Advance Analytical Course

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Lecture No # 02

The next step or the next method is accelerated solvent extraction, ASE.

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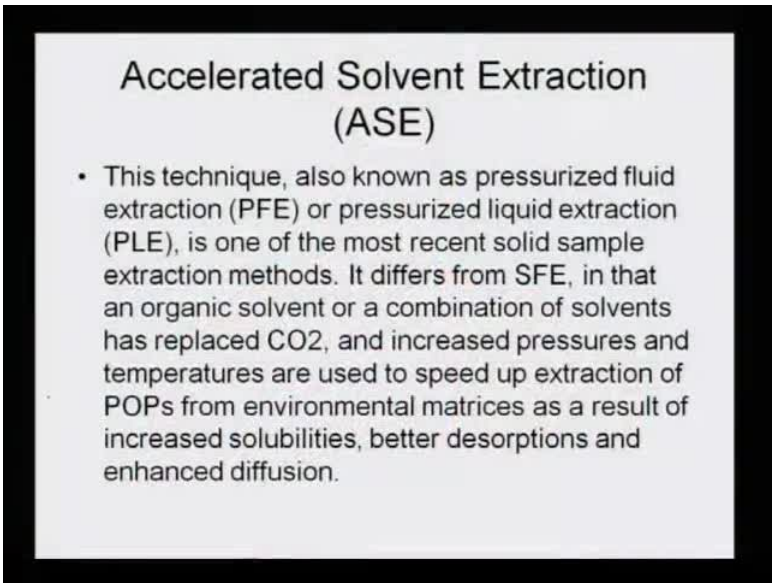

This technique, also known as pressurized fluid extraction (PFE) or pressurized liquid extraction (PLE), is one of the most recent solid sample extraction methods. Again and again, I am emphasizing on various methods of extraction and their differently named methodologies, because if you come across different terms, like in place of ASE, if you find PFE or PLE, it is one and the same method, which means that there is an accelerated for doing the extraction process. It differs from SFE, in that an organic solvent or a combination of solvents has replaced liquefied carbon dioxide, and increased pressures and temperatures are used to speed up the extraction of the POPs from environmental matrices as a result of increased solubilities, better desorptions and enhanced diffusion. So, we came to know about two fast methods of extraction: one is the supercritical fluid extraction and the second one is called the accelerated solvent extraction.

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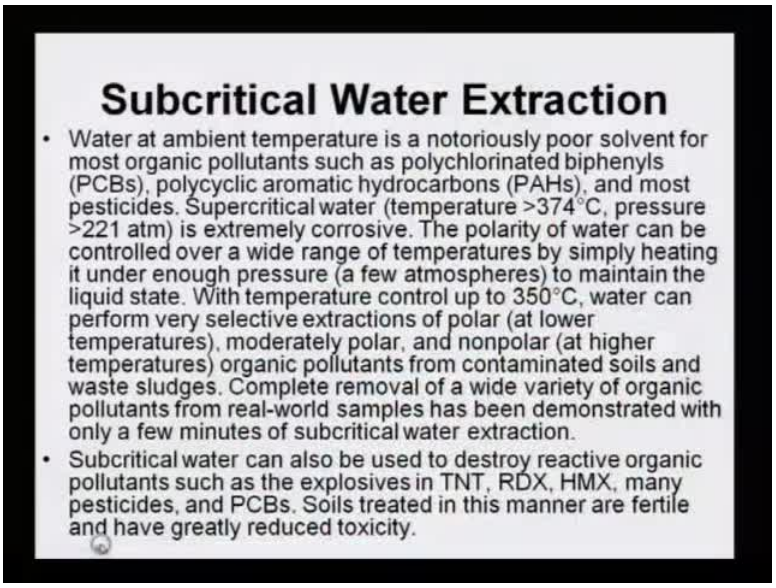

Another type of extraction where subcritical water extraction is used is also a newer technique as compared to the ones, which I have just mentioned. Water at ambient temperature is a notoriously poor solvent for most organic pollutants such as polychlorinated biphenyls, polycyclic aromatic hydrocarbons and most of the other pesticides. Polychlorinated biphenyls or PCBs, polycyclic aromatic hydrocarbons or PAHs are very injurious to human health; that is where their importance and their analysis play a very vital role. Supercritical water temperature is above 374 degree Celsius and at pressure 221 atm, is extremely corrosive. The polarity of water can be controlled over a wide range of temperatures by simply heating it under enough pressure, a few atmospheres to maintain the liquid state. With temperature control up to 350 degree Celsius, water can perform very selective extractions of polar at low temperatures, moderately polar, and non-polar at high temperatures, organic pollutants from contaminated soils and waste sludges. Complete removal of a wide variety of organic pollutant from real-world samples have been demonstrated with only a few minutes of subcritical water extraction. So, one can see that if one wants to really speedup the analysis, then the subcritical water extraction process is one of the fastest methods to do that.

Subcritical water can also be used to destroy reactive organic pollutant such as the corrosives and explosives, which are TNT, RDX, HMX, many pesticides, and PCBs. Soils treated in this manner, are fertile and have greatly reduced toxicity. So, one can do

this kind of extraction for soil samples very efficiently, because from matrix to matrix, the extraction process varies. It is not a hard and fast rule to use all the time same extraction method; for ground water, it could be liquid-liquid extraction process, but for ground soil, it is another type of extraction process.

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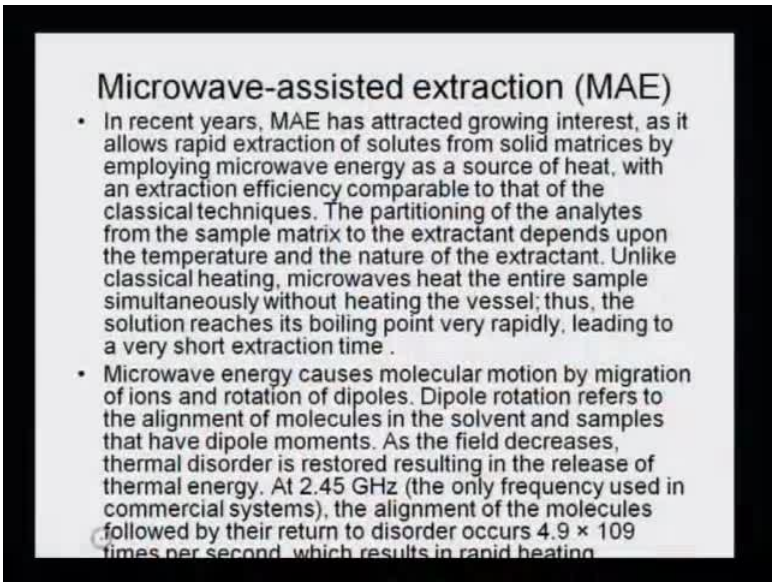

The third, other important type of extraction process is microwave-assisted extraction or MAE. In recent years, microwave-assisted extraction has attracted growing interest, as it allows rapid extraction of solutes from solid matrices by employing microwave energy as a source of heat, with an extraction efficiency comparable to that of classical techniques. The partitioning of the analytes from the sample matrix to the extractant depends upon the temperature and the nature of the extractant. Unlike classical heating, microwaves heat the entire sample simultaneously without heating the vessel; thus, the solution reaches its boiling point very rapidly, leading to a very short extraction time. Now, you must have seen microwaves being used in houses, but they are mostly for cooking purposes. However, the same or similar phenomena can also be used to extract the analyte. As what mentioned earlier, the extraction process is much faster because it utilizes the microwave energy; the microwave energy only heats up the material and not the vessel.

Microwave energy causes molecular motion by migration of ions and rotation of dipoles. The dipolar rotation refers to the alignment of molecules in the solvent and samples that

have dipole moments can only be heated in the system. As the field decreases, thermal disorder is restored resulting in the release of thermal energy. At 2.45 gigahertz, the only frequency used in commercial microwave systems, the alignment of the molecules followed by their return to disorder occurs 4.9 into 10 to the power 9 times per second, which results in rapid heating. So, that is what actually cause the molecular excitation and that is how the heat is generated in this case.

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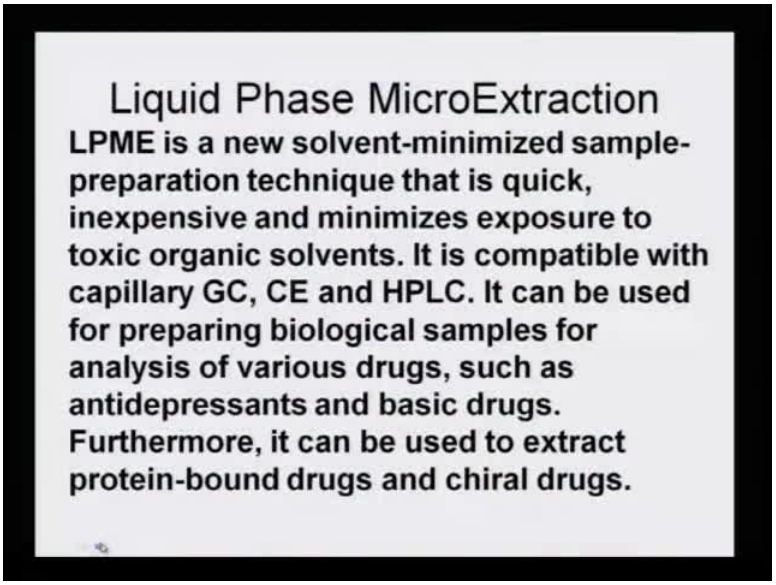

Liquid phase micro extraction – I am coming to more and more recent methods of extraction for the simple reason that now, many new techniques have come up and they depend on the fact that as and when whatever was the need, and if the classical methods were not one of the best methods for extraction, a newer method was devised; one such new method is called liquid phase micro extraction. As the word micro would be very well understood by you all that it relates to smaller and minute quantities of the analyte being extracted. So, it is a more sensitive process than the ones, which I have mentioned earlier.

LPME or liquid phase micro extraction is a new solvent-minimized sample-preparation technique that is quick, inexpensive and minimizes exposure to toxic organic solvents. It is compatible with the capillary GC, with capillary E and HPLC. It can be used for preparing biological samples for analysis of various drugs, such as antidepressants and basic drugs. Furthermore, it can be used to extract protein-bound drugs and chiral drugs.

So, now you can see that how specific the extraction is becoming and how newer techniques are being used.

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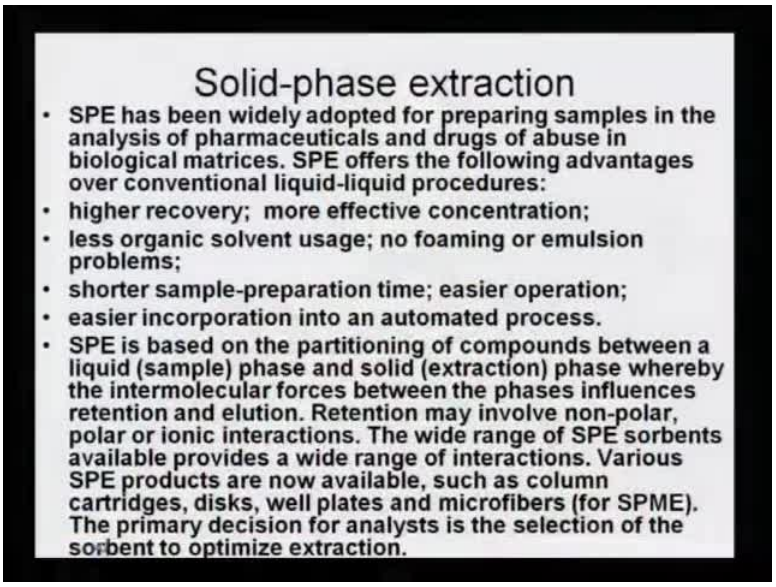

Similarly, there is something called solid-phase extraction. As the name suggest that there is no solvent; it is all solid-solid-analyte-solid analysis. SPE has been widely adopted for preparing samples in the analysis of pharmaceuticals and drugs of abuse in biological matrices. SPE or solid-phase extraction offers the following advantages over conventional liquid-liquid procedures, because as what I mentioned earlier that every new method must have an advantage over the existing classical methods; otherwise, there would not be a need for a new method. It has high recovery; more effective concentration method; less organic solvent usage; no foaming, no emulsion problem; shorter sample-preparation time; easier operation; easier incorporation into an automated process. Because of these advantages, it came into existence and it was used for the purpose of extraction.

SPE is based on the partitioning of compounds between a liquid sample phase and a solid extraction phase whereby the molecular forces between the phases influences retention and elution. Retention may involve non-polar, polar or ionic interactions. A wide range of SPE sorbents available provides a wide range of interactions. Various SPE products are now available, such as column cartridges, disks, well plates, microfibers for SPME, that is, micro extraction. The primary decision for analyst is the selection of the sorbent

to optimize extraction. The bottom line still remains that the analyst has to decide what the analyte is and how to extract it, whether it is in macro quantity or whether it is in micro quantity, and which method of the methods that I have mentioned, must be applied for an efficient extraction process.

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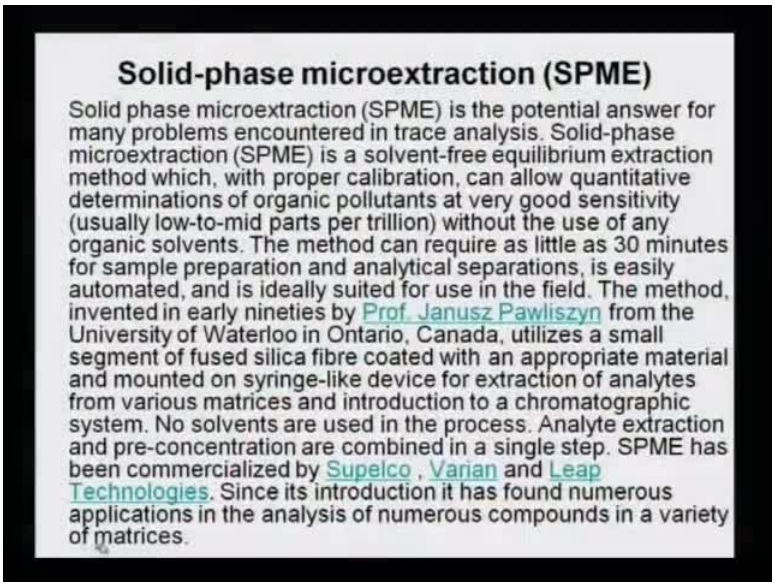

Solid-phase microextraction is similar to the solid-phase extraction, except that it is primarily meant for smaller quantities as the word micro is incorporated. SPME is the potential answer for many problems encountered in trace analysis. Solid-phase microextraction is a solvent-free equilibrium extraction method with proper calibration, can allow quantitative determinations of organic pollutants at very good sensitivity; usually, low-to-mid parts per trillion can be separated without the use of any organic solvents. Parts per trillion means ppt; we only deal with ppm, parts per million commonly; here is a process, which can actually extract parts per trillion even.

The method can require as little as 30 minutes for sample preparations and analytical separation, is easily automated; it is ideally suited for the use in the field. The method was first invented in early nineties by Professor Janusz Pawliszyn from the University of Waterloo in Ontario, Canada. It utilizes a small segment of fused silica fiber coated with an appropriate material and mounted on syringe-like device for extraction of analytes from various matrices and introduction to a chromatographic system. No solvents are used in this process; so, it is truly a solid-phase microextraction as the name suggest.

Analyte extraction and pre-concentration are combined in a single step. SPME has been commercialized by Supelco, Varian, Leap Technologies; these are some of the companies' names, who are commercializing such micro extractants. Since its introduction, it has found numerous applications in the analysis of numerous compounds in a variety of matrices.

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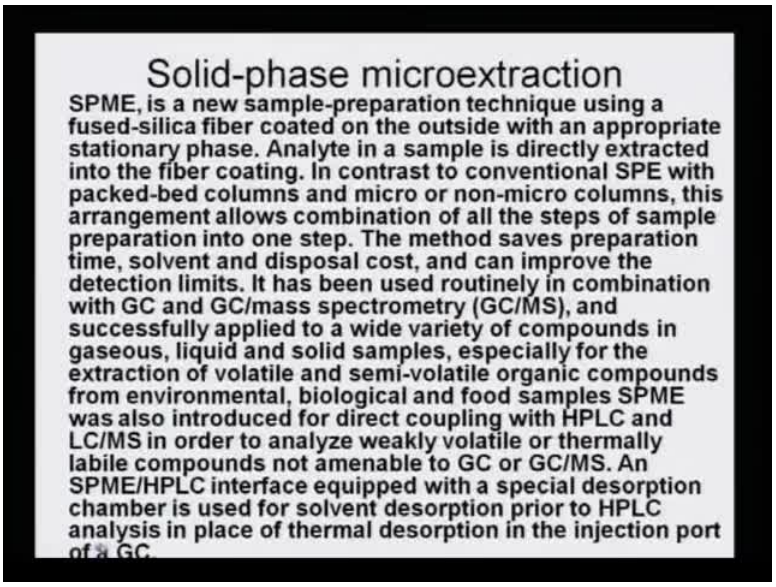

We have just spoken in detail about the solid-phase microextraction; it can be directly linked up with GC MS, that is, gas chromatography mass spectrometry, and that is what it has an advantage that there is no contamination. The analyte in the sample is directly extracted into the fiber coating. In contrast to conventional solid-phase extraction with packed-bed columns and micro and non-micro columns, this arrangement allows combination of all the steps of sample preparation in just one step.

The method saves preparation time, solvent and disposal cost, and can improve the detection limits. It has been used routinely in combination with gas chromatography or GC and gas chromatography mass spectrometry, that is, GC MS, and is successfully applied to a variety of compounds in gaseous, liquid and solid samples, especially for the extraction of volatile and semi-volatile organic compounds from environmental, biological and food samples. That is why it has an added advantage. Even it can be connected to HPLC, where it is interfaced with a chromatographic machine, so that it can be separated and used further on. So, the extractant is directly connected to that.

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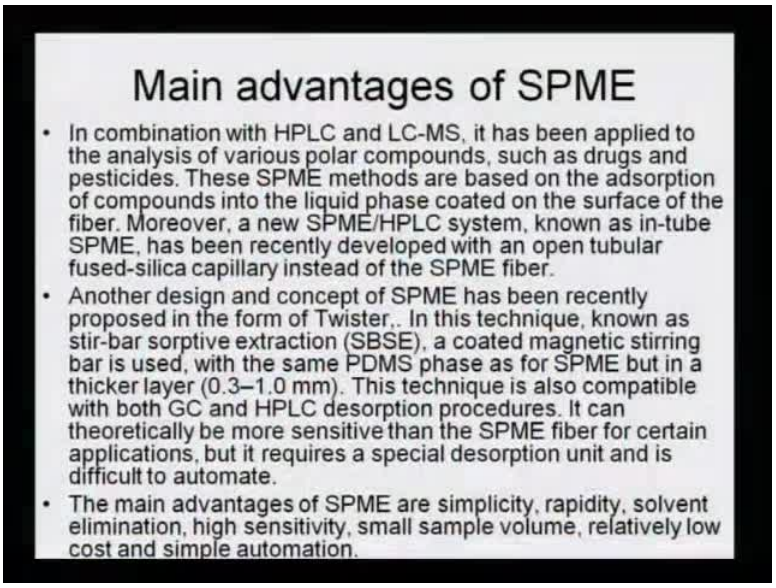

Main advantages of solid-phase microextraction – I am repeatedly talking about this and I am making so much of emphasis on this, because this is one of the most recent techniques in extraction, and it can be combined with HPLC, with LCMS, with GC and GC MS. It has been applied to analysis of various polar compounds, such as drugs and pesticides. These SPME methods are based on the adsorption of compounds into the liquid phase coated on the surface of the fiber. Moreover, a new SPME HPLC system, known as in-tube SPME, has been recently developed with an open tubular fused-silica capillary instead of the SPME fiber. So, more and more and more recent advances have actually taken place according to the need of the extraction.

Another design and concept of SPME has been recently proposed in the form of Twisters. In this technique, known as stir-bar sorptive extraction, a coated magnetic stirring bar is used, with the same PDMS, which I mentioned a little while ago, and this is acting as a phase for SPME, but in a thicker layer; that is, if the layer of that PDMS is almost 0.3 to 1 millimeter, this technique is also compatible with both GC and HPLC desorption procedures. It can be theoretically be more sensitive than the SPME fiber for certain applications, but it requires a special desorption unit and is difficult to automate. So, according to the need of the analysis, newer machines, newer adoptability into the existing solid-phase micro extraction has been brought about. The main advantages of SPME are simplicity, rapidity, solvent elimination, high sensitivity, small sample volume, relatively low cost and simple automation.

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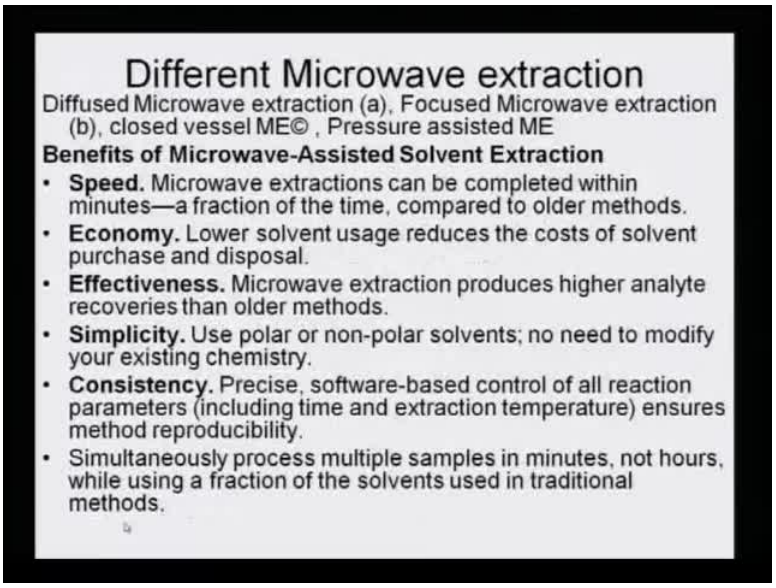

Different microwave extraction: now, a little while ago, I had told you about microwave extraction, but I forgot to mention that there are different modules. I am just briefly mentioning these here in order to keep you aware that it is not just one model that is proposed for microwave extraction, there is a model called diffused microwave extraction; there is another model, which is called focused microwave extraction; there is a third model, which is closed vessel microwave extraction; there is a 4th model, which is pressure-assisted microwave extraction. As the name suggest, if it is diffused, that means it is a very general kind of a microwave extraction; if it is focused, the microwave energies are particularly focused on a particular point; if it is a closed vessel microwave extraction, the vessel is completely tightly closed; if it is a pressure assisted, that means there is a special pressure acting upon the microwave extraction.

Benefits of microwave-assisted solvent extraction: it is very speedy; microwave extractions can be completed within a few minutes – a fraction of a second; sometimes even that compared to the older methods. It is economical; that means lower solvent usage reduces the costs of solvent purchase and disposal. Effectiveness – microwave extraction produces higher analyte recoveries than older methods. It is simple because it has simplicity; use of polar or non-polar solvents; no need to modify the existing chemistry. It is also showing a lot of consistency; precise, software-based control for all reaction parameters including time of extraction and extraction temperature; assures repeatability and reproducibility. Simultaneous processes multiple samples in just being

extracted in a few minutes, not hours; while using a fraction of the solvents, is not it a better method than traditional methods? Definitely, it is a better method.

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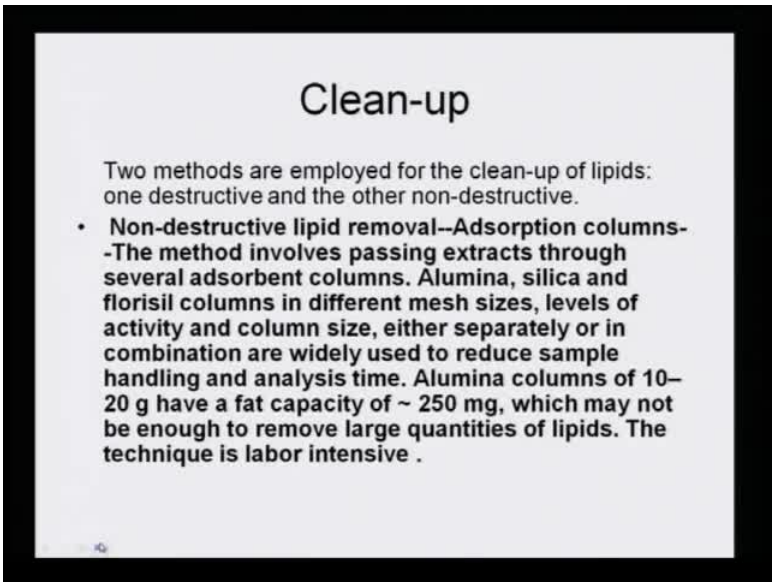

However, sometimes, we have to remember that there are extractants, which have to be cleaned up. Two methods are employed for cleaning up the lipids, because lipids cause interference in the analysis; so, they have to be removed from the extractant. One method is the destructive method and the other one is the non-destructive method. The non-destructive liquid removal is usually carried out by the use of absorption columns. The method involves passing extracts through several adsorbent columns made out of alumina, silica or florisil columns in different mesh sizes, levels of activity and column size, either separately or in combination are widely used to reduce sample handling and analysis time. Alumina columns of 10 to 20 gram have a fat capacity of 250 milligram, which may not be enough to remove large quantities of lipids. The technique is labor intensive; that means if there are large amounts of fat or lipids that need to be removed, one has to use bigger columns and more columns.

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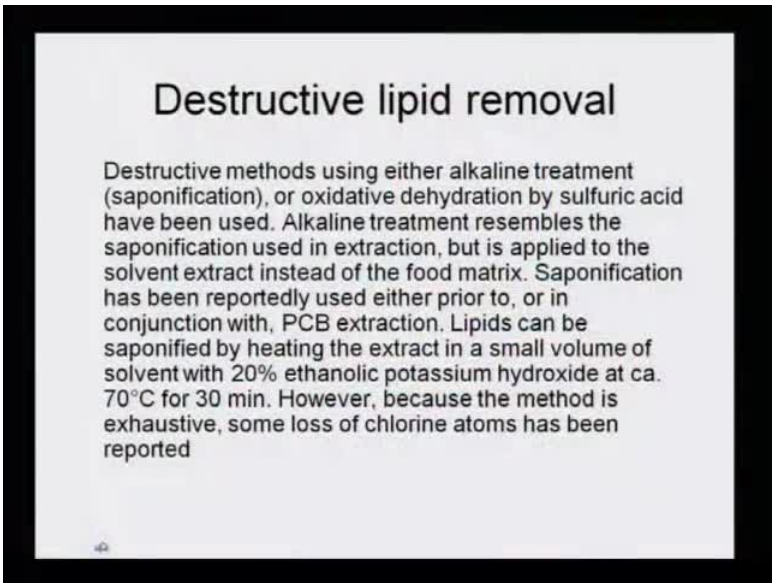

Destructive lipid removal is the second option. The destructive methods using either alkaline treatment or we call it saponification, or oxidative dehydration by sulfuric acid have been used. Alkaline treatment resembles the saponification used in extraction, but is applied to the solvent extract instead of the food matrix. Saponification has been reportedly used either prior to, or in conjunction with the PCB extractions. So, these methods are actually very compound specific. Whether we should use the destructive method or the non-destructive method has to be decided as to what is the analyte that is to be extracted. Liquids can be saponified by heating the extract in a small volume of solvent with 20 percent ethanolic potassium hydroxide at 70 degree Celsius for just 30 minutes. However, because the method is exhaustive, some losses of chlorine atoms also are reported. So, one has to weigh and outweigh, which process to carry out in order to get maximum extract.

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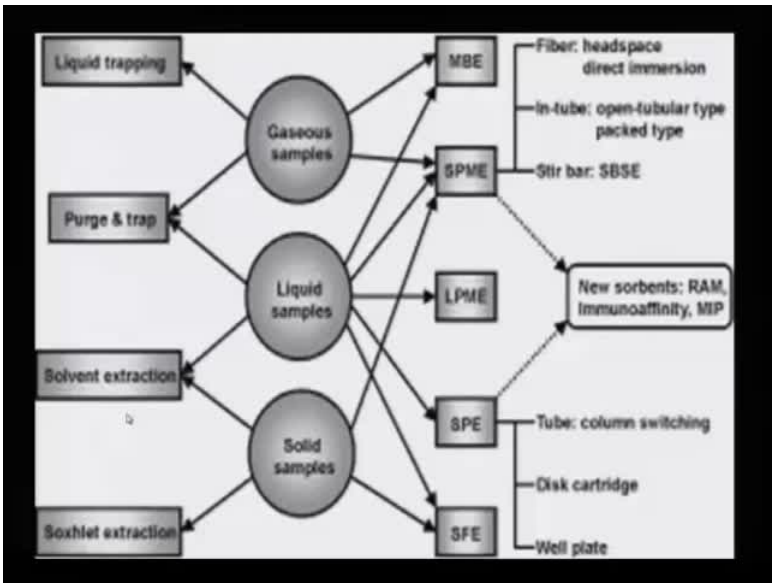

This is an overview where different types of gaseous liquid and solid samples are shown; whether they should be done by liquid trapping, by purge or trap, or solvent extraction, or soxhlet extraction, and whether one should use microwave extraction, or one should use solid phase micro extraction or liquid phase micro extraction, and various other options have been given. So, this gives an overview what all we can do if the samples are gaseous, or if they are liquid, or if they are solid.

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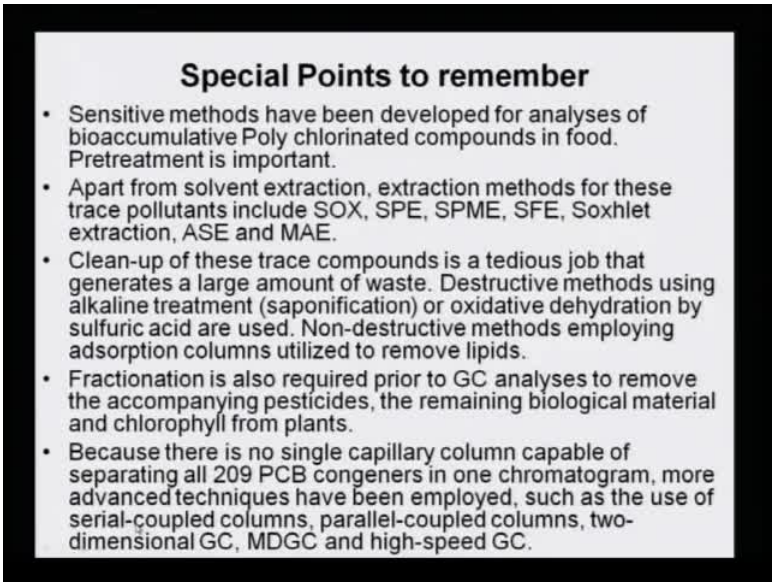

The special points that need to be remembered in this lecture are the following. Sensitive methods have been developed for analyses of bioaccumulative poly chlorinated compounds in food. Pretreatment is important. Apart from solvent extraction, extraction methods for these trace pollutants include the SOX – that is, the soxhlet, solid-phase extraction, solid-phase micro extraction, supercritical fluid extraction, soxhlet extraction, accelerated solvent extraction and microwave accelerated extraction. Clean-up of these trace compounds is a tedious job that generates a large amount of waste. Destruction methods using alkaline treatment, that is, saponification or oxidative dehydration by sulfuric acids are used. Non-destructive methods by employing adsorption columns, utilizes to remove the lipids.

Fractionation is also required prior to GC, that is, the gas chromatographic analyses to remove the accompanying pesticides, the remaining biological material and chlorophyll from the plants. Because there is a no single capillary column capable of separating all 209 PCB congeners in one chromatogram, more advanced techniques have been employed, such as the use of serial-coupled columns, parallel-coupled columns, two-dimensional GC, mid-dimensional GC and high-speed GC. However, there are many new processes still awaiting to be discovered, because from sample to sample, from analyte to analyte, one needs to take a judgment as to what has to be extracted and how it has to be extracted.