**Advance Analytical Course**

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**Lecture No. # 11**

Different types of microwave extraction systems have been used for PCBs.

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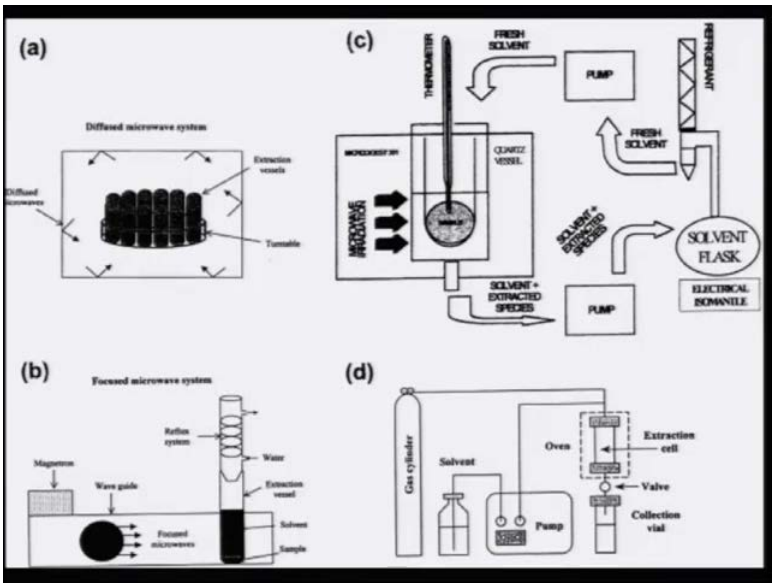


And, several layouts have been shown in this slide.

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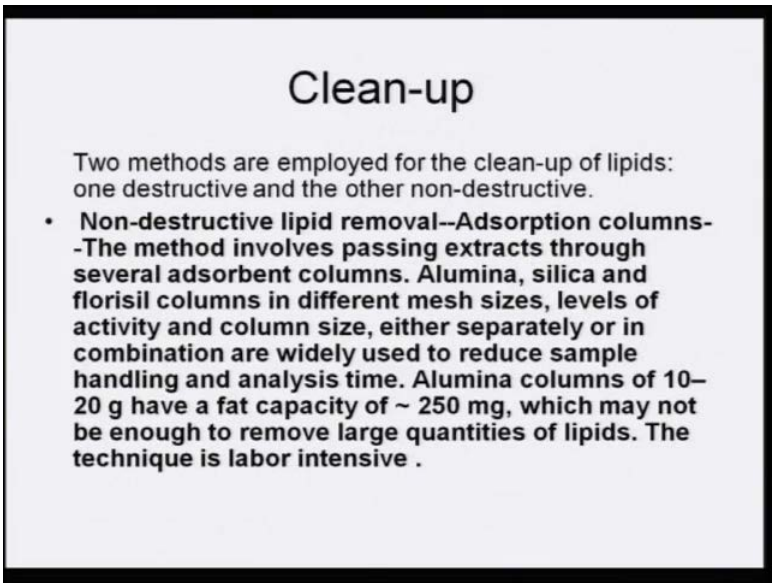


However, PCBs need to be cleaned up. And, the clean-up methods – there are two distinct methods for clean-up. The two methods are employed for the clean-up of lipids: one is a destructive method and the other is a non-destructive method. The non-destructive method lipid removal, as the name suggests, it is in the adsorption columns – the method involves passing the extracts through the several adsorbent columns. It could be alumina, silica and florisil columns in different mesh sizes, levels of activity and column size, either separately or in combination are widely used to reduce the sample handling and analysis time. Alumina columns of 10 to 20 gram have a fat capacity of almost 250 milligrams, which means that they can adsorb 250 milligrams of fat, which may not be enough to remove large quantities of lipids. The technique is labor intensive. That means it has to be done again and again on different types of columns like for alumina column, then silica column and then florisil column. So, it is a labor intensive process.

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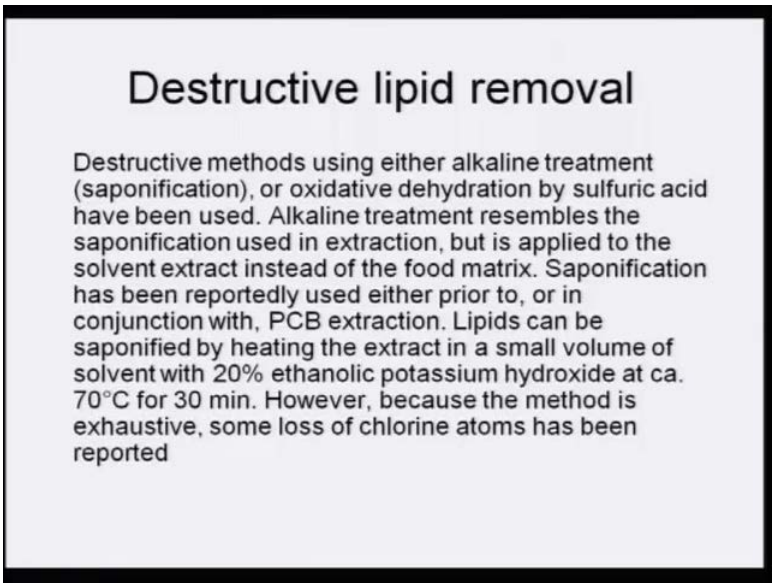


Destructive lipid removal – destruction methods using either alkaline treatment, which means saponification, or oxidative dehydration by sulfuric acid have been used. Somehow, the lipid has to be removed; otherwise, the PCBs will not be able to come out of the extraction medium. Alkaline treatment resembles 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 PCB extraction. Lipids can be saponified by heating the extract in a small volume of solvent with 20 percent ethanolic potassium hydroxide at 70 degrees for 30 minutes. So, just by agitating with 20 percent ethanolic potassium hydroxide at 70 degrees for half an hour, one can get rid of the lipids and one can get the PCB in the extract. However, because the method is exhaustive, some loss of chlorine atoms has also been reported. However, this method has a little trickiness that sometimes, in some congeners, the chlorine may be cleaved off. So, that will give wrong results. So, one has to optimize and not overdo this particular destructive removal of lipids.

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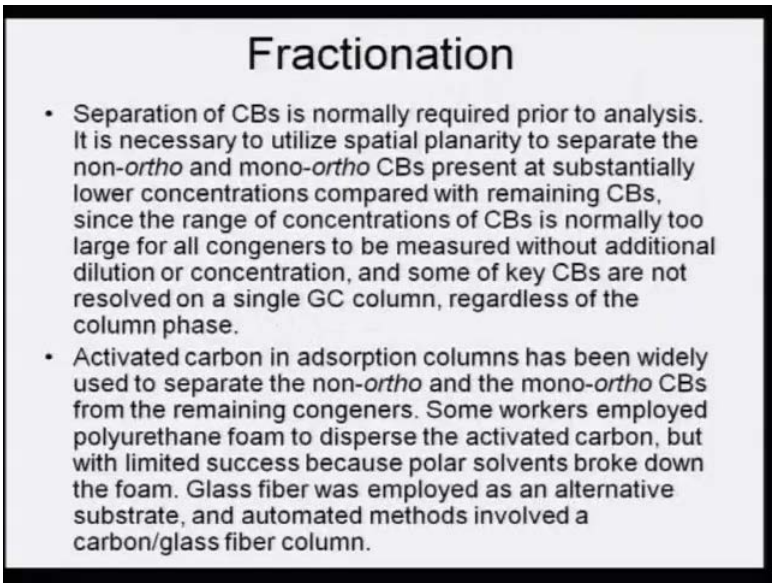


Fractionation – separation of several CBs is normally required prior to analysis. It is necessary to utilize spatial planarity to separate the non-ortho and the mono-ortho CBs present at substantially lower concentrations compared with remaining CBs, since the range of concentrations of CBs is normally too large for all congeners to be measured without additional dilution or concentration, and some key CBs are not resolved in just one single GC column, regardless of the column phase. So, because these compounds are so close in their structures, it is important to manipulate the process in such a manner that all the congeners should be separated properly.

Activated carbon in adsorption columns has been widely used to separate the non-ortho and the mono-ortho CBs from the remaining congeners. So, there was a need to be able to separate the volatiles, non-congeners, non-ortho congeners and mono-ortho congeners, and that is how activated carbon was utilized for this purpose. Some workers employed polyurethane foam to disperse the activated carbon, but with limited success because polar solvents broke down the foam. Glass fiber was employed as an alternative substrate, and the automated methods involved a carbon/glass fiber column. So, many manipulations had to be done in order to be able to extract the maximum number of congeners from the extract or the matrix.

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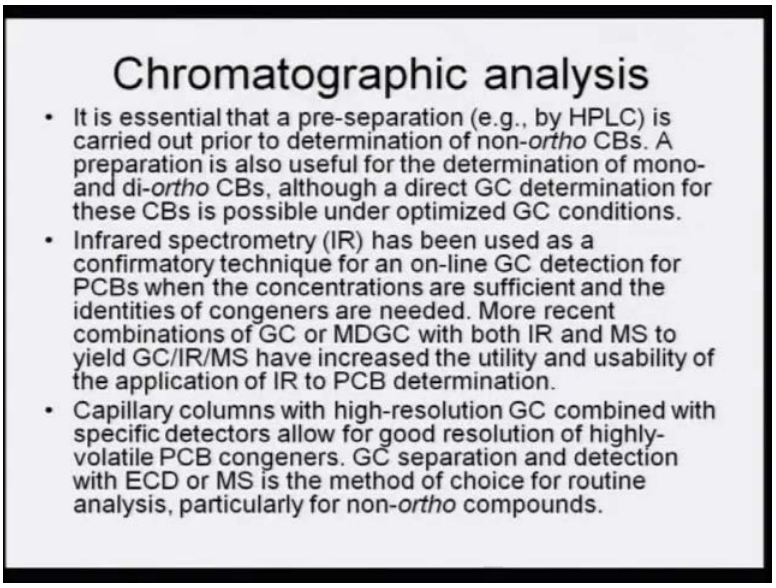


Chromatographic analysis – it is essential that a pre-separation, that is, by HPLC, is carried out prior to determination of non-ortho CBs. A preparation is called carefully and useful for the determination of mono- and di-ortho CBs, although a direct GC determination for these CBs is possible under optimized GC conditions. So, if a little bit of pre-separation is carried out with the help of HPLC, then a normal GC machine can be utilized. Infrared spectrometry or IR has also been used as a confirmatory technique for an on-line GC detection for PCBs when the concentrations are sufficient and the identities of congeners are needed.

Infrared spectrometry comes very handy. The information imparted by infrared is very good. More recent combinations of GC or MDGC with both IR and MS to yield GC/IR/MS have increased the utility and usability of the application of IR to find out the PCB determination.

Capillary columns with high-resolution GC combined with specific detectors allow for good resolution of highly-volatile PCB congeners. GC separation and detection with ECD or MS is the method of choice for routine analysis, particularly for non-ortho compounds. Non-ortho means that the chloro groups are not next to each other; that is what is meant by non-ortho.

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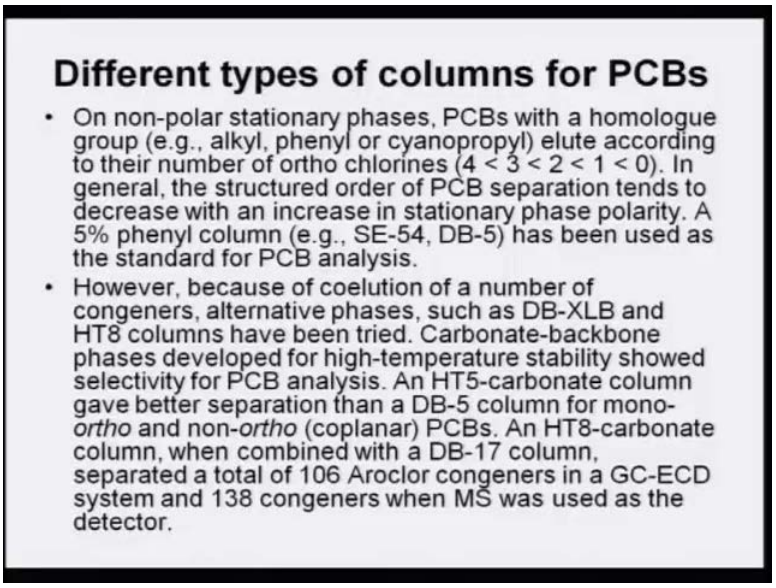


Different types of columns have been utilized for the analysis of polychlorinated biphenyls, that is, the PCBs. On non-polar stationary phases, PCBs with homologous group – alkyl, phenyl, cyanopropyl elute according to their number of ortho chlorines, that is, 4 is greater than 3; and 3 is greater than 2; and 2 is greater than 1; and 1 is greater than 0. In general, the structured order of PCB separation tends to decrease with an increase in stationary phase polarity. A 5 percent phenyl column, that is, DB-5 of a commercial name, has been used as the standard for PCB analysis.

Now, let me tell you one thing at this point of time that columns are of different polarities, as what I mentioned when I was talking about GC and its working and the different types of columns that were used in GC. The different companies have different prefixes, that is, DB or SE may change, but the number that follows the hyphen, that is, 1, 5, 17 shows the polarity level of the column. So, that remains the same; whether the manufacturer is a Perkinelmer manufacturer or a DB company manufacturer, it does not matter. The polarity of DB-5 and PE-5 will be the same.

However, because of coelution of a number of congeners, alternative phases, such as DB-XLB and HT8 columns have also been tried out. Carbonate-backbone phases developed for high temperature stability showed selectivity for PCB analysis. An HT5-carbonate column gave better separation than a DB-5 column for mono-ortho and non-ortho coplanar PCBs. An HT8-carbonate column, when combined with a DB-5 column,

separated a total number of 106 Aroclor congeners in a GC-ECD system and 138 congeners when MS was used as the detector. So, you can see immediately that in my earlier lecture also, I had mentioned, that GC-ECD and GC-MS have different sensitivity, and here also, the Aroclor congeners were identified in different numbers, which shows that ECD detector is less sensitive as compared to the MS detector.

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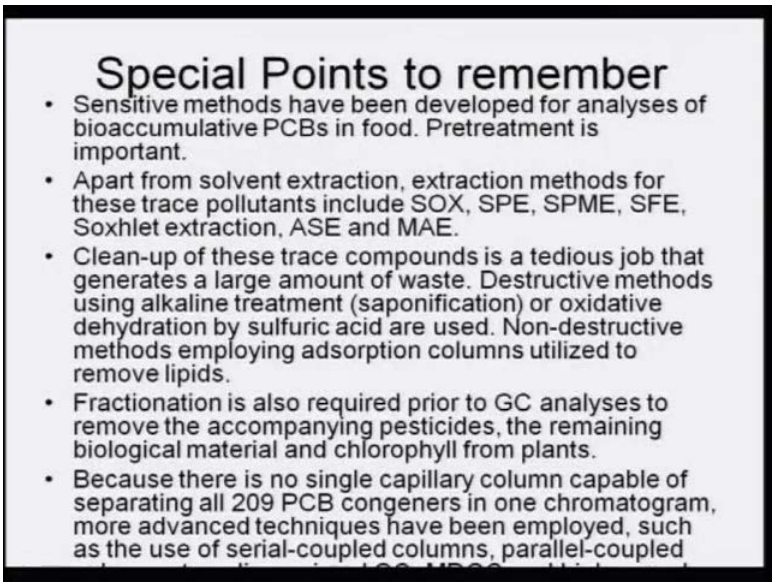


Special points that need to be remembered when one is analyzing PCBs. Sensitive methods have been developed for analyses of bioaccumulative PCBs in food. Pretreatment is important. Apart from solvent extraction, extraction methods for these trace pollutants include SOX, PSE, SPME, SFE, Soxhlet extraction, ASE and MAE. Clean-up of these trace compounds is a tedious job that generates a large amount of waste. Destructive methods using alkaline treatment or saponification or oxidative dehydration by sulfuric acid are used. Non-destructive methods employing adsorption columns utilized to remove lipids as well. So, one can use either a destructive method, where there is a possibility of losing out of some chlorine atoms or a non-destructive method by employing adsorption columns.

Fractionation is also required prior to GC analyses to remove the accompanying pesticides and other chemicals, which are not required to be analyzed in the system, the remaining biological material and chlorophyll from the plants. Because there is no single capillary column capable of separating all the 209 PCB congeners in one chromatogram,

more advanced techniques have to be employed, such as the use of serial-coupled columns, parallel columns and so on. So, one can understand that the more the intricate the analysis, the more care has to be taken for designing the methodology. I am again coming back to modern gas chromatography, because so far, we have studied so many adaptations of gas chromatography, and I feel that a more intense treatment once again needs to be done for the modern gas chromatography and the recent developments that have been brought about in this chromatographic technique.

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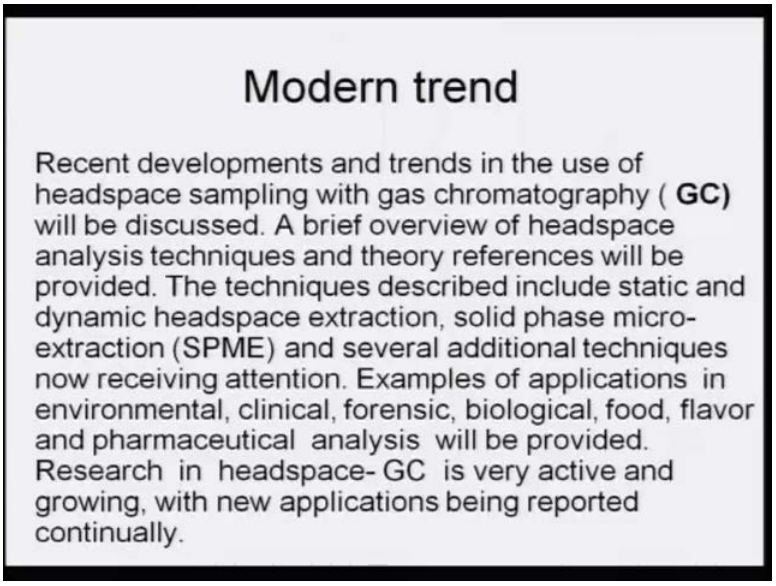


Modern trend has been that in the recent developments and trends, there is the use of headspace sampling with gas chromatography. I have spoken the word headspace earlier, but I thought that I should spend some special dedicated lecture on the headspace adaption of the GC machine. A brief overview of headspace analysis technique and theory references will be provided in this lecture. The technique describes that it includes static and dynamic headspace extraction, solid-phase microextraction (SPME) and several additional techniques now receiving attention. Examples of applications in environmental, clinical, forensic, biological, food, flavor and pharmaceutical analysis will be provided. Research in headspace-GC is very active and growing, with new applications being reported continuously.

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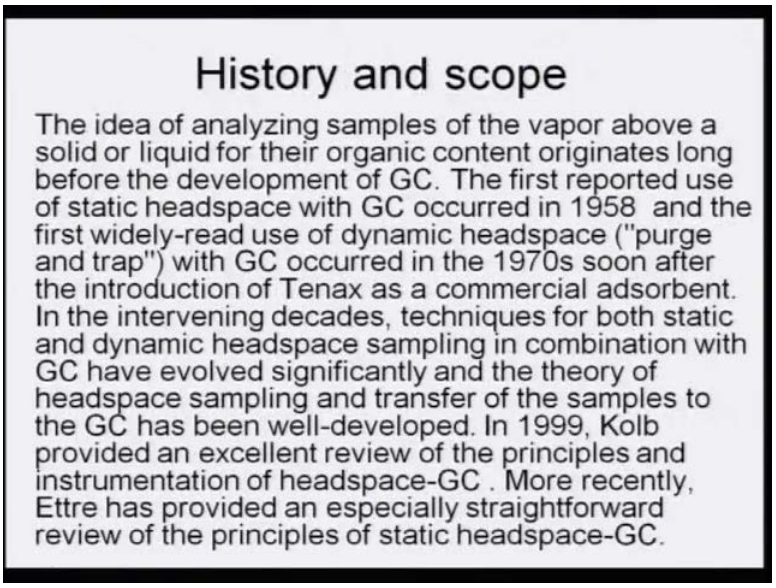


I will give you a little history and its scope, because this is the most modern trend of the GC machine and the use of the headspace has come to be in notice very recently. That is why I am spending special time on this particular new technique. The idea of analyzing samples of the vapor above a solid or liquid for their organic content originates long before the development of GC. The first reported use of static headspace with GC occurred in 1958 and the first widely-read use of dynamic headspace, that is, the purge and the trap method with GC occurred in the 1990s; so, it is not very old; soon after the introduction of Tenax as a commercial adsorbent. In the intervening decades, techniques for both static and dynamic headspace sampling in combination with GC have evolved significantly and the theory of headspace sampling and transfer of the samples to the GC has been well-developed. In 1999, Kolb provided an excellent review of the principles and instrumentation of headspace-GC. More recently, Ettre has provided an especially straightforward review the principle of static headspace-GC. So, you see a lot of research has gone into whether headspace should be static or dynamic. As the name suggests, static means it is not moving and only the adsorption and desorption is taking place, whereas in the dynamic, the adsorption is also taking place and the extraction is also taking place. So, as the name suggests, these two methods have their own significant role to play while analyzing these on the GC machine.

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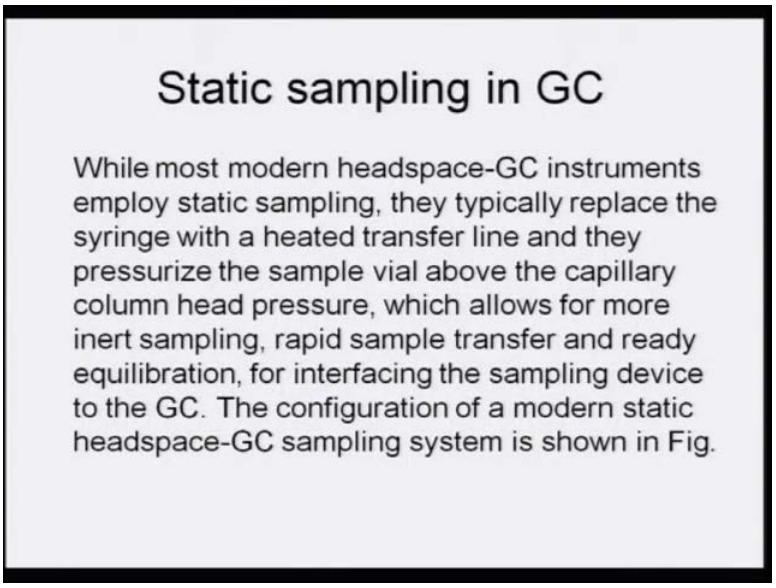


Static sampling in GC – while modern headspace-GC instruments employ static sampling, they typically replace the syringe with a heated transfer line and they pressurize the sample vial above the capillary column head pressure, which allows for more inert sampling, rapid sample transfer and ready equilibration, for interfacing the sampling device to the GC machine. The configuration of a modern static headspace is given in the next figure. So, what happens? There is a direct on the column head itself; capillary column, there is a pressurized on-line transfer.

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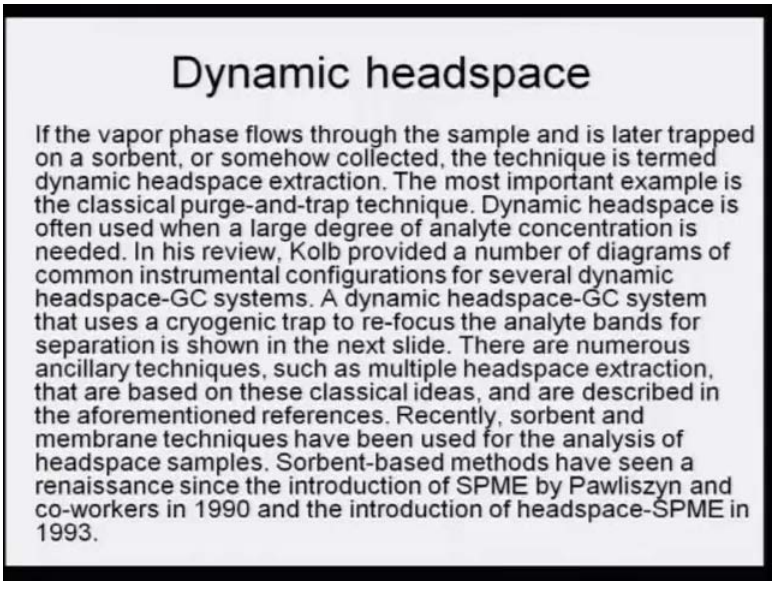

Dynamic headspace – if the vapor phase flows through the sample and is later trapped on a sorbent, or somehow collected, the technique is termed as dynamic headspace extraction, as what I mentioned a while ago. I said, there is a simultaneous transfer and extraction, both occurring, and that is happening at the headspace of the GC. So, that is why the name dynamic headspace. The most important example is the classical purge–and-trap technique. Dynamic headspace is often used when a large degree of analyte concentration is needed. In this review, Kolb provided a number of diagrams of common instrumental configuration for several dynamic headspace-GC systems. A dynamic headspace GC system that uses a cryogenic trap to re-focus the analyte bands for separation is shown in the next slide. There are several ancillaries and numerous techniques, such as multiple headspace extraction, that are based on these classical ideas, and are described in the aforementioned references. Recently, sorbent and membrane techniques have also been used for the analysis of headspace samples. Sorbent-based methods have been seen as a renaissance since the introduction of SPME was done very recently in 1990 and the introduction of headspace SPME in 1993. So, you can see that how the development has taken place. From 1958 to 1993, the headspace, both static and dynamic, came into existence and was used by the analysts.

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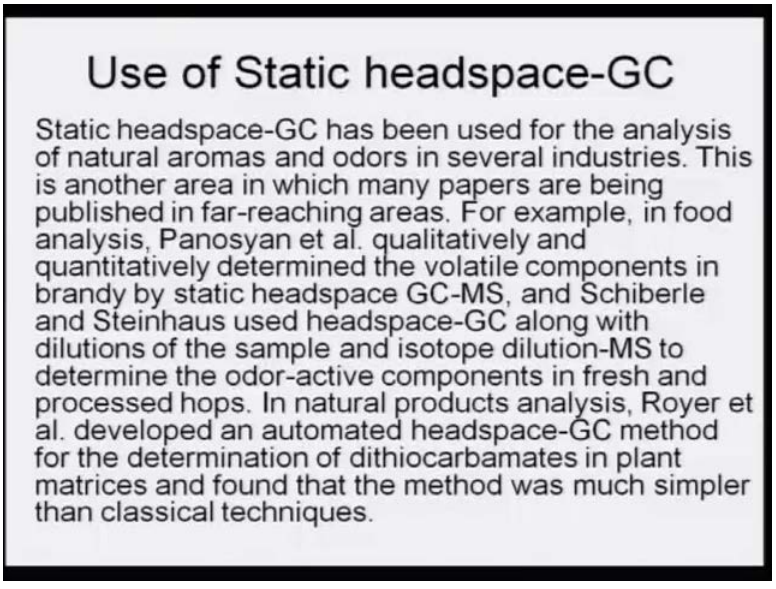


Use of static headspace-GC – static headspace-GC has been used for the analysis of natural aromas and odors in several industries. This is another area in which many papers are being published in far-fetching areas. For example, in food analysis, Panosyan et al.

quantitatively and qualitatively determined the volatile components in brandy by static headspace GC-MS, and Schiberle and Steinhaus used headspace-GC along with dilutions of the sample and isotope dilution-MS to determine the odor-active compounds in fresh and processed hops. In natural products analysis, Royer et al. developed an automated headspace-GC method for the determination of dithiocarbamates in plant matrices and found that the method was much simpler than the classical techniques. So, in many cases, the static headspace-GC turned out to be much simple to operate as compared to the conventional or the statistical techniques.

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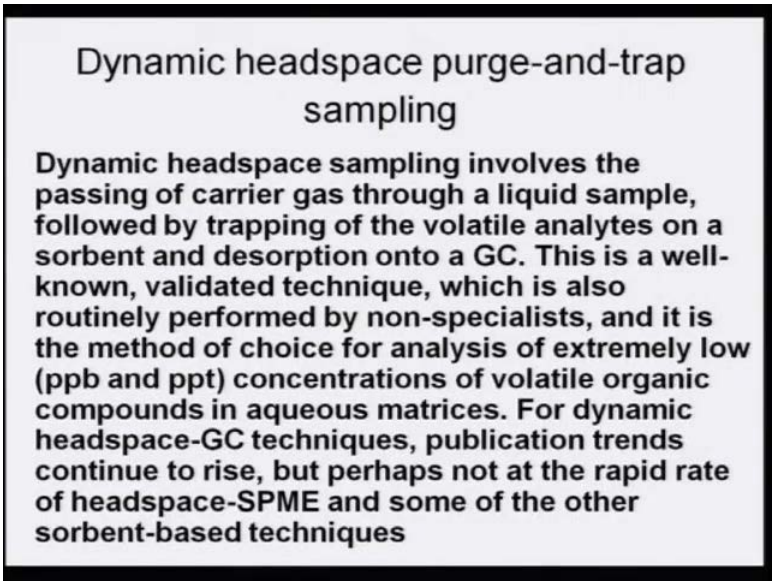


Dynamic headspace purge-and-trap sampling – dynamic headspace sampling involves the passing of carrier gas through a liquid sample, followed by trapping of the volatile analytes on a sorbent and desorption onto the GC. As the name suggests, it is moving; it is getting extracted; and it is getting desorbed on the GC capillary column. This is a well-known validated technique, which is also routinely performed by non-specialists and is the method of choice for analysis of extremely low, that is, in the ppb and ppt (parts per billion and parts per trillion) concentrations of the volatile organic compounds in aqueous matrices. For dynamic headspace-GC techniques, publication trends continue to rise, but perhaps not at the rapid rate of headspace-SPME and some of the other sorbent-based techniques, which means that the more common and the more trendy mechanism is the headspace with solid-phase microextraction, which is connected to the GC machine.

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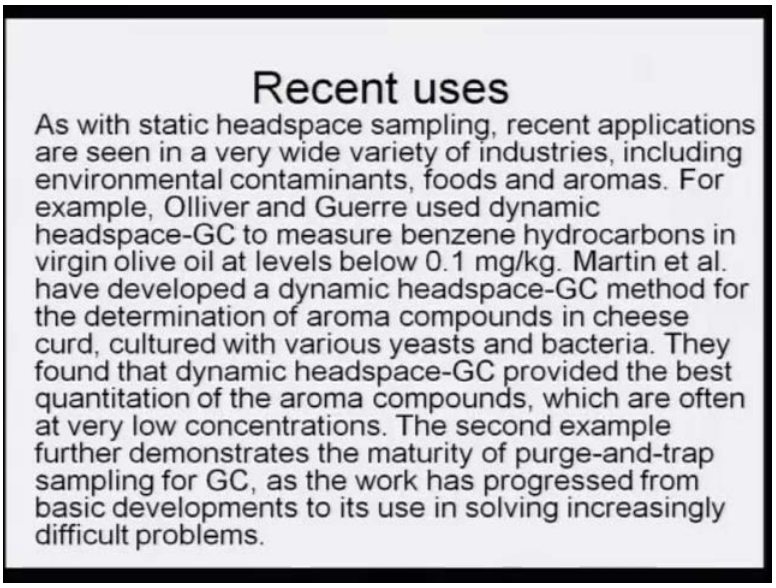


Recent uses – as with static headspace sampling, recent applications are seen in a very wide variety of industries, including environmental contaminants, foods and aromas. For example, Olliver and Guerre used dynamic headspace-GC to measure benzene hydrocarbons in virgin olive oil at levels below 0.1 milligram per kilogram. Martin et al. have developed a dynamic headspace-GC method for the determination of aroma compounds in cheese, curd, cultured with various yeasts and bacteria. They found that dynamic headspace-GC provided the best quantitation of the aroma compounds, which are often at very low concentrations. The second example further demonstrates the maturity of the purge-and-trap sampling for GC, as the work has progressed from basic developments to its use in solving increasingly difficult problems. So, you see, as the recent trends have proceeded, more and more intricate systems and more and more intricate headspace systems have been used.

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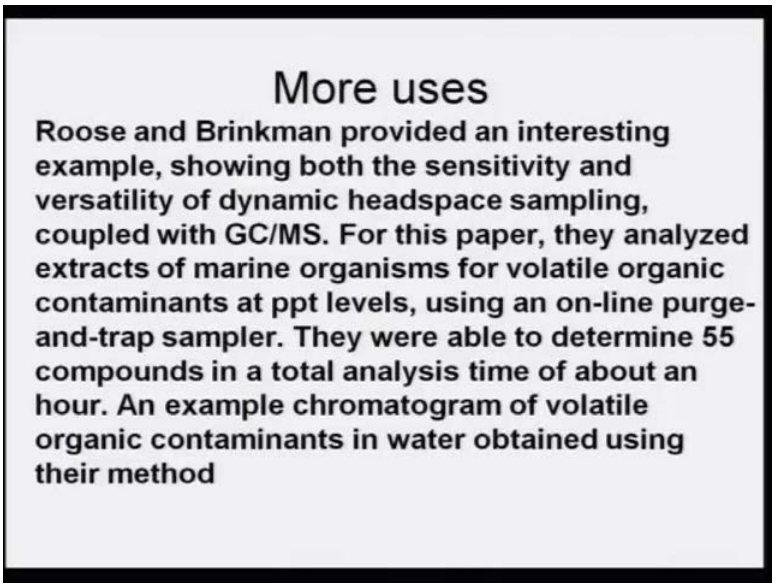


More uses – Roose and brinkmen provided an interesting example, showing both the sensitivity and versatility of dynamic headspace sampling, coupled with GC/MS. For this paper, they analyzed extracts of marine organism for volatile organic contaminants at ppt levels (parts per trillion), using an on-line purge-and-trap sampler. They were able to determine 55 compounds in a total analysis time of about an hour. An example chromatogram of volatile organic contaminants in water obtained using their method, can be seen in this research paper. So, you see that in such a complicated system, where there are so many volatile organic contaminants that to add the ppt level, they have been identified and they have been analyzed very carefully.

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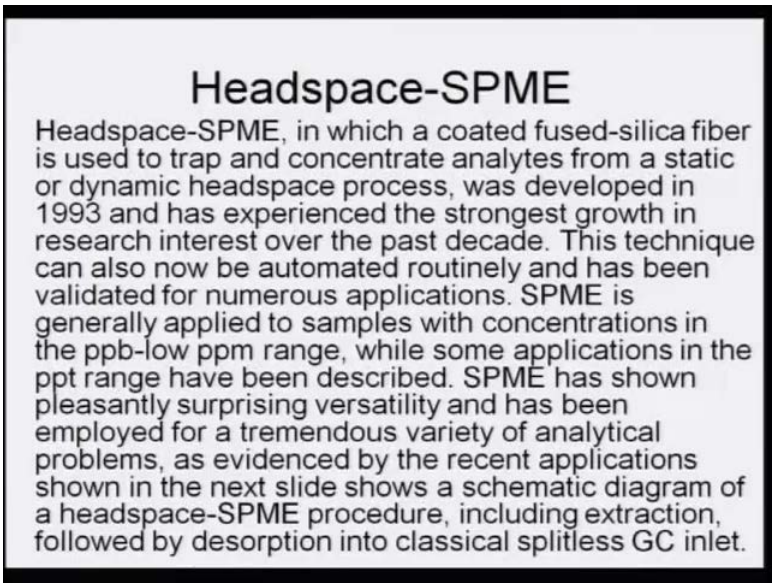


Headspace-SPME – headspace-SPME, in which a coated fused-silica fiber is used to trap and concentrate the analytes from a static or dynamic headspace process, was developed in 1993 and has experienced the strongest growth in the research interest over the past decade. This technique can also now, be automated routinely and has been validated for numerous applications. SPME is generally applied to samples with concentrations in the ppb-low ppm range, while some applications in the ppt range have been described as well. SPME has shown pleasantly surprising versatility and has been employed for a tremendous variety of analytical problems, as evidenced by the recent applications, and one can see that the headspace-SPME, when connected or desorbed on the classical splitless GC, can work very well.

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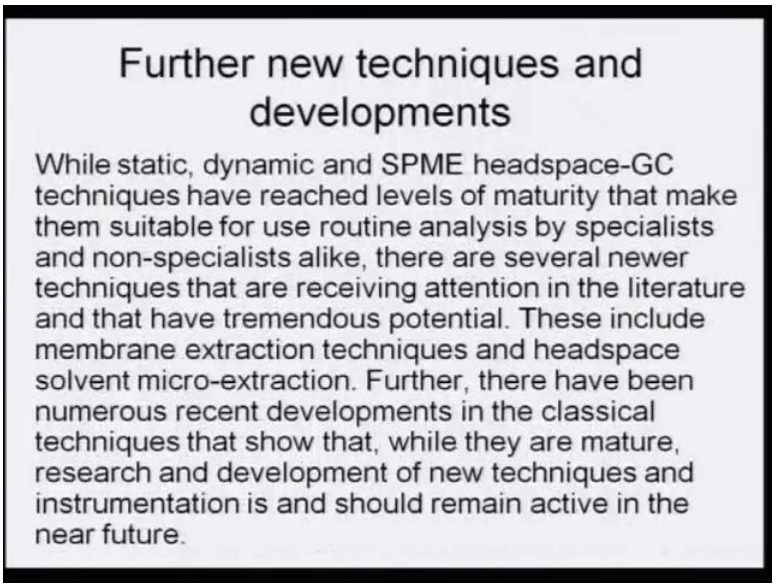


Further new techniques and developments – while static, dynamic and SPME headspace-GC techniques have reach levels of maturity that make them suitable for the use routine analysis by specialists and non-specialists alike, there are several newer techniques that are receiving attention in the literature and that have tremendous potential. So, as what I told, continuously, I have been talking about the advancement. Every time a method is developed, there is always a need to go ahead and do something more specialized; and that is what the scientists are doing world over. They want to find a better and a better and a still better method for the analysis; and that is what give rise to lot of research work. These include membrane extraction techniques and headspace solvent micro-extraction. Further, there have been numerous recent developments in the classical techniques that show that, while they are mature, research and development of new techniques and instrumentation is and should remain active in near future, because one cannot say that this is it; there has to be… Research is an ongoing process. Newer and newer methods are developed for very compounds-specific need.

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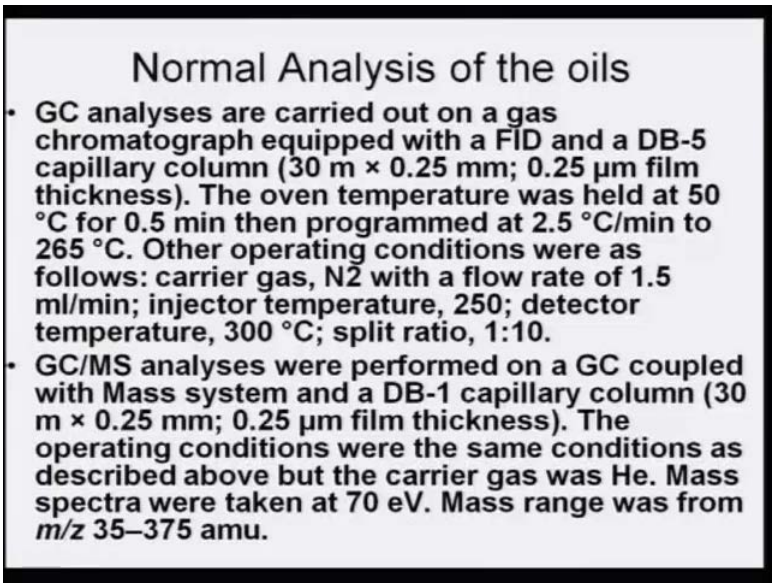


Normal analysis of oils – GC analyses are carried out on a gas chromatograph equipped with FID and a DB-5 capillary column having 30 meters length, 0.25 millimeter ID and 0.25 micrometer film thickness. The oven temperature was held at 50 degrees for 0.5 minutes then programmed; there was a ramp given at 2.5 degrees centigrade per minute to 265 degrees. Other operating conditions were the same. The carrier gas, that is, the nitrogen flow rate and injector temperature, detector temperature and the split ratio, were all designed for this kind of analysis.

The GC/MS analyses were performed on GC coupled with mass system with a DB-1 capillary column having the similar specification. The operating conditions were the same conditions as described above, but the carrier gas in GC/MS was the helium instead of nitrogen, as what it was in GC-FID. Mass spectra were taken at 70 electro volt. Mass range was from 35 to 375 amu. And, all the essential oils could be analyzed on these.

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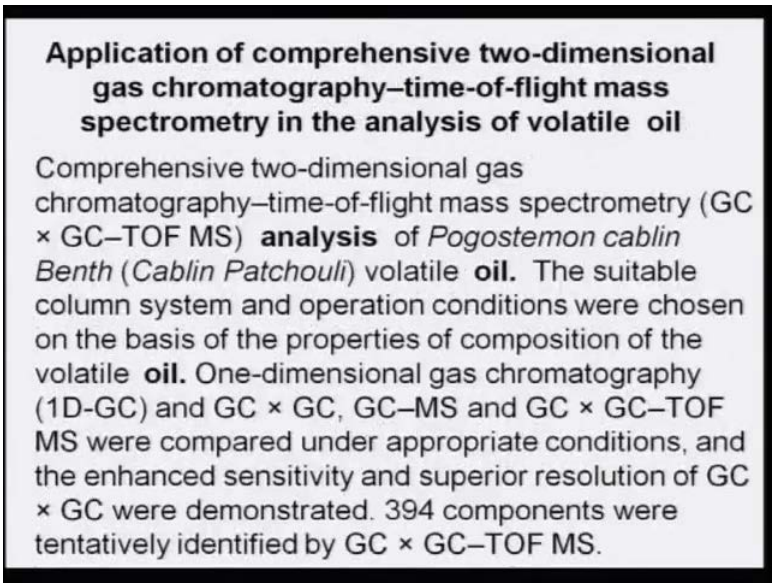


Application of the comprehensive two-dimensional gas chromatography with time-of–flight – I have already talked about this particular analysis in details in the previous lecture.

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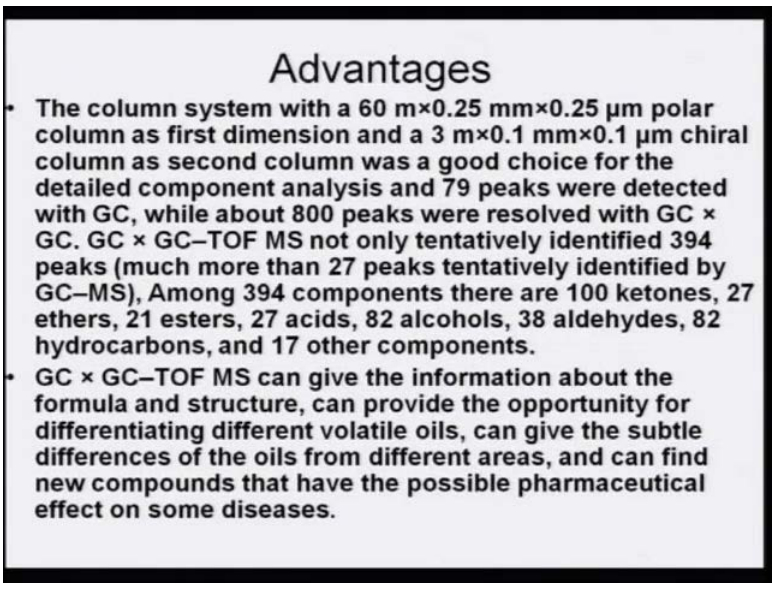


Now, the advantages that are there, one has to keep in mind that these compounds are an array of large number of compounds. Almost 394 compounds would be identified and among that 394, 100 were ketones, 27 were ethers, 21 were esters, 27 were acids, 82 were alcohols, 38 were aldehydes and 82 were hydrocarbons. So, you see how specific

this analysis is. With a minor adaptation, one can do the analysis to the minutes detail; even the VOCs can be analyzed.

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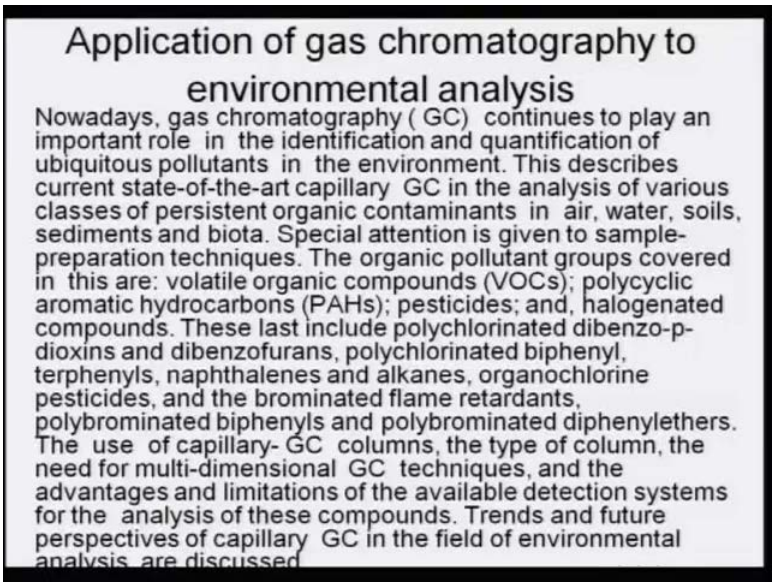


Application of gas chromatography for environmental analysis – nowadays, gas chromatography continues to play an important role in the identification and quantification of ubiquitous pollutants in the environment. And, all the volatile organics, polycyclic aromatic hydrocarbons, can be analyzed on the GC machine with great specification.

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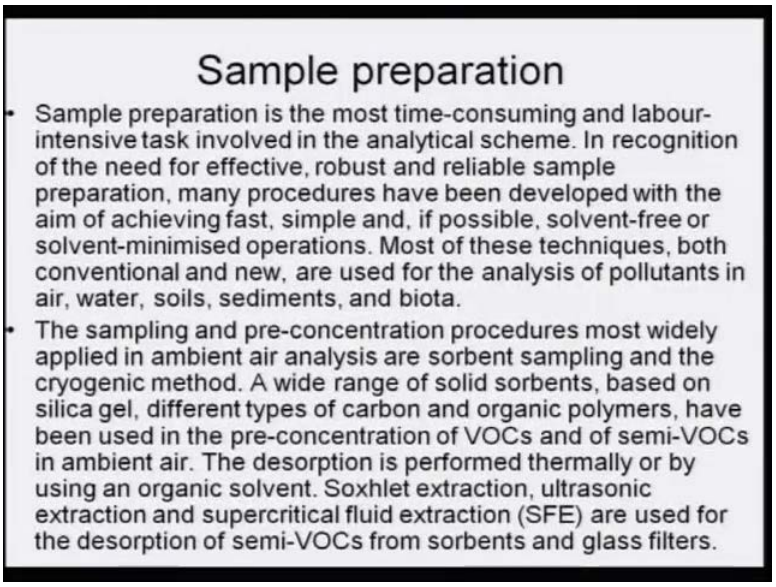

Sample preparation – of course, as I said, is always very item-specific. That means that sample preparation depends on which type of compound is to be analyzed. And so, sampling and pre-concentration procedures most widely applied in ambient air analysis are sorbent sampling and the cryogenic method. A wide range of solid sorbents, based on silica gel, different types of carbon and organic polymers, have been used in the pre-concentration of volatile organic carbons, which is VOCs and semi-volatile organic carbons in ambient air. The desorption is performed thermally or by using an organic solvent. Soxhlet extraction, ultrasonic extraction and supercritical extractions are also used for the desorption of the semi-VOCs from sorbents and the glass filters, because they have to be desorbed on the GC capillary column and then only, the analysis can take place very efficiently.

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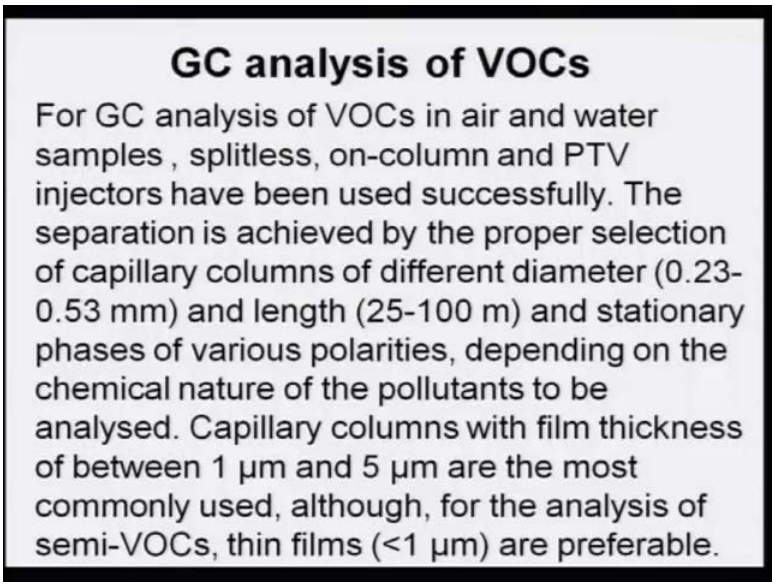


GC analysis of the VOCs – for GC analysis of the VOCs in air and water samples, splitless, on column and PTV injectors have been used successfully. The separation is achieved by the proper selection of capillary columns of different diameter and length and stationary phases of various polarities, depending on the chemical nature of the pollutants to be analyzed. Capillary columns with film thickness of between 1 micrometer to 5 micrometer are the most commonly used, although, for the analysis of semi-VOCs, thin films are preferred.

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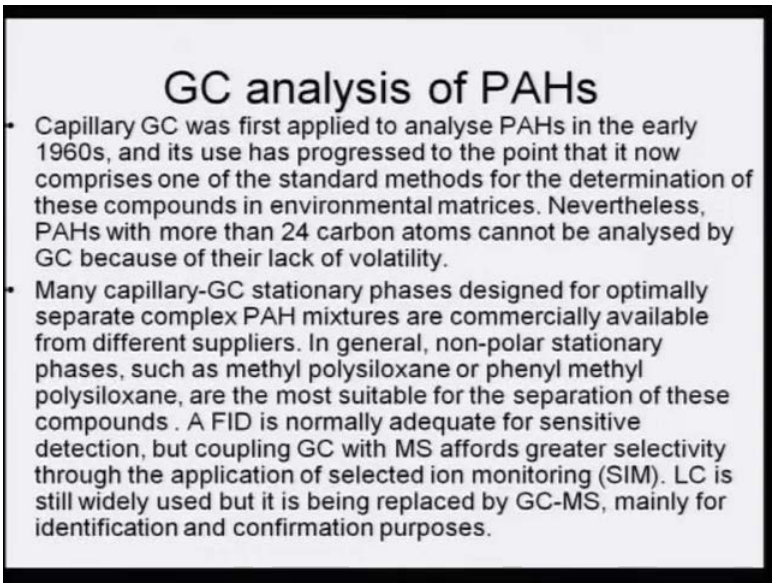


GC analysis of PAHs – I have just discussed a while ago. So, I will not repeat, but I will only say that a GC with an FID, can do the needful, but of course, a GC/MS is always superior for polycyclic aromatic hydrocarbons.

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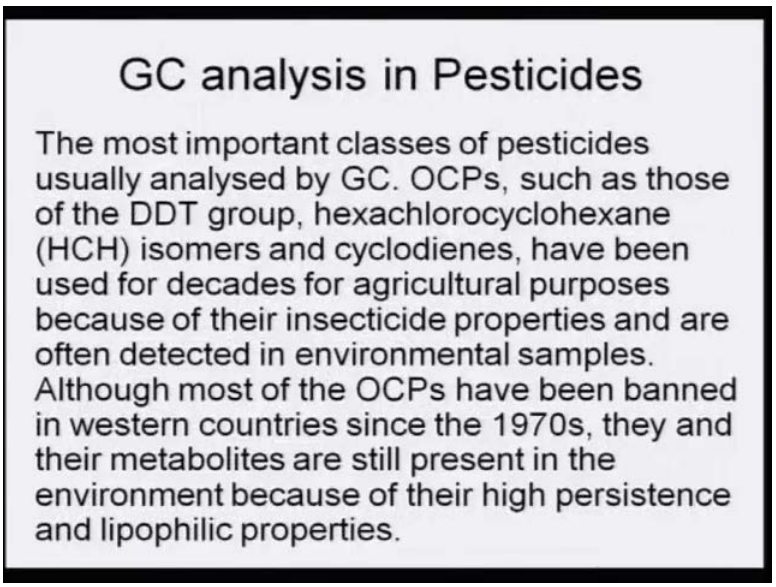


GC for pesticide is again a very important area for analysis of environmental samples – the most important classes of pesticides usually analyzed by GC. The OCPs, that is, the organochlorine pesticides, such as those of the DDT group, hexachlorocyclohexane (HCH) isomers and cyclodienes, have been used for decades for agricultural purposes,

because of their insecticide properties and are often detected in environmental samples. Although most of the OCPs have been banned in western countries since the 1970s, they are there still present in their metabolites form and are still present in the environment, because of their high persistence and lipophilic properties; and, that is precisely, why they are called as POPs (persistent organic pollutants).

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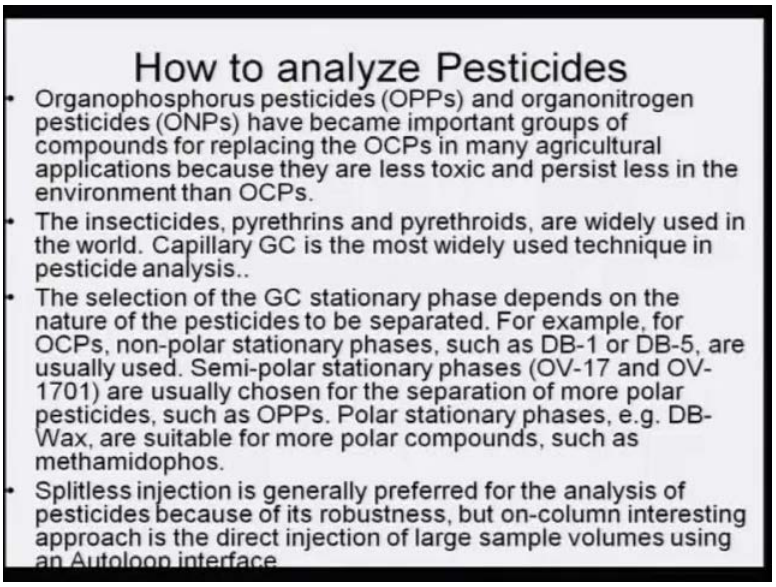


How to analyze pesticides? Organophosphorus pesticides and organonitrogen pesticides have become important groups of compounds for replacing the OCPs in many agricultural applications, because they are less toxic to humans and persist less in the environment than as compared to the organochlorine pesticides. The insecticides, pyrethrins and pyrethroids, are widely used in the world. Capillary GC is the most widely used technique in pesticide analysis.

The selection of the GC stationary phase depends on the nature of the pesticides to be separated. For example, for OCPs, that is, organochlorine pesticides, non-polar stationary phases like DB-1 or DB-5, are usually used. Semi-polar stationary phases like OV-17 or OV-1701, are usually chosen for the separation of more polar pesticides, such as OPPs. Polar stationary phases like DB-Wax, are suitable for more polar compounds, such as methamidophos. Splitless injection is generally preferred for the analysis of pesticides because of its robustness, but on-line interesting approach is the direct injection of the large sample volumes using an autoloop interface. It means that a splitless injection is

preferred for pesticide analysis and on the column, it is important to have an autoloop interface, so that the compound can be trapped in that auto loop and only release into the column when required to do so.

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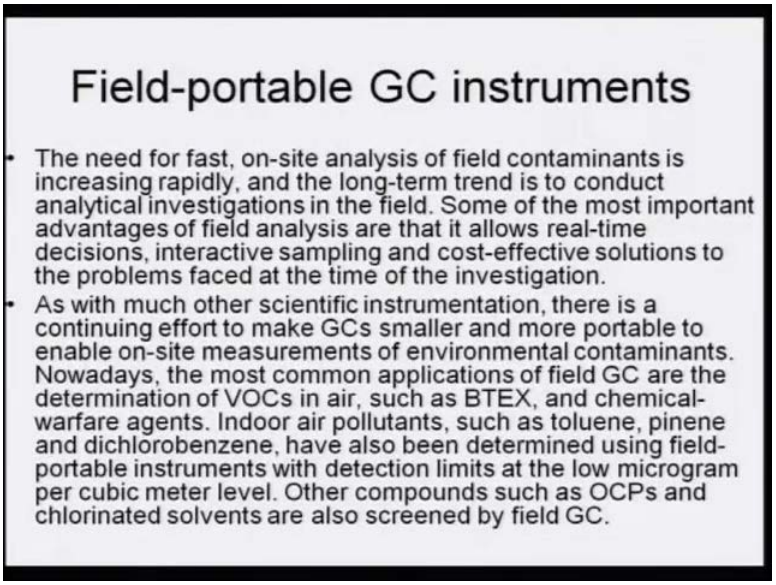


Field-portable GC instruments – now, because the pesticides have become such a mess, one needs to have field-portable GC machines as well. The need for fast, on-site analysis of field contaminants is increasingly and rapidly being used, and for the long term trend, is to conduct analytical investigations in the field itself. Some of the most important advantages of field analysis are that it allows real-time decisions, interactive sampling and cost-effective solutions to the problems faced at the time of investigation.

As with much other scientific instrumentation, there is a continuing effort make GCs smaller and more portable to enable on-site measurements of environmental contaminants. Nowadays, most common applications of field GC are the determination of VOCs in air, such as BTEX, and chemical-warfare agents, indoor air pollutants, such as toluene, pinene and dichlorobenzene, have also been determined using field-portable instruments with detection limits as low as microgram per cubic meter level. Other compounds, such as organochlorine pesticides and chlorinated solvents are also screened by field GC. So, these are smaller versions, smaller machines, but they can do the job of analyses of environmental contaminates very effectively.

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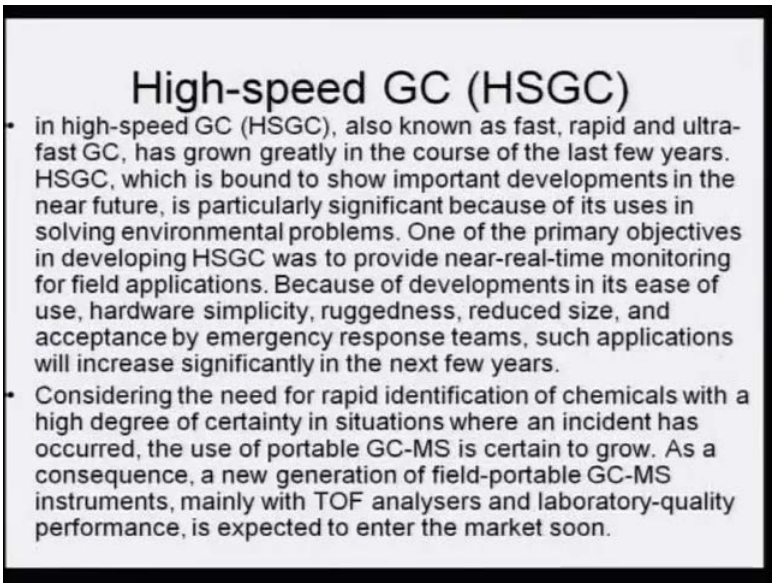


High-speed GC – in high-speed GC, as the name suggests, it is fast, rapid, ultra-fast GC, and has grown greatly in the course of the last few years. High-speed GC, which is bound to show important developments in the near future, is particularly significant because of its uses in solving environmental problems. One of the primary objectives in developing the high-speed GC was to provide near-real-time monitoring for field application.