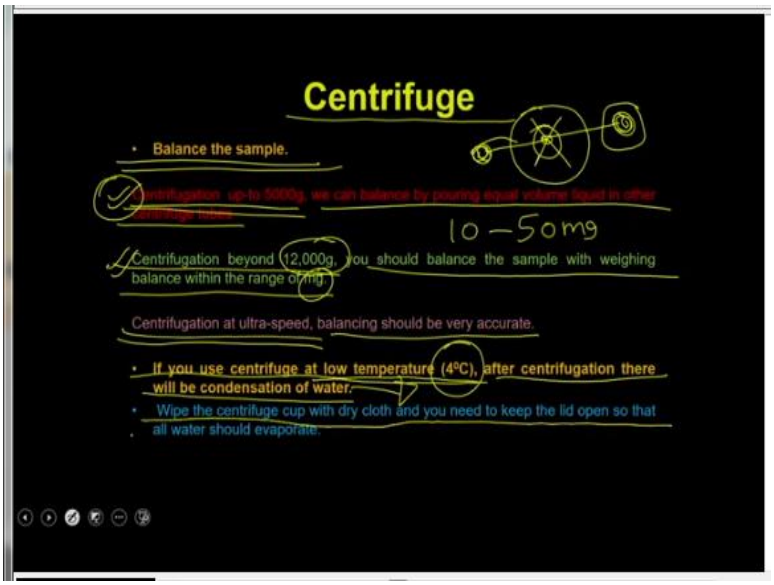

When you perform the centrifuge or when you do the centrifugations, you have to consider many aspects like you have to balance the samples. In a practical way you cannot balance a sample as you know very accurately because that is very, very time consuming. So, there is a set rule that if you are doing a centrifugation up to the 5000g, you can balance simply by pouring the equal amount of the liquid into the other centrifuge tubes.

So, that should be good enough actually to balance the 2 tubes, but if you are doing the centrifugation which is beyond 12000g. You should balance a sample with a weighing balance within the range of milligrams which means, if there will be a range if there will be a difference of 10 to 50 mg between the balance as well as the sample. Then it will not cause any problem to the centrifuge.

But if you are doing a ultra speed or you are doing a centrifugation at a very, very high speed. like 35,000 rpms or more than 1 lakhs, then the balancing should be very, very accurate which means even though 10 to 50 milligram differences is going to create trouble if you are doing the centrifugation at a very, very high speed. And how the balancing is important because when you do not do the balance, what happen is at a central axis you have 2 samples, one is sample, the other one is your balance.

So, if the centrifugal force is different or if the movement of these molecule is going to be different, then what will happen is that there will be a vibration of the central axis. So, central axis is actually going to vibrate and when it will vibrate, it actually going to vibrate, it will actually going to translate that vibration into the rotor as well. And because of that it is actually going to create trouble into your centrifugations or it is actually going to damage your centrifuge.

Because if this vibration will be too high, it is actually going to break the central axis and that is how it is actually going to damage the centrifuge. If you use the centrifuge at very low temperature like 4 degree, after the centrifugation there will be a condensation of the water. So, if you are doing the centrifugation at a very low speed because your sample is you know sensitive for temperature.

Remember that when you do a centrifugation, you actually going to increase the temperature of that particular chamber. Because when you are spinning a rotor, the rotor is going to spin the air around it also and because the air is going to cause the friction, the friction is actually going to increase the temperature of that particular rotor. So, that is why it is important that you run the centrifuge at a very, very low temperature.

So, if you run the centrifuge at low temperature and then you, so ice will going to form inside the cup ok. And then if you leave it, it is actually going to cause the condensation of the water and eventually there will be a water which is going to be formed within that cup and that water is actually going to damage the sensors, what is been placed just below the rotor. So, just below the rotor, there are sensor, in a sophisticated centrifuges you have the sensors which are being placed to monitor the speed of that particular rotors.

And if you allow the condensation of the water or filling of the water that actually is going to corrode the sensors as well as the just below the sensor you are going to have the electronic circuits and that actually also going to be damage. So, that is why it is recommended that you wipe the centrifuge cup with a dry cloth. And you have to keep the lid open, so that all the vapor should evaporate as soon as you are done with the 4 degree centrifugation.

And there is nobody in the lab who is going to use this centrifuge, then you just open the lid, keep the centrifuge open and then you wipe the cup, the centrifuge cup where you are actually having the rotor housed. And you just leave it open so that all the water what is being condensed even after your wiping should evaporate.

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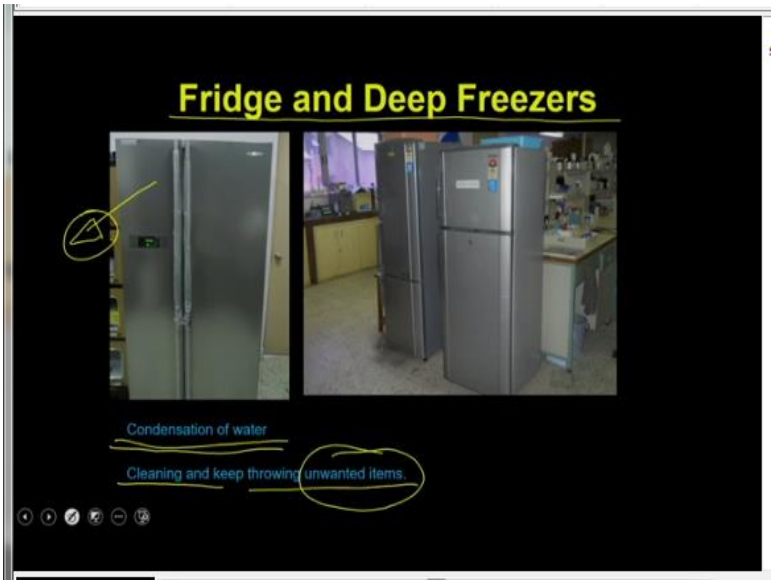

Then we have the fridges and the deep freezers with you know that the fridges and the deep fridges are actually been connected to the compressors. And these compressors actually runs the machines so that there will be a temperature. So, you can have the deep freezers like - 20 and - 80 or you can have the normal fridges. The fridge maintenance are going to be the remain the same as what you do in your home as well.

Except that here the frequency of opening and closing of these fridges are very high compared to your home. So, and because as many times you were going to open the fridge there will be going to be a condensation of the water because the fridge is cold but outside is air is hot. So, as soon as you open this hot air goes inside the fridge and then it actually contains some moisture.

So, that moisture get condense inside the fridge and eventually what will happen it is actually start building up the ice inside and that actually is going to compromise or it is actually going to give the extra load on to the compressors. And so if you want to keep the you know the life of your fridge for very, very long time because these fridges are required to maintain your to keep

the store the you know perishable chemicals and all that you should clean the fridges and keep throwing the unwanted material.

Because if you have the unwanted material in your fridge, the fridge is actually a compressor is going to work longer because all these material has to be bring at 4 degree after every opening. So, if you have unwanted material, you should remove that on a periodic basis you have to clean the fridge and you have to turn off the fridge and let you know fridge to get thaw because whatever the ice is being built should be removed.

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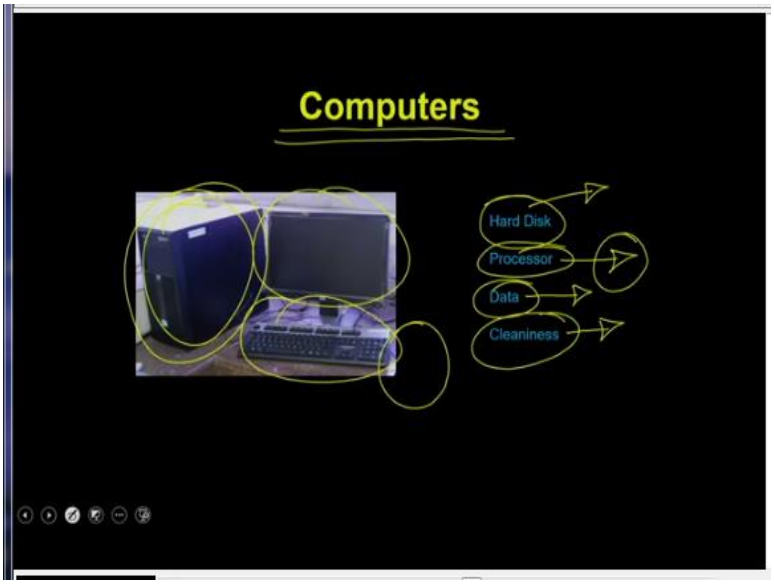

Now apart from that every lab normally contains the computers. So, this is a typical computer where you have this is the CPU, this is the monitor, you have the keyboard and then you have mouse here also. Most of the computers are actually either if it is connected to the instruments or if it is been used simply for your own personal use like browsing the net or you know reading that research articles you have to consider or you have to very, very careful about the hard disk, the processors, the data and the cleanliness.

So, all these you know the processor or all these desktops are having the fans and the fans are connected to a cabinet. So, this cabinet has to be cleaned on a periodic basis, so that the fan will get the clean air then the hard disk you have to keep taking the data backup of because and you have to keep defragmenting the hard disk. So, that the hard disk will not going to damaged.

The processor also need to be you know you have to work on the processor as well as the data you have to take the periodic data. And then you have to do the cleaning of the computers what is been present in your lab and you have to maintain the cleanliness.

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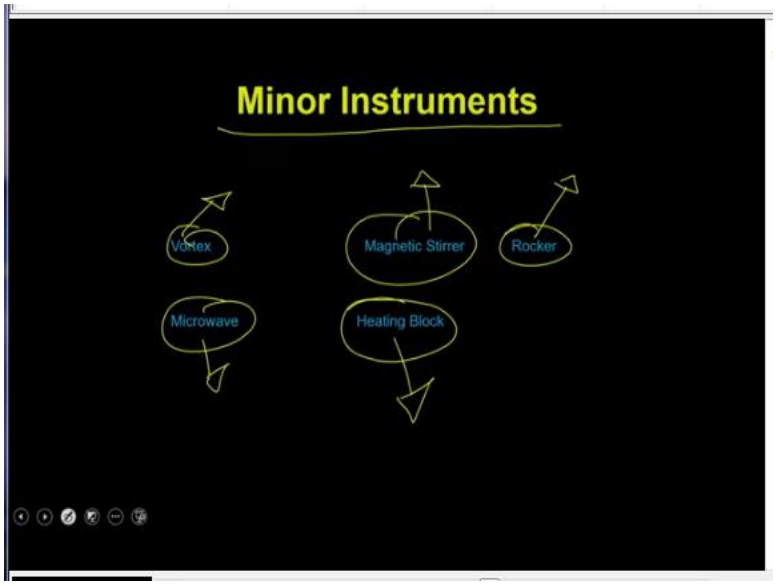

So, then these are the minor instruments like vortex magnetic stirrers, rockers, microwaves and the heating blocks. So, these are the minor equipments which does not require much maintenance but all these are very, very essentials. Like for example the vortex are been used to mixing the materials, magnetic stirrers are being use to prepare the solutions, rocker is been used to rock the sample.

So, that it actually can mix the sample like you have to use the rocker for performing the gel staining as well as for the western blotting. Similarly you require the microwave in case you want to heat up the samples like if you want to prepare the agarose gels or any other kind of you know the you want to make the LB agars and all those kinds of thing. And the heating blocks are required to prepare the SDS samples and you have to whatever the, you have to heat up to 100 degree and all that.

So, these are the minor equipment they do not require the special care but you have to be little care that you have to clean them you have to turn off if you are not in using them and all that.

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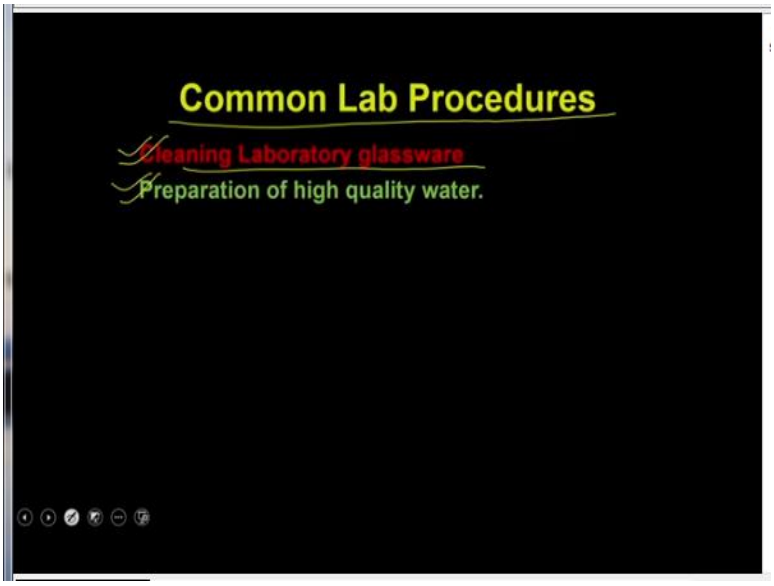

So, far what we have discuss about the common lab instruments. Now we will discuss about the common lab procedures. So, what we are going to discuss are the 2 procedures like the cleaning the laboratory glassware as well as the preparation of the high quality water, why there is a need to clean the glassware.

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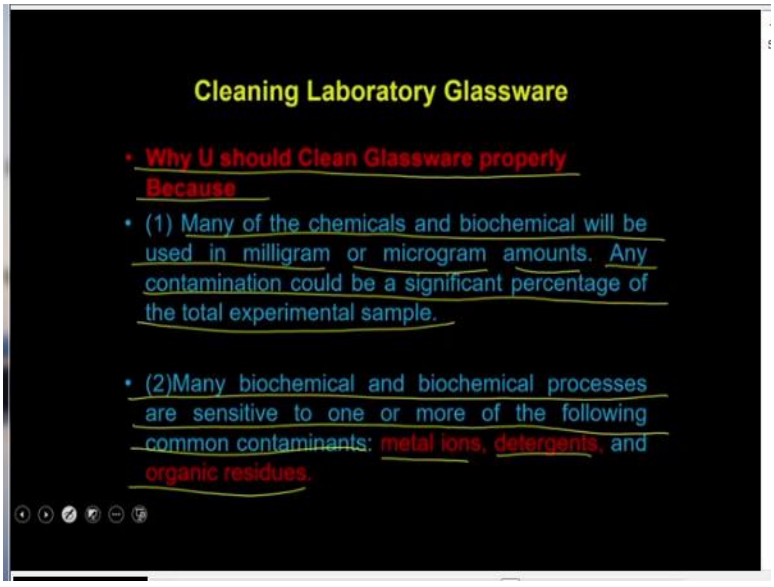

Because many of the chemicals and biochemicals we will used in the milligram or the microgram range. Any contaminations could be a significant percentage of the total experimental sample. So, you know that the biochemical reactions or whatever we perform in our laboratory normally uses the chemicals in the range of milligrams or the microgram range. So, if you have a

very small even a small quantity of contamination of your previous experiment that could be a significant percentage of the existing sample.

So, that could actually interfere in reactions, it could be possible that you may have some leftover chemical from your previous reaction and it could be a inhibitor of your enzyme. And you are trying to perform the enzyme acid and it is not working because you have some of the contaminants. For example, if you have just simple detergent and you are trying to perform some experiments, the detergent might be just killing the cells and they are doing some other kind of artifacts.

The many biochemical and biochemical processes are sensitive to one or more of the following common contaminants like metal ions, detergents and the organic residues. So, it is since the biochemical reaction as well as the life sciences like cells and all that are very sensitive even for the minor contamination of these materials. We have to be very, very careful that we should be clean the glasswares very thoroughly before you use them.

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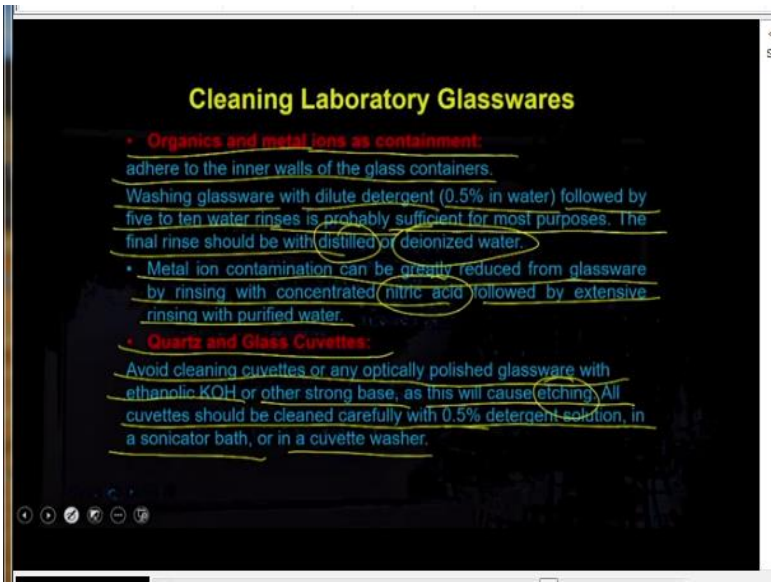

The cleaning of the glassware also depends on the contaminations. For example, if you have the organics as well as the metal ion as a contaminant which actually adheres to the inner wall of the glass containers. You have to wash the glasswares with the dilute detergent like 0.5% in water

then you have to followed by the 5 to 10 times water rinsing and that actually should be good enough to remove the organics as well as the metals.

You remember that you have to always rinse with the distilled or the deionized water because ultimately you want to remove all the material what is been. So, that you know there should be no case that you are actually removed the organic as well as the metal ions. But instead of that now the detergent is being present which you have used for cleaning. So, the cleaning is important but or the removal of the contaminant is important.

But at the same time you should not have the cleaning agent to be remained there because then it becomes the contaminant again. So, you have to very thoroughly wash the glasswares with the distilled water as well as the deionized water. The metal ion contamination can be greatly reduce from glassware by rinsing with the concentrated nitric acid followed by the extensive rinsing with the purified water.

Apart from that, when you have the quartz or the glass cuvettes you can avoid cleaning the cuvette or any optically polished glassware with ethanolic KOH or other strong base, as this will cause the etching or it will actually make the surfaces very rough. All cuvettes should be cleaned carefully with 0.5% detergent solutions or in sonicator bath or in a cuvette washer. So, with the cuvette whether it is a quartz cuvette or the normal glass cuvettes you have to be very, very careful that you cannot use the harsh chemicals like the alcoholic KOH.

You have to use the mild detergent and then you can use the sonicators or you can use the sonic bath because if you keep them in a sonic bath, all the contaminating material is going to be removed.

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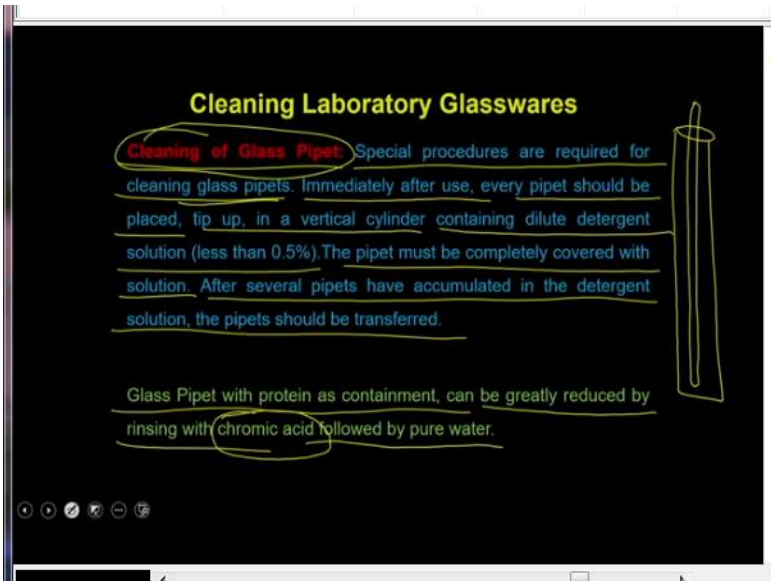

Then the cleaning of the glass pipette, so if you remember in the previous section we have discuss about how to you know use the liquid handling system. So, once those liquid handling systems are going to be dirty then you have to use them. So, the special procedures are required for cleaning the glass pipettes as soon as you are done with the glass pipette because glass pipettes the ends are very narrow.

So, that is why immediately after use every pipette should be placed tip up in a vertical cylinder containing dilute detergent solutions. The pipette must be completely covered with the solution, so as soon as you are done with the pipette you have to take a glass beaker and then you dip your glass pipette keeping the tip down. After several pipettes have been accumulated in the detergent solution, the pipette should be transferred and then you can use a pipette washer and that can be actually used to clean the pipettes.

If you have a glass pipette with protein as contaminant, it can be readily reduced by rinsing with the chromic acid. So, chromic acid is a special acid what you can use to pure the glass pipette if you are using the protein samples. Like for example, if you are using the glass pipette for cell culture purposes, see in those cases you are taking the serum and all other kind of materials. So, that actually going to coat the inner surface of the glass pipette and that will not go simply by the detergent.

So, what you have to do is, you have to just first dip the glass pipette into a chromic acid solution. So, chromic acid is actually going to damage all the proteins into you know it is actually going to oxidize the protein and that is how it is actually going to give you the proper cleaning.

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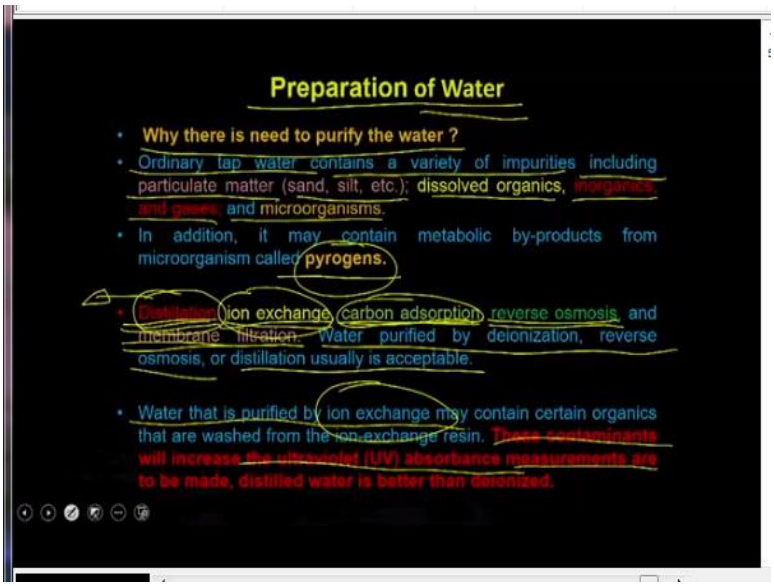

Then you have to prepare the water, so why there is a need to prepare the purified water because the ordinary tap water which actually comes in our home and that actually you use simply by the simple purification steps contains a number of impurities like the particulate matters like sand, slit and all that. Then you it has the dissolved organics, then it has the inorganic material and gases and then ultimately it also contains the microorganism.

In addition to that it also contains the pyrogens, the pyrogens are the metabolic byproducts or by waste what we produced by the bacteria or other kind of microorganisms and these pyrogens are very problematic. For suppose you want to use this water for your cell culture purposes, then these pyrogens are going to interfere in the cell propagation. There are many material what you can many ways in which you can be able to use to prepare the water.

For example distillations ion exchange, carbon adsorption, reverse osmosis and membrane filtrations. Distillation we have just discussed like how to use the water distillation units, you can use the ion exchange cottages. So, that actually is going to remove the positively as well as a

negatively charged ions what is been present in the water. And that actually is going to make the deionized water and that is going to use for most of the biochemical experiments.

Then you can use the carbon adsorption is also going to do the same as the ion exchange materials. Then you can do the reverse osmosis as well as the membrane filtrations. The water purified by the deionization reverse osmosis or distillation is very much acceptable because it gives you the pure form of water. There is a problem if you are preparing the water by ion exchange method.

As I said you know if you are making a deionized water that actually is going to because when you are passing the water through a ion exchange matrix, some of the chemicals are been leached from the ion exchange matrix and that actually. So, it makes the water deionized but at the same time the chemical what is been least from the ion exchange column actually contaminates the water with this organic substance.

And this organic substance are actually increases the ultraviolet absorption of that particular water. That is why if you are doing any experiment where you are actually doing the absorption experiments like if you are measuring the absorption of that particular solutions prepared in the water. Then you should not use the deionized water instead you can use the water which is been prepared by the distillations.

Because that actually is not going to add these organic substances and that actually is not going to increase the absorbance of the water into the UV range. So, with this I would like to conclude my lecture here, thank you.