Environmental Quality:
Monitoring and Analysis
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Lecture No. 23
Analysis Methods – Overall Methodology for Organics

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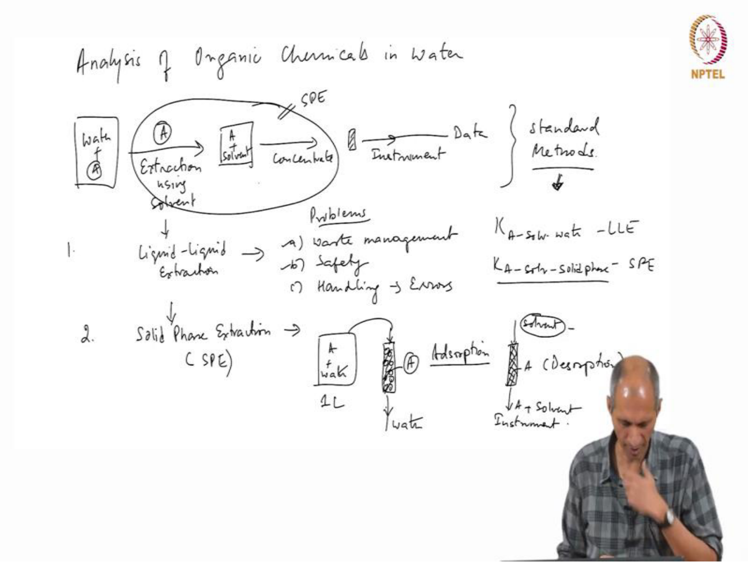

So, last class we were discussing the analysis. We started discussing the analysis of organic chemicals, in water. So, we were looking, let's say we have a water sample with A, we remove the A here, by extraction and then we also have another process we concentrate to make this a smaller volume and this goes into the analytical instrument to get concentration data on that.

So, we discussed one method of this and we also looked at some standard methods, that are reported. They are listed in the regulatory agencies for the analysis of different types of components in water. So, here, the one method that we use for extraction is what is called as liquid-liquid extraction. And this usually involves the adding of some amount of solvent to the water sample and then shaking it to extract by whatever means you can bring the solvent.

And it is done so that there is an efficient exchange between the water and the solvent. So the idea is to use a solvent that has very good capacity to hold the solute that you are interested in.

So, that is a matter of experience and some people have collected this data and recommend a particular solvent for the analysis of solvent or solvents, several solvents that are applicable for the extraction of one class of chemicals, A from the water.

And so we looked at some of the solvents so there is no point in me giving you a list of solvents by and large chlorinated organic solvents are very strong for any of these purposes, but they also have a problem they are themselves listed as hazardous chemicals. So, you have to worry about their disposal. And so, people always look at some alternative, but if extraction is the main concern, people still use these chemicals because they are not banned you just have to take care of their disposal properly, if you are using it. So there are several problems in using liquid-liquid extraction, one is the waste management. Second is safety itself while extracting and concentration. What do I mean by concentration is you are evaporating solvent which means the solvent is coming out and it is potentially released into the environment then the analyst themselves can get exposed to this solvent. So, we have to be careful about doing all that. So, in the standard methods, if you are doing liquid-liquid extraction, you will also see the safety methods that you have to follow for safe handling which includes the concentration to be done inside what is called as a fume hood. So, I will not go into details of it, I suggest you to read some of these methods in detail, we will read some of it there.

So, there are two issues here waste management, safety and third is the amount of handling can lead to a lot of errors. So, you are manually taking sample, extracting or taking out solvent, concentrating it, and taking it to the instrument all this can also lead to additional errors in sample loss in the process. So, in order to circumvent all of this, there is another method that people use now is called solid-phase extraction.

This cuts the problem in a little bit but it does not remove the problem completely. But what this solid-phase extraction means is instead of using a solvent to extract we are sending the entire thing into a solid, A + water into solid. The water comes out and the A is retained on the solid column so, essentially we are doing another mass transfer process is called adsorption.

We are adsorbing all A from the water onto the solid phase which is usually a packed column and all the A is retained in the solid phase. So, if you are processing 1 liter of water or a large quantity, you send it through the solid phase and whatever quantity of A that is there in the water gets retained on this small solid column and water is going through and there is no solvent involved here.

And in this, safety issues are less, then you take this solid-phase which contains A. You have to take it out to bring it to the instrument again which means that I have to remove the A from solid, and then go to the instrument for analysis. There are very few methods that people have, especially for the organics that we are interested in. The direct analysis of A on a solid phase is not possible.

There is not an easy method that exist for it, as of now so, you should have to remove it out of the solid phase. So, you have to extract it somehow and this process is called desorption. You have to use desorption to get it out. So, depending on for water usually, the instrument that we are going to be using is typically takes some solvent. So, one way is to send a solvent. Now, this solvent interaction with chemicals with reference to water is very different from its interactions reference to the solid.

So, we are talking about the partition, in this case, liquid-liquid extraction and about a partition constant of A between the solvent and water in a liquid-liquid extraction, in the case of the desorption we are talking about the solvent and the solid phase SPE. So, in desorption you have to select a suitable solvent because now the organic solids that are there in the SPE column itself must be able to take its properties and should be very similar to that of the solvent that you are using in the first place which means if you are using other solvent to take it out of SPE it must be really strong. So, the partitioning of the chemical from the solid phase to the solvent must be really good. So there are recommendations for the selection of solvents for a particular class of chemicals from a particular solid. So there is adsorption and desorption, this is mass transfer and the equilibrium of a chemical between 2 phases in this kind of systems.

So, what we are effectively doing is 1 example is, say, you have 1 liter of sample it goes into a small cartridge, which contains maybe a few milligrams, 100 milligrams or maybe a gram few grams of the solid and all A is there then you take a small amount of solvent, maybe 10 ml of solvent and these all will bring out all the A and it comes into this solvent. So, you are essentially doing the same thing what you di. So what you did in this zone is equivalent to what we are doing in the SP except that we are not using a solvent we are not using liquid-liquid extraction, we are using the solid-phase extraction.

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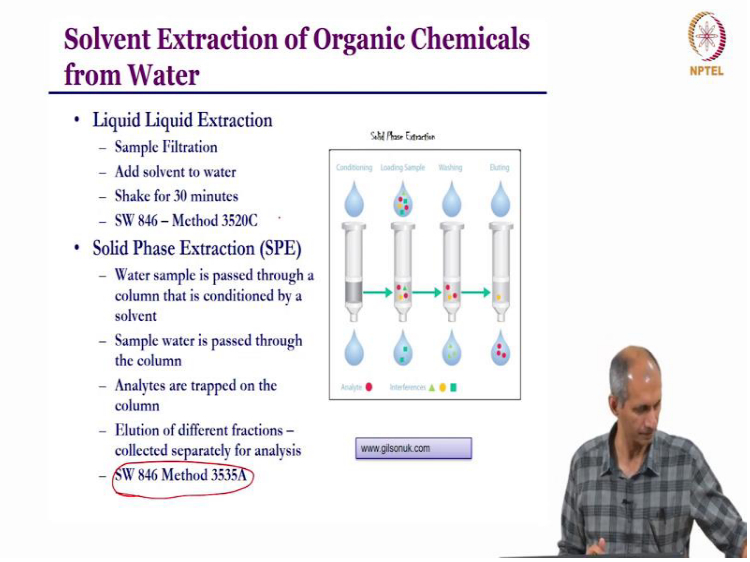

Solvent Extraction of Organic Chemicals from Water

- Liquid Liquid Extraction
  - Sample Filtration
  - Add solvent to water
  - Shake for 30 minutes
  - SW 846 – Method 3520C
- Solid Phase Extraction (SPE)
  - Water sample is passed through a column that is conditioned by a solvent
  - Sample water is passed through the column
  - Analytes are trapped on the column
  - Elution of different fractions – collected separately for analysis
  - SW 846 Method 3535A

So, I have an image showing that this is a solid-phase extraction. So, essentially what we do is we have a cartridge that has a solid and this cartridge then allows the material to be sent through and the analyte is received initially restored here and then we elute it and there is a method for that, there is a standard method for that also. So, yesterday we talked about solid waste standard methods, one of them was for solid waste.

So, there are a large number of methods which talk about different extraction methods and there is standard order for extraction, so, you can do the quality control for extraction, how much is the recovery efficiency how much is the loss during for different groups of compounds with different kinds of methods and all that. So, what you will get in the method? You will get very specific instructions as to what is the flow rate you need to use? What is the time that you need to have? What is the purity of solvents? How much of solvent you should use? And what is the mass of

SPE you should use? And same in liquid-liquid extraction also you have for a given volume of water, what is the solvent volume you should use? How long should you shake? How should you shake it? What is rpm? all those instructions are given. You can modify it whichever way you want, for that you have enough sufficient justification for doing it.

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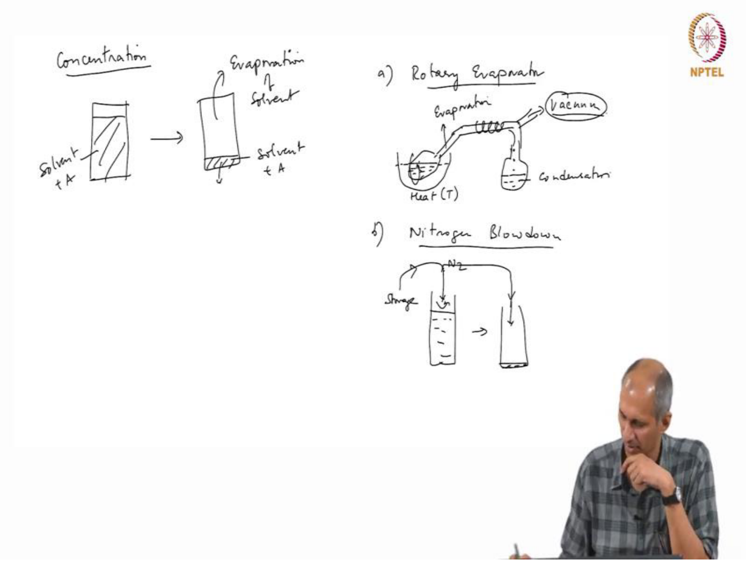

So, the next step is a concentration step. Sometimes when you extract, you extract a large volume of solvent, this is sometimes possible that you only can use 20 or 30 ml or sometimes it is even larger. So, concentration essentially means, you are reducing the volume, solvent volume is going from large to very small, which means that the solvent is evaporating reducing the volume of solvent assuming and hoping that all the A that is in the system is retained in this second set also. So, that concentration is higher in this case. And two ways of doing it, one is if you have a very large amount of solvent, you can use equipment that is used for it, we can use what is called a rotary evaporator. Rotary evaporator essentially is a system. But essentially what we do is we have a sample that is kept inside a container.

And this is kept inside a particular temperature heating bath or mantle or something and then the vapor that comes out is condensed and collected. But here, we are also applying this vaporization, evaporation and condensation to collect to recover this we use vacuum. So, at a particular temperature by using a certain vacuum, you are influencing it, this is equivalent to boiling except we do not boil it, we do not increase temperature but we change the pressure and we reduce the pressure and through that, evaporation occurs at a lower temperature. So, at room temperatures you have certain amounts of vacuum you need to apply for certain solvents. So, there is a list of that. So at 35 degrees, this is a vacuum which you apply for different solvents and it is based on the vacuum that they evaporate. So this is rotated there and it is called rotary evaporator because this is rotated, there is this portion which is rotated.

And so that the level keeps coming down. So beyond a certain volume, you cannot reduce this. So, if you have 100 ml of solvent, you can reduce it to a 5 ml or so. Beyond that the effect of vacuum is not felt. it goes up, condense and come back straight away. It cannot reduce it all the way but it is very good. We will use it for various reasons, but in environmental science sciences we do it for concentration.

The advantage of this method is you also can recover the solvent. Now, how pure the solvent is a different question you may have to check that because if are multiple things, few things will go out, several things will go but it is like distillation which means that it is the basis of evaporation which is the separation on basis of the volatility of that particular thing and there we are influencing volatility by applying different vacuum. This is a more sophisticated way of doing it and it is used for large volumes.

But for small volumes, let's say this is only 10 ml or 5 ml as in the case of SPE. Your volume of the extract from the SPE column is 10 ml or 6 ml or something like that. But you still want to reduce it, so, you can`t use a rotary evaporator, as it is too much. So we use what is called a Nitrogen Blowdown which is very straightforward, and is a very simple. Sample is placed here. And then you have flow of nitrogen on the surface. So it is essentially evaporating just like that.

So it is very precise. So you have a needle that is supplying nitrogen from nitrogen cylinder storage and it evaporates, so you can keep on bringing this needle inside as the level keeps going down. So this will go down. Nitrogen is used because it is inert. That is the reason why we use nitrogen. It would not react and it also would not cause other problems in the system. There are commercially available nitrogen load on the system but it's nothing this is what it means. So there are instruments that you can use and do it and all in automated manner and all that, but essentially it is evaporation. So the quality control issues here are during evaporation, you will lose some solute of your interest depending on what is the volatility of that also, while you are doing naphthalene analysis, naphthalene has reasonable volatility.

1 of the main problems is, what do you call as co-evaporation effect so, if say dichloromethane or hexane is a solvent, very volatile solvent. The reason we choose a particular solvent in for extraction is that if that solvent has high vapor pressure, high volatility, it is easy for us to concentrate. If you take a solvent, which is not easy to evaporate, you cannot concentrate it easily. You have to spend a lot of energy to evaporate.

And in the process, you may lose a lot of the chemical itself. So you always have to worry about quality control and recovery of your analyte, and main chemical is critical here and sometimes you will have a lot of losses. So, losses can occur as a way of incomplete extraction during the extraction process, it also can be lost during this concentration process. So, what you recover in the instrument is lesser than what is there in original sample for these reasons. These are the 2 possible ways in which we do nitrogen blow down, if you are doing solid samples.

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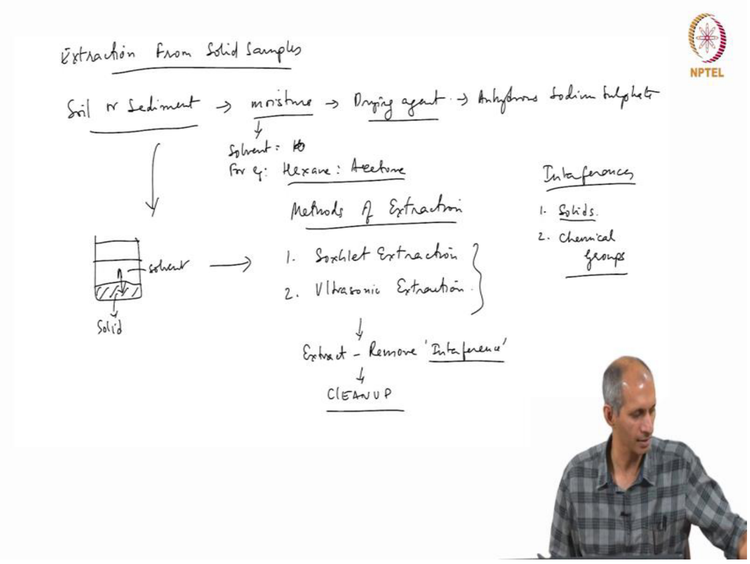
Extraction from Solid Samples

Soil or Sediment → moisture → Drying agent → Anhydrous sodium sulphate

Solvent: ~~Ho~~
For eg: Hexane : Acetone

Methods of Extraction
1. Soxhlet Extraction
2. Ultrasonic Extraction

Extract - Remove 'Interference'
CLEANUP

Interferences
1. Solids
2. Chemical groups

We will talk about the extraction process of solid samples. Solid samples are more complex matrices and for that you need something more rigorous. You can’t do liquid-liquid extraction that kind of things, you can add solvent and mix it but one of the main problems is, because of sediment or soil contain moisture it does not mix very well and you are adding an organic salt, as water is there it will not mix. So, you need to use the solvent which must be a sufficient mixture where it can allow water. That is one way of doing so sometimes people use a solvent which is a mixture.

So for example we have a mixture of hexane and acetone. Both of them are good organic solvents but one of them is soluble in water. One of them mixes with water well the other one does not, hexane does not mix well with water. But you still need a phase by acetone and hexane mix well. The water will get into a little bit of acetone and all that will happen but what this will help us is that the solvent and the solid matrix can mix well. We need good mass transfer that is one, and second is we will also add a drying agent.

So, there is moisture and we add a drying agent like anhydrous sodium sulfate, which will absorb moisture, it will absorb moisture and this sample will become powdery and easy for us to do mass transfer, shaking. So when you have soil or sediment, so essentially what we do is we take some sample solid sample then we add the solvent then we have to bring these two in contact with each other and pull out the A that is sitting.

So, originally where is the chemical sitting on the solid this? It is with the organic matter or some such phase or it is sitting as bulk phase/pure phase. So, you have to pull that out into this and it is a solid matrix. So, by doing all this moisture reduction and drying agent, it becomes particles that are easily you can mix it well with the solvent, and therefore, extraction efficiency is higher. Okay, But in some cases, so, one way of doing this is to you can just mix but mixing itself is not sometimes enough.

So, in the methods of extraction, one older method is Soxhlet extraction. This is an old method and this is used to extract all kinds of solid matrices, anything sludge, soil, sediment from anything any solid, big chunk whatever you want.

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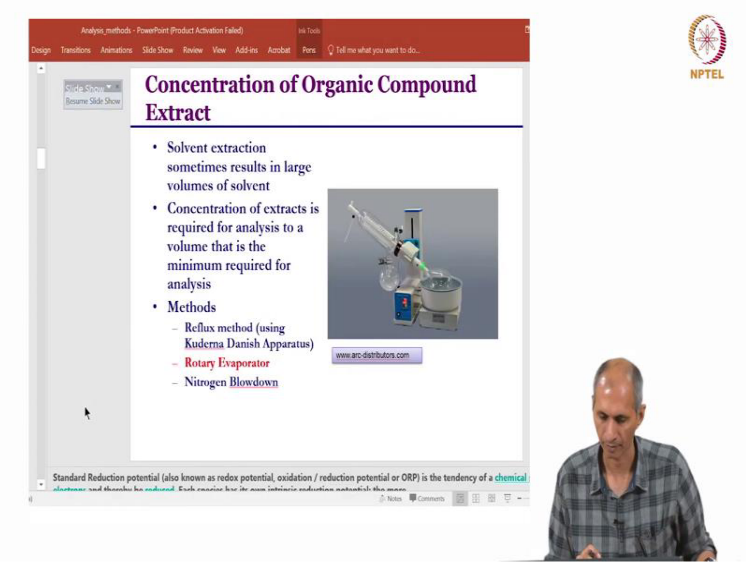

Concentration of Organic Compound Extract

- Solvent extraction sometimes results in large volumes of solvent
- Concentration of extracts is required for analysis to a volume that is the minimum required for analysis
- Methods
  - Reflux method (using Kuderna Danish Apparatus)
  - Rotary Evaporator
  - Nitrogen Blowdown

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Standard Reduction potential (also known as redox potential, oxidation / reduction potential or ORP) is the tendency of a chemical

this is a Rotary evaporator that I showed you.

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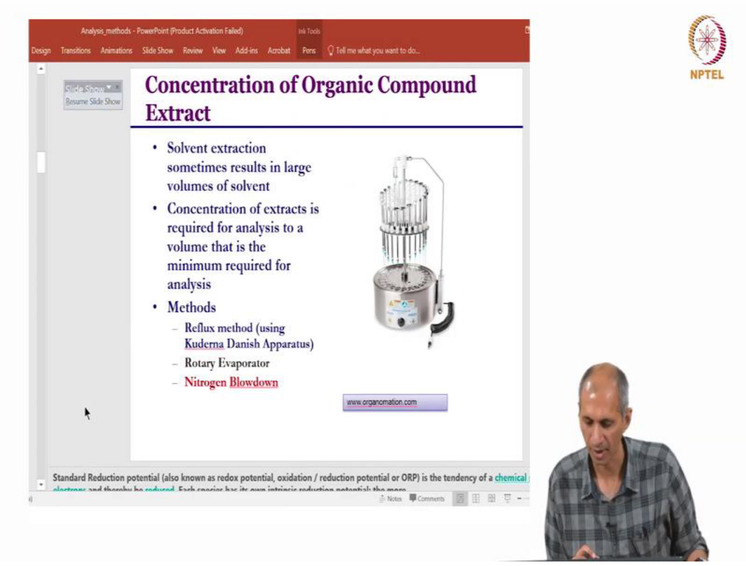

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Standard Reduction potential (also known as redox potential, oxidation / reduction potential or ORP) is the tendency of a chemical

This is a nitrogen system, which we looked at.

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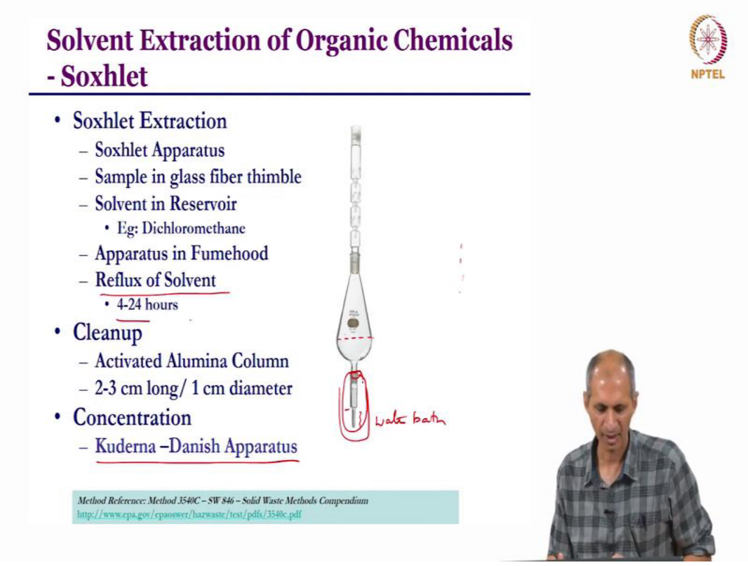

Solvent Extraction of Organic Chemicals - Soxhlet

- Soxhlet Extraction
  - Soxhlet Apparatus
  - Sample in glass fiber thimble
  - Solvent in Reservoir
    - Eg: Dichloromethane
  - Apparatus in Fumehood
  - Reflux of Solvent
    - 4-24 hours
- Cleanup
  - Activated Alumina Column
  - 2-3 cm long/ 1 cm diameter
- Concentration
  - Kuderna –Danish Apparatus

Method Reference: Method 3540C – SW 846 – Solid Waste Methods Compendium
http://www.epa.gov/epaoswer/hazwaste/test/pdfs/3540c.pdf

This is what is called Soxhlet apparatus. What we do is, there is this small thing that you see here on the right-hand side. This small thing is a thimble here, this is a sample holder and it is like a cup. One of the other things in extraction is that solid extraction is like liquid extraction after you finish the extraction you have to separate the solid and the extract the solid. So here what they use is this thimble and it is a filter.

It is made of filter material, you put the solid into that and then it has to be brought in contact with the solvent. So, this is the apparatus, the solvent is placed here, this is a solvent. So this solvent is placed on a heating bath and it is kept at close to its boiling point. It starts boiling, it goes up. It goes up here you see this is condensing. This thing goes up, condenses, and drops down into the thimble.

So it is dropping close to its boiling point, very close to its boiling point. So it is hot. So it is a hot extraction and it drops down and it is now in contact with the solid and it fills up. So, when it fills up this outer layer also fills up and when it reaches this level there is you see a small system here, it goes beyond that and it creates a suction effect and the entire liquid level. The entire liquid solvent that is there in this chamber is now transferred back into the main reservoir.

So this happens in cycles, it takes about let's say 30 minutes for the boiling to happen and drops into this thing. And it fills up when it fills up everything empties. So it is like one cycle of extraction for the thing you are extracting, if at that temperature with a certain volume of solvent for 45 minutes, and then you do another one and then you do another one, so you can look at it. It is called the reflex of solvents and it is done for 4 to 24 hours depending on how hard the matrix is, so this is very rigorous, very harsh extractions, it high temperature.

It is very time consuming, and it is also expensive, expensive in a sense, in terms of energy and it is a bit laborious, but once you pack it in, it is done, it keeps going, it keeps going round and round. So, you will get at the end of the process, the extract is here, and you take out everything and the extract is taken for further processing.

So, in this method, you need a large amount of solvent you cannot do it with 20 ml and 30 ml, you may need 150 ml or so because it has to evaporate, go down still something must be there here. So in this kind of situation we have to use a rotary evaporator or some. If you do not have a rotary evaporator, there is another option that is an instrument that is used it is called as this, this called as Kuderna-Danish Apparatus. So this is placed inside a water bath this is again reflux. The idea behind is this is an evaporator basically, but it does not evaporate very rapidly.

What it does is, it evaporates, the sample is placed here, the initial sample level, maybe here, in the bottom you have a small attachment which is like a test tube and as you can see the graduations, the graduations here are much smaller than here. So you can reduce it to about 1 ml this is about 1 ml and so 250 ml is somewhere here and you are boiling it, so, it goes up.

You can see that these do not allow the sample to just go escape, they go up and they condense they fall back they go up condense fall back some of it escapes the pressure becomes enough they keep escaping. This is a very old technique and it is to prevent the loss of analyte because one of the problems as I was talking about is, if you rapidly evaporate for concentration, solvent will evaporate very fast because it is at the boiling point.

The rate of evaporation is so fast that it will take a lot of other things and go with it co-evaporation will occur and loss will occur and a lot of losses will occur. So, this was seen as a way of reducing it a little bit. But this is a very time-consuming method and it will also not entirely reduce but you will reduce it to some volume here. The same problem with a rotary evaporator is that you cannot reduce it below a certain volume.

So but the idea is once you get it into inside this tube, you can take this tube and do the nitrogen blowdown, reduce it to whatever small volume you want.

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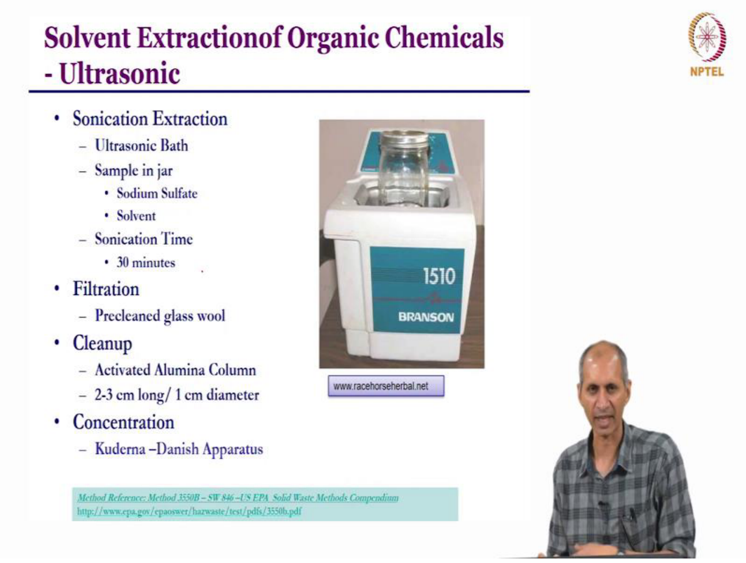

Solvent Extractionof Organic Chemicals - Ultrasonic

- Sonication Extraction
  - Ultrasonic Bath
  - Sample in jar
    - Sodium Sulfate
    - Solvent
  - Sonication Time
    - 30 minutes
- Filtration
  - Precleaned glass wool
- Cleanup
  - Activated Alumina Column
  - 2-3 cm long/ 1 cm diameter
- Concentration
  - Kuderna –Danish Apparatus

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Method Reference: Method 3550B – SW 846 –US EPA Solid Waste Methods Compendium
http://www.epa.gov/epaoswer/hazwaste/test/pdfs/3550b.pdf

So for solids, the other option that we have is a more recent method, that is using an ultrasonic bath. Soxhlet extraction is a very painful and long process to do. So, a new method was developed which is called ultrasonic extraction. Ultrasonic extraction takes a sample and you add all the moisture reducing sodium sulfate and the solvent in it, mix it well and put it in ultrasonic bath and what it does is, at the frequency that is suggested it breaks particles and it makes it more of these things.

So, this ultrasonics have different enhancement of mass transfer effects and also it breaks particles. So, both these things allow it to be effective as extraction methods. In both these methods, once you get an extract in solid-phase, extraction from solid samples like soil it will contain a lot of things, it will contain organic matter an other things, if you are extracting from the soil the extract and the solvent is colorless, but the extract will look yellow or sometimes brown, dark brown, which means it contains a lot of other things, some of which may be analyte of interest, some of which may not be.

So we were discussing this the other day we will call this as an interference, whether it is an interference or not it is determined by you depending on what you want to analyze. So it contains the extract to remove what we call as interferences. You have to filter the sample, process the sample. So, we do what is called a cleanup before we go any further so, one of the main interferences is solid itself.

Because even though we use a filter thimble, the thimble or filter material, or even an ultrasonic extraction, there is no filter and so, you still have to filter it. So, doing this filtration is for interferences, possible interferences. One is solid with other certain chemical groups that we are talking about. So there are cleanup methods to remove different types of interferences, okay, whatever you want, whatever. So, essentially it looks like this.

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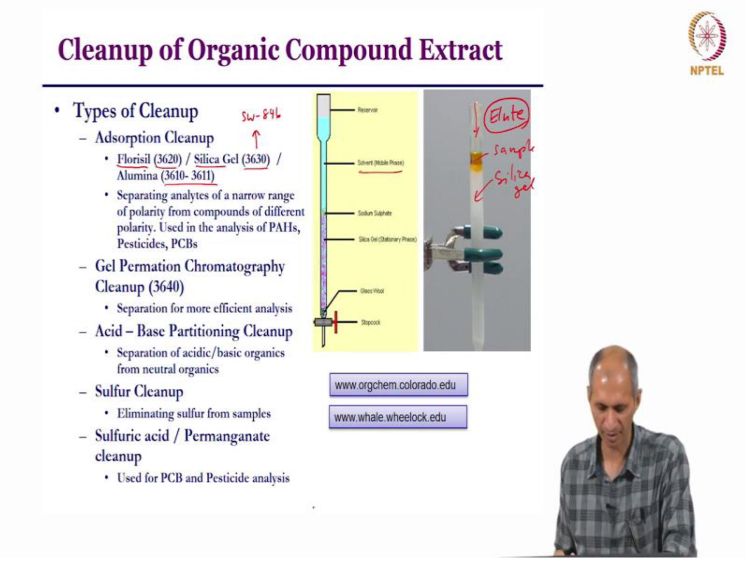

Cleanup of Organic Compound Extract

- Types of Cleanup
  - Adsorption Cleanup
    - Florisil (3620) / Silica Gel (3630) / Alumina (3610- 3611)
    - Separating analytes of a narrow range of polarity from compounds of different polarity. Used in the analysis of PAHs, Pesticides, PCBs
  - Gel Permation Chromatography Cleanup (3640)
    - Separation for more efficient analysis
  - Acid – Base Partitioning Cleanup
    - Separation of acidic/basic organics from neutral organics
  - Sulfur Cleanup
    - Eliminating sulfur from samples
  - Sulfuric acid / Permanganate cleanup
    - Used for PCB and Pesticide analysis

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So, one type of cleanup you have is an adsorption. We have cleanup for these and are all standard methods the numbers you see here 3620 are solid waste 846 method numbers, okay, these are solid waste is SW-846, last class we saw this solid waste 846 methods and these, are the type of material that is used for the cleanup, for example, you have silica gel cleanup, alumina cleanup, florisil cleanup they are different materials.

So, what happens here is, it is like a filter, it is just a bed, your sample is added in the top and allowed to go through this column and it is a filter, it retains anything that you do not want and depending on what you want. So, this is a silica gel column. Silica gel is not the silica gel that you use in the lab here, but it is the silica gel it is used for this particular process.

And it is not just the solid, you can see that it is the gel and it becomes like this when you saturate it with solvent, if you add solvent, it becomes a liquid-solid gel. And on top of it, you add the sample, this is a sample, you can see and the sample goes through, if you just keep the sample, it is not enough you have to elude the sample. And this term of elusion is very specific. Elusion is a term that is used in chromatography.

And we will come to chromatography in the next topic. So we are doing what is called column chromatography here in some sense, but if you are just filtering then we are using that as filtration, but in the case of silica gel, it also does what is called liquid chromatography. So, the oldest form of chromatography which means that you should have a mobile phase here you can see that there is a mobile phase it is moving through when you add this here.

And we will discuss chromatography after this, and you will understand more. So, basically you must have a continuous flow of this going through. So, you have various kinds of cleanup procedures for cleaning up Sulfur, cleaning up acid-base, and all kinds of things. So, depending on where your sample is? What you expect to be as a significant interference? We remove many of these things. Come back to this. Any questions on this so far, in terms of what we are doing.