Advance Analytical Course

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Lecture No. # 14

I was talking about this microwave vessels and the focus microwave technology.

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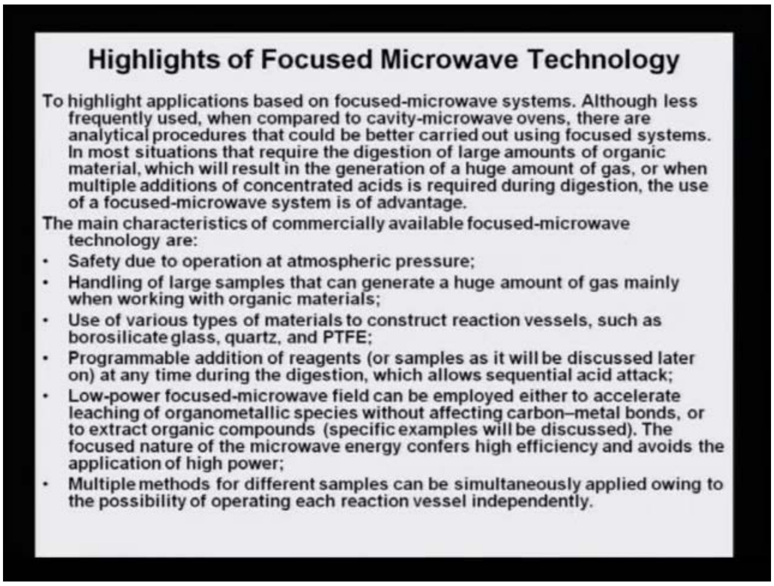

Highlights of Focused Microwave Technology

To highlight applications based on focused-microwave systems. Although less frequently used, when compared to cavity-microwave ovens, there are analytical procedures that could be better carried out using focused systems. In most situations that require the digestion of large amounts of organic material, which will result in the generation of a huge amount of gas, or when multiple additions of concentrated acids is required during digestion, the use of a focused-microwave system is of advantage.

The main characteristics of commercially available focused-microwave technology are:

- Safety due to operation at atmospheric pressure;
- Handling of large samples that can generate a huge amount of gas mainly when working with organic materials;
- Use of various types of materials to construct reaction vessels, such as borosilicate glass, quartz, and PTFE;
- Programmable addition of reagents (or samples as it will be discussed later on) at any time during the digestion, which allows sequential acid attack;
- Low-power focused-microwave field can be employed either to accelerate leaching of organometallic species without affecting carbon–metal bonds, or to extract organic compounds (specific examples will be discussed). The focused nature of the microwave energy confers high efficiency and avoids the application of high power;
- Multiple methods for different samples can be simultaneously applied owing to the possibility of operating each reaction vessel independently.

Now, in this, what happens that, the tough the material of the digestive vessel is, the better it is, so that there is no leaching from the vessel and that is why a borosilicate glass or quartz vessel, or poly-teflon vessel is most ideally suited for this kind of digestive vessels.

Similarly, you know, there can be a programmable method of adding different acids to this vessel, and these different acids should not react with the vessel material. So, that is why it is a very very unique kind of machine which is in the latest trend to be used for metal/metalloid extraction, but there are certain difficulties because no method is full proof; if there are advantages, there are certain disadvantages and difficulties with any analytical methodology that is developed.

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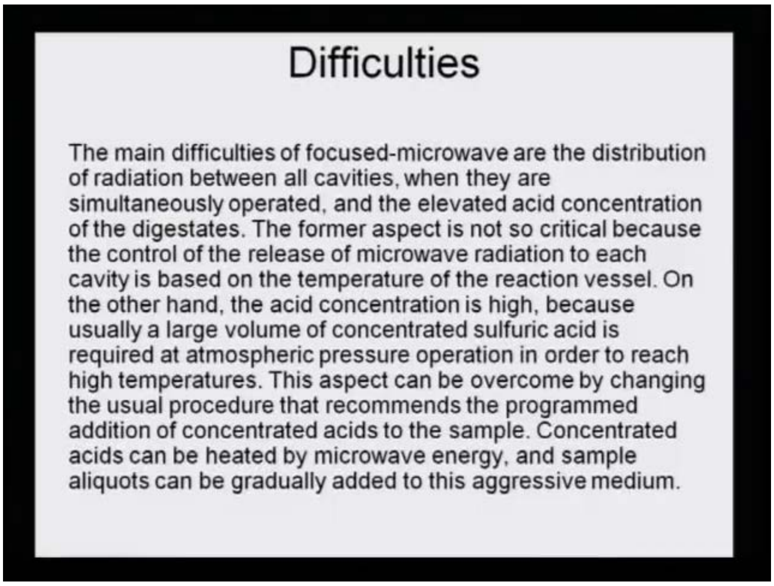

Difficulties

The main difficulties of focused-microwave are the distribution of radiation between all cavities, when they are simultaneously operated, and the elevated acid concentration of the digestates. The former aspect is not so critical because the control of the release of microwave radiation to each cavity is based on the temperature of the reaction vessel. On the other hand, the acid concentration is high, because usually a large volume of concentrated sulfuric acid is required at atmospheric pressure operation in order to reach high temperatures. This aspect can be overcome by changing the usual procedure that recommends the programmed addition of concentrated acids to the sample. Concentrated acids can be heated by microwave energy, and sample aliquots can be gradually added to this aggressive medium.

The main difficulties of focused-microwave are the distribution of radiation between all cavities, when they are simultaneously operated, and the elevated acid concentrations of the digestates. The former aspect is not so critical because the control of the release of microwave radiation to each cavity is based on temperature of the reaction vessel. On the other hand, the acid concentration is high because usually a large volume of concentrated sulphuric acid is required at atmospheric pressure operation in order to reach high temperatures. This aspect can be overcome by changing the usual procedure that recommends the programmed addition of concentrated acids to the sample. Concentrated acids can be heated by microwave energy, and the sample aliquots can be gradually added to this aggressive medium.

So, now, it is not necessary that all the acids should be added in one go and then microwave is passed through it. The microwave energy can heat it up very largely and it would create lot of fumes of the acid; instead, this can be programmed in such a manner that small volumes of acids will be added and the efficient digestion will still take place without taking a bulk of the acid.

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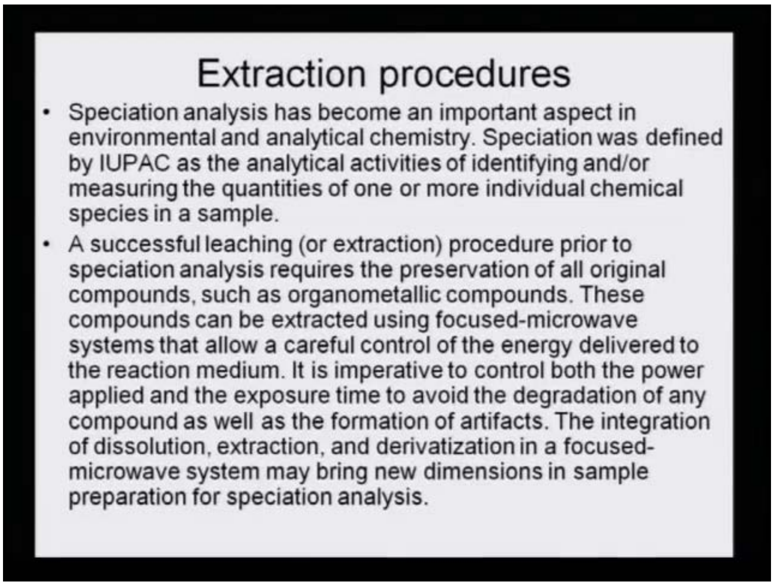

Extraction procedures

- Speciation analysis has become an important aspect in environmental and analytical chemistry. Speciation was defined by IUPAC as the analytical activities of identifying and/or measuring the quantities of one or more individual chemical species in a sample.
- A successful leaching (or extraction) procedure prior to speciation analysis requires the preservation of all original compounds, such as organometallic compounds. These compounds can be extracted using focused-microwave systems that allow a careful control of the energy delivered to the reaction medium. It is imperative to control both the power applied and the exposure time to avoid the degradation of any compound as well as the formation of artifacts. The integration of dissolution, extraction, and derivatization in a focused-microwave system may bring new dimensions in sample preparation for speciation analysis.

Extraction procedures - speciation analysis has become an important aspect in environmental and analytical chemistry. Speciation was defined by IUPAC as the analytical activities of identifying and/or measuring the quantities of one or more individual chemical species in a sample. So, the word speciation which I have been using all along, you should also understand the meaning of this; it means that, we are trying to identify one or more individual chemical component in the sample.

A successful leaching or extraction procedure prior to speciation analysis requires the preservation of all original compounds, such as organometallic compounds. These compounds can be extracted using focused-microwave system that allows a careful control of the energy delivered to the reaction medium. It is, therefore, imperative to control both the power applied and the exposure time to avoid the degradation of any compound as well as the formation of artifacts. The integration of dissolution, extraction, and derivatization in a focused-microwave system may bring new dimension in sample preparation for speciation analysis.

So, now, you see that it is so well designed that it mildly reacts with organometallic species without disturbing the organic part of the molecule, which can be later on analyzed. It only specially takes out the metal and does the speciation of the metal.

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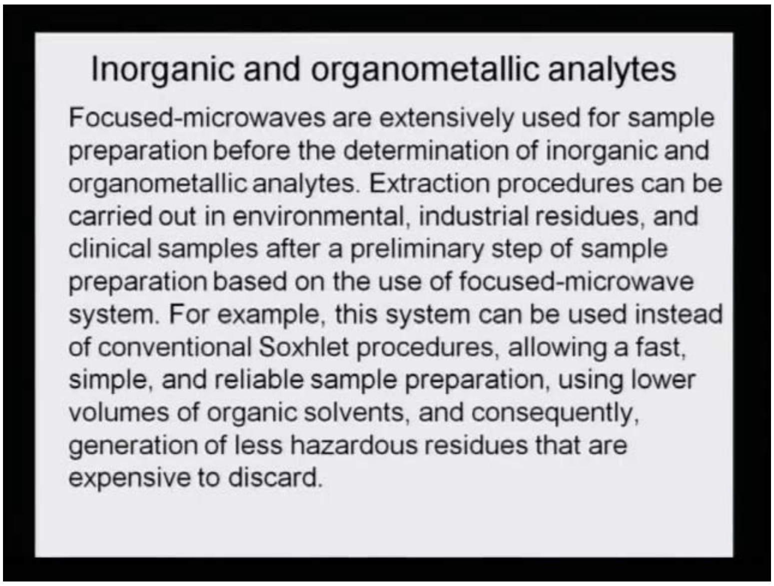

Inorganic and organometallic analytes

Focused-microwaves are extensively used for sample preparation before the determination of inorganic and organometallic analytes. Extraction procedures can be carried out in environmental, industrial residues, and clinical samples after a preliminary step of sample preparation based on the use of focused-microwave system. For example, this system can be used instead of conventional Soxhlet procedures, allowing a fast, simple, and reliable sample preparation, using lower volumes of organic solvents, and consequently, generation of less hazardous residues that are expensive to discard.

Inorganic and organometallic analytes – focused-microwaves are extensively used for sample preparation before the determination of inorganic and organometallic analytes. Extraction procedures can be carried out in environmental, industrial residues, and clinical samples after a preliminary step of sample preparation based on the use of focused-microwave system.

So, you see that they can be used for environmental sample, for industrial residues, and for clinical samples. So, there is a large variety where the focused-microwave can be used and it can be used for inorganic samples as well as organometallic samples.

For example, this system can be used instead of conventional Soxhlet procedure, allowing a fast, simple, and reliable sample preparation, using low volumes of organic solvents, and consequently, generation of less hazardous residues that are expensive to discard. So, it has an advantage that, it not only uses very small quantities of organic solvents for dissolution, and therefore, the disposal also is not very difficult

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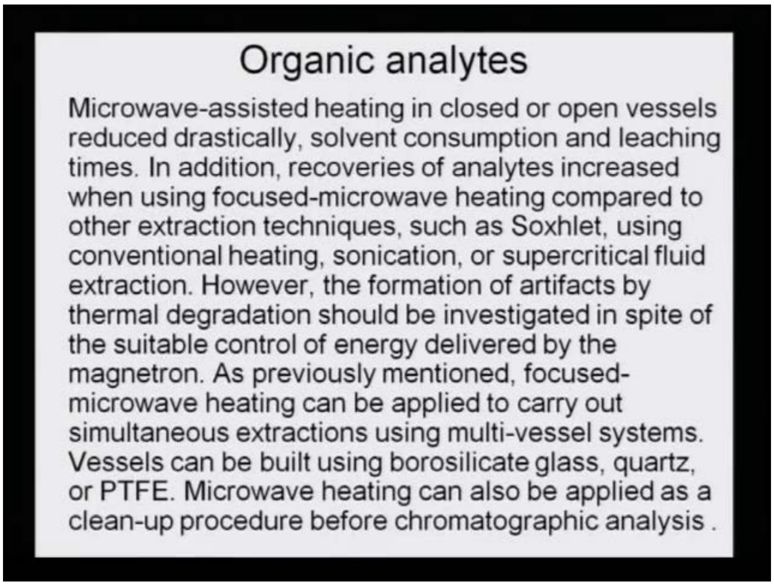

Organic analytes

Microwave-assisted heating in closed or open vessels reduced drastically, solvent consumption and leaching times. In addition, recoveries of analytes increased when using focused-microwave heating compared to other extraction techniques, such as Soxhlet, using conventional heating, sonication, or supercritical fluid extraction. However, the formation of artifacts by thermal degradation should be investigated in spite of the suitable control of energy delivered by the magnetron. As previously mentioned, focused-microwave heating can be applied to carry out simultaneous extractions using multi-vessel systems. Vessels can be built using borosilicate glass, quartz, or PTFE. Microwave heating can also be applied as a clean-up procedure before chromatographic analysis .

Organic analytes also can be treated. Microwave-assisted heating in closed or open vessel reduced drastically, solvent consumption and leaching times. In addition, recoveries of analytes increased when using focused-microwave heating compared to other extraction techniques, such as Soxhlet, using conventional heating, sonification, or supercritical fluid extraction.

However, the formation of artifacts by thermal degradation should be investigated in spite of the suitable control of energy delivered by the magnetron. It is to be understood that, whether it is organic analyte, inorganic analyte, organometallic analyte, every time there is a new sort of parameters that need to be controlled, there is no hard and fast one method, that will dissolve all the species or different types of samples. And that is why, I have taken up one by one, so that you can understand, very carefully, that if there are inorganic and organometallics, what is the condition that should be applied and if the samples are organic in nature, what is the condition that needs to be applied, and what are the parameters that needs to be taken into consideration.

As previously mentioned, focused-microwave heating can be applied to carry out simultaneous extraction using multi vessel system. Vessels can be built up using borosilicate glass, quartz, or PTFE that is polytetrafluoroethylene, which is kind of a white plastic which is very very sturdy. Microwave heating can also be applied as a cleanup procedure before chromatographic analysis. So, even there, microwave heating helps to discard a lot of junk material, before taking to chromatographic techniques. So, it can also be used as a cleanup methodology.

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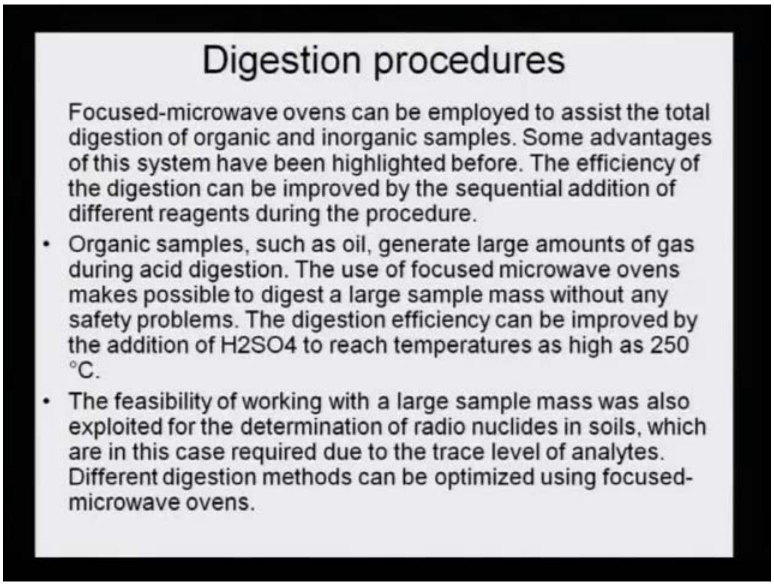

Digestion procedures

Focused-microwave ovens can be employed to assist the total digestion of organic and inorganic samples. Some advantages of this system have been highlighted before. The efficiency of the digestion can be improved by the sequential addition of different reagents during the procedure.

- Organic samples, such as oil, generate large amounts of gas during acid digestion. The use of focused microwave ovens makes possible to digest a large sample mass without any safety problems. The digestion efficiency can be improved by the addition of H_2SO_4 to reach temperatures as high as 250 °C.
- The feasibility of working with a large sample mass was also exploited for the determination of radio nuclides in soils, which are in this case required due to the trace level of analytes. Different digestion methods can be optimized using focused-microwave ovens.

Digestion procedures - focused-microwave ovens can be employed to assist the total digestion of organic and inorganic samples. Some advantages of this system have been highlighted before. The efficiency of the digestion can be improved by the sequential addition of different reagents during the procedure.

Now, I will give you an example. What happens is that, when there are certain metal or metalloids which are very well embedded in the biotic material, they do not come out with just using one acid, and combination of acids and sequential addition of acid needs to be done in the reaction vessel, and focused-microwave is being subjected to these vessels. What happens is that, in every step, more and more gets digested, and at the end of the such design recipe, the maximum amount of speciation takes place.

Organic sample, such as oil, generate large amount of gas during acid digestion. The use of focused-microwave ovens makes possible to digest large sample mass without any safety problems. The digestion efficiency can be improved by the addition of sulphuric acid to reach temperatures as high as 250 degrees centigrade. And since it is a closed system and it has a vent which is thrown off out of the laboratory, it is much same because those fumes are not entering the laboratory.

The feasibility of working with a large sample mass was also exploited for the determination of radio nuclea, nuclei, nuclides in soils, which are in this are required to be done at a trace level of the analytes. Different digestion methods can be optimized using focused-microwave ovens.

Now, radioactive compounds, when they have to be extracted from soil, even then this focused-microwave works very efficiently and very well.

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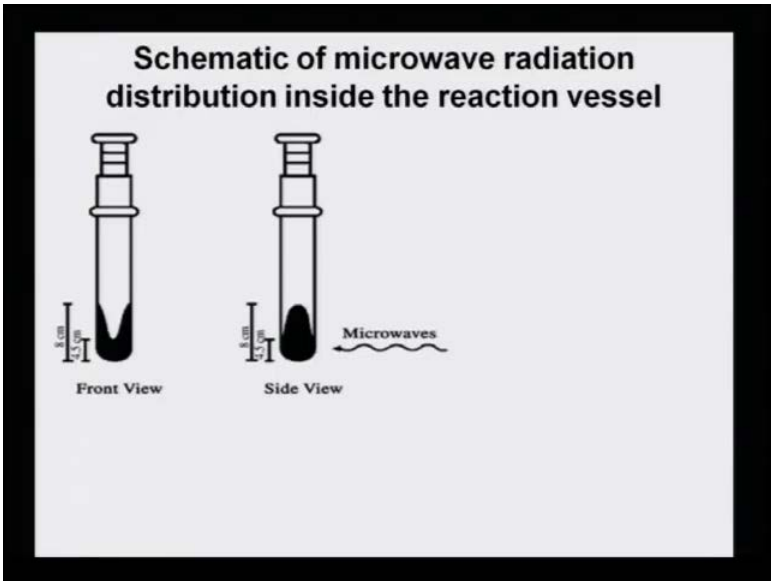

Schematic of microwave radiation distribution inside the reaction vessel

This is how, the schematic microwave radiation distribution inside a vessel looks like. You see, this is the frontal view, this is the side view, and from the side, the microwave energy is going on bombarding it or reacting with it.

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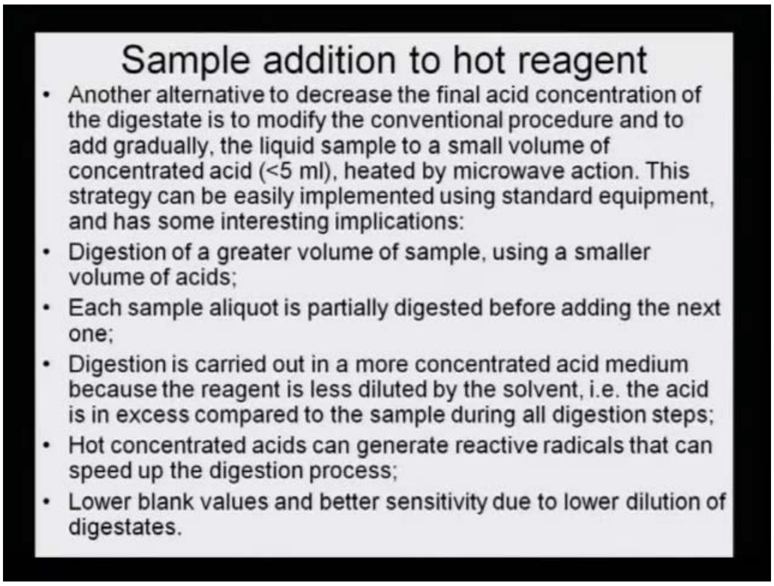

Sample addition to hot reagent

- Another alternative to decrease the final acid concentration of the digestate is to modify the conventional procedure and to add gradually, the liquid sample to a small volume of concentrated acid (<5 ml), heated by microwave action. This strategy can be easily implemented using standard equipment, and has some interesting implications:
- Digestion of a greater volume of sample, using a smaller volume of acids;
- Each sample aliquot is partially digested before adding the next one;
- Digestion is carried out in a more concentrated acid medium because the reagent is less diluted by the solvent, i.e. the acid is in excess compared to the sample during all digestion steps;
- Hot concentrated acids can generate reactive radicals that can speed up the digestion process;
- Lower blank values and better sensitivity due to lower dilution of digestates.

Sample addition to hot reagent - another alternative to decrease the final acid concentration to digestate, is to modify the conventional procedure and to add gradually, the liquid sample to a small volume of concentrated acid. So far, we were taking the sample and adding the acid into it. Now, this is another method, that already heated acid is taken in the teflon vessel or the borosilicate vessel of the microwave digester, and to that, the sample is gradually added.

Small volumes of concentrated acids are taken. They are heated by microwave action. This strategy can be easily implemented using standard equipment and has some interesting implication.

Now, what happens when we do this? Reverse addition digestion of greater volume of sample using a smaller volume of acid is possible because already the acid is heated up, and so, it has a better digestive power as compared to a cold acid which is gradually added to the hot sample.

Each sample aliquot is partially digested before adding the next one. Digestion is carried out in a more concentrated acid media because the reagent is less diluted by the solvent. The acid is in excess compared to the sample during all digestion steps. Hot concentrated acids are can generate reactive radicle that can speed up the digestion process. Lower blank values and better sensitivity due to lower dilution of digestates is possible.

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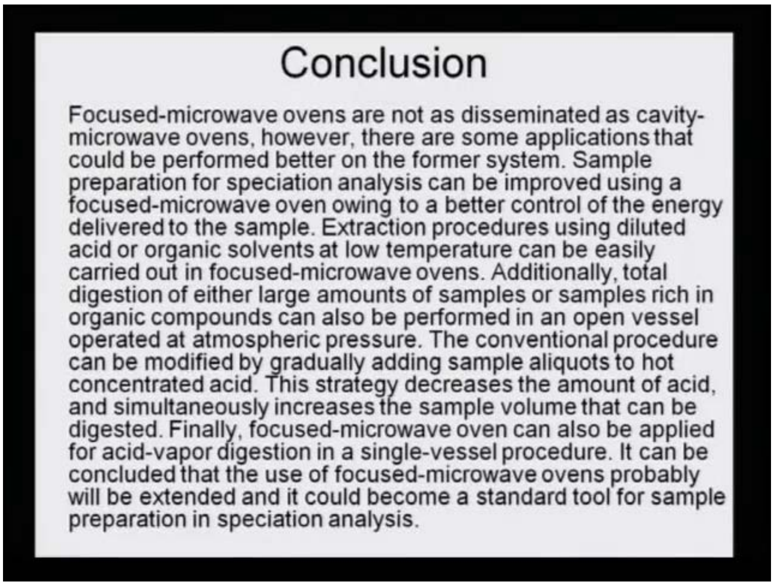

Conclusion

Focused-microwave ovens are not as disseminated as cavity-microwave ovens, however, there are some applications that could be performed better on the former system. Sample preparation for speciation analysis can be improved using a focused-microwave oven owing to a better control of the energy delivered to the sample. Extraction procedures using diluted acid or organic solvents at low temperature can be easily carried out in focused-microwave ovens. Additionally, total digestion of either large amounts of samples or samples rich in organic compounds can also be performed in an open vessel operated at atmospheric pressure. The conventional procedure can be modified by gradually adding sample aliquots to hot concentrated acid. This strategy decreases the amount of acid, and simultaneously increases the sample volume that can be digested. Finally, focused-microwave oven can also be applied for acid-vapor digestion in a single-vessel procedure. It can be concluded that the use of focused-microwave ovens probably will be extended and it could become a standard tool for sample preparation in speciation analysis.

So you see there is also an advantage in this reverse addition. So, we can conclude that focused-microwave ovens are not as disseminated as cavity-microwave ovens. However, there are some applications that could be performed better on the former system, that is focused-microwave is sometimes better than the cavity-microwave ovens.

Sample preparation for speciation analysis can be improved using a focused-microwave oven owing to a better control of the energy delivered to the sample. Extraction procedures using diluted acid or organic solvents at low temperature can easily be carried out in focused-microwave ovens. Additionally, total digestion of either large amounts of samples or samples rich in organic compounds can also be performed in open vessel operated at atmospheric pressure. The conventional procedure can be modified by gradually adding sample aliquots to hot concentrated acids also. There are lot of permutation combinations that can be carried out using the focused-microwave ovens.

This strategy decreases the amount of acid and simultaneously increases the sample volume that can be digested. So, you see there is a distinct advantage this helps to use lesser amount of acid; at the same time, the sample volume can be the large sample volume can be digested.

Finally, focused-microwave oven can also be applied for acid-vapor digestion in a single-vessel procedure also. It can be concluded that the use of focused-microwave ovens probably will be extended and it can become a standard tool for sample preparation in speciation analysis. So, if we have to target speciation analysis, focused-microwave ovens are the answer for the analyst and the choice of method for analyst.

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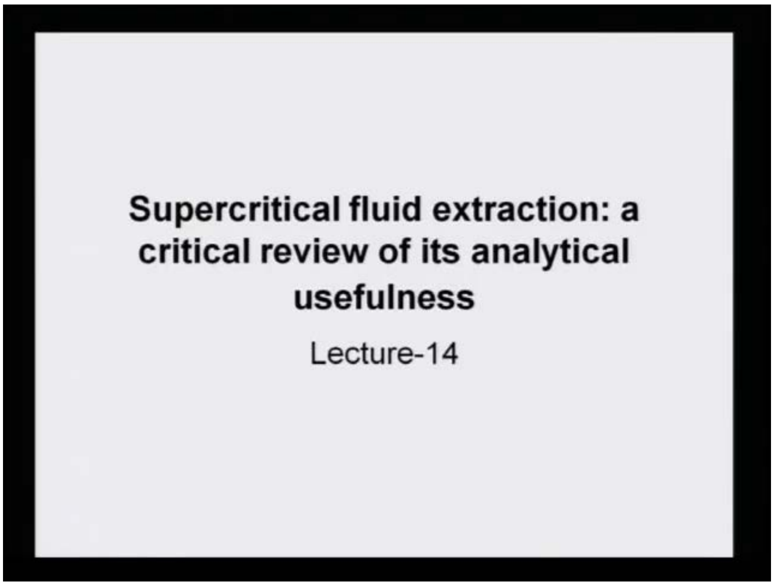

Supercritical fluid extraction: a critical review of its analytical usefulness

Lecture-14

We have been talking about supercritical fluid extraction: a critical review of its analytical usefulness. I am going to talk about this because in the last few lectures, I have been talking about one very major effect that the for extraction. Supercritical fluids is one of the best methods, but let us try to look, why it is better than others; how is it better because just by merely understanding or knowing the name, it is not enough. This is one of the most recent trends of extraction. And as the name suggests, supercritical fluid extraction, it is a very useful analytical tool for analyzing not only inorganic sample or organometallic samples, but also organic and inorganic samples.

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Supercritical fluid extraction

(SFE)

The evolution of **supercritical fluid extraction** (SFE) since 1990 in order to pinpoint the reasons for its rare implementation by routine **analytical** laboratories despite its high **analytical** potential. We identify various reasons, and we propose ways to overcome the shortcomings behind them. We also discuss the great **analytical** potential of SFE and justify its use for routine work.

Supercritical fluid extraction (SFE) - it will be referred as that all along. The evolution of supercritical fluid extraction is very recent, since 1990 in order to pinpoint the reason for its rare implementation by routine analytical laboratories despite its high analytical potential. We identify various reasons, and we propose ways to overcome the shortcomings behind them. We also discuss the great analytical potential of the SFE and justify its use for routine work.

I will give you an example. We were facing a lot of problem in the extraction of a natural dye from Eucalyptus bark, and whenever we were boiling the eucalyptus bark, we were only getting dark brown extract. Then, we thought, why not to put it and do solvent extraction, but again, in the solvent extraction, that is the Soxhlet extraction, we were getting only dark brown extract. So, the extract was also giving dark brown and the solvent extract was also giving dark brown, but when the solvent extract was column chromatograph, there was a band which was coming as yellow color. So, we thought that, along with the tannings which are brown in color, there must be a yellow compound.

Now, how to specially analyze or extract this yellow? We were just pondering over this extraction challenge, when we came across this supercritical fluid extraction. We did not have this extractor in our laboratory. So, I sent the sample to IIT Bombay, and there, we were able to extract and get beautiful yellow dyes especially only with that.

So, that means that, it is one of the methods of uniquely separating the one specific compound, when there are arrays of compound. And rightly so, the woman who works at IIT Bombay, the scientist, also has written up a book, and she has shown that, from one particular plant material which may be having essential oils as well as dyes as well as other terpenoids, it can specifically and extract one by one, at different parameters from the same plant material. So, by stepwise extraction at different parameters, one can get different compounds, and thus, from one plant material, very useful value addition products can be obtained.

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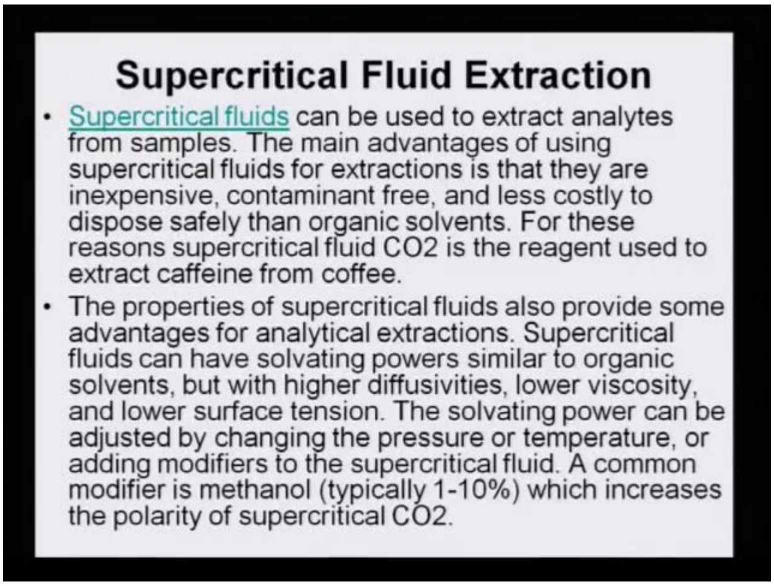

Supercritical Fluid Extraction

- Supercritical fluids can be used to extract analytes from samples. The main advantages of using supercritical fluids for extractions is that they are inexpensive, contaminant free, and less costly to dispose safely than organic solvents. For these reasons supercritical fluid CO2 is the reagent used to extract caffeine from coffee.
- The properties of supercritical fluids also provide some advantages for analytical extractions. Supercritical fluids can have solvating powers similar to organic solvents, but with higher diffusivities, lower viscosity, and lower surface tension. The solvating power can be adjusted by changing the pressure or temperature, or adding modifiers to the supercritical fluid. A common modifier is methanol (typically 1-10%) which increases the polarity of supercritical CO2.

Supercritical fluid extraction - supercritical fluids can be used to extract analytes from samples. The main advantages of using supercritical fluids for extraction are that, they are inexpensive, contaminant free, and less costly to dispose safely than organic solvents. For these reasons, supercritical fluid Carbon dioxide is the reagent used to extract caffeine from coffee.

Now, you will think that Carbon dioxide is a gas; how is it that, I am saying that, it is a supercritical fluid? Yes. When Carbon dioxide is compressed, the gaseous body is then compressed into liquid, and further if it is compressed, it becomes solid.

The properties of supercritical fluid also provide some advantages for analytical extraction. Supercritical fluids can have solvating powers similar to organic solvents, but with higher diffusivity, lower viscosity, and lower surface tension. The solvating power can be adjusted by changing the pressure or temperature, or adding modifiers to the supercritical fluid. A common modifier is methanol; typically, 1 to 10 percent Methanol can be added to this liquefied Carbon dioxide which increases the polarity of the supercritical Carbon dioxide.

So, whenever, as what I mentioned, the different types of compounds are actually differentiated among on the terms of their polarity. So, at particular condition, only supercritical Carbon dioxide can extract; at another condition, the 1 percent of Methanol addition to the supercritical Carbon dioxide can extract; another situation can be that 5 percent of Methanol when added to supercritical Carbon dioxide can extract the third type of compound.

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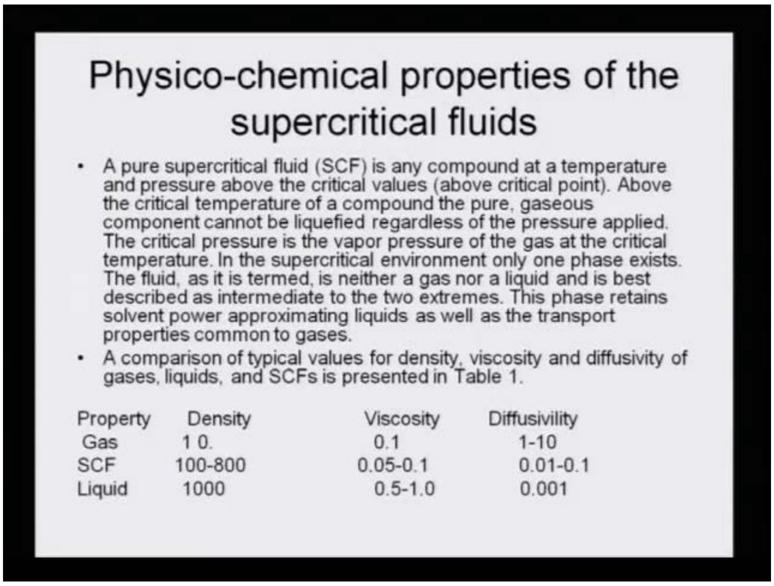

Physico-chemical properties of the supercritical fluids

- A pure supercritical fluid (SCF) is any compound at a temperature and pressure above the critical values (above critical point). Above the critical temperature of a compound the pure, gaseous component cannot be liquefied regardless of the pressure applied. The critical pressure is the vapor pressure of the gas at the critical temperature. In the supercritical environment only one phase exists. The fluid, as it is termed, is neither a gas nor a liquid and is best described as intermediate to the two extremes. This phase retains solvent power approximating liquids as well as the transport properties common to gases.
- A comparison of typical values for density, viscosity and diffusivity of gases, liquids, and SCFs is presented in Table 1.

Property	Density	Viscosity	Diffusivility
Gas	1 0.	0.1	1-10
SCF	100-800	0.05-0.1	0.01-0.1
Liquid	1000	0.5-1.0	0.001

Physicochemical properties of the supercritical fluids - now what makes them different from normal fluids? A pure supercritical fluid is any compound at a temperature and pressure above its critical value or critical point. Above the critical temperature of a compound, the pure, gaseous component cannot be liquefied regardless of the pressure applied. The critical pressure is the vapor pressure of the gas at the critical temperature. Even the supercritical environment, only one phase exists. The fluid phase, as it is termed, it is neither a gas nor a liquid, but it is a fluid, and is best described as an intermediate of the two extremes; that means, it is not a gas, it is not a liquid, but it is an intermediate or an in between stage of the two, and this happens only at a particular temperature and pressure, for a particular compound.

This phase retains solvent power approximately, as like liquids as well as transports properties like the common gases. A comparison of typical values for density, viscosity, and diffusivity of gases liquids and supercritical is presented.

So, you see that the density also increases, the viscosity also increases, and diffusivity also decreases. diffused It is more diffused; so, it decreases. And it is thus between the gaseous and the liquid, as what is shown in this slide.

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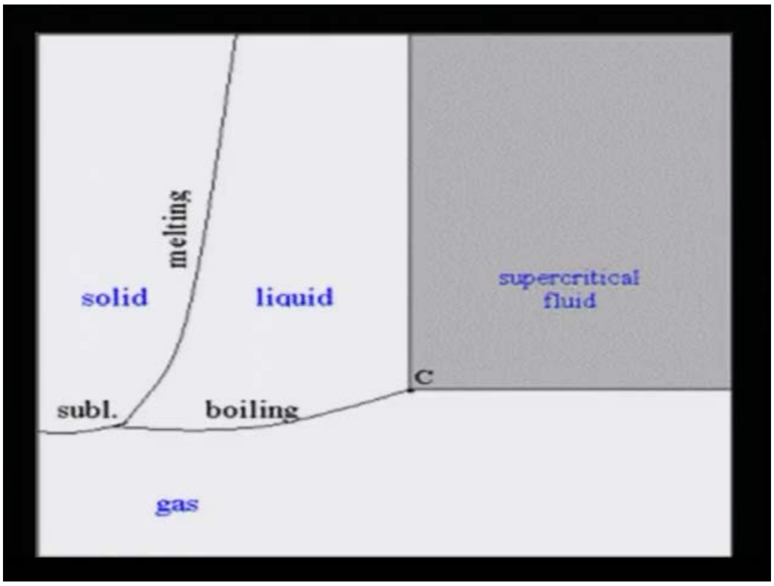

Now, what happens? That this is a very typical supercritical graph where liquid and gaseous are shown. So, this is the stage at which the supercritical fluid exists.

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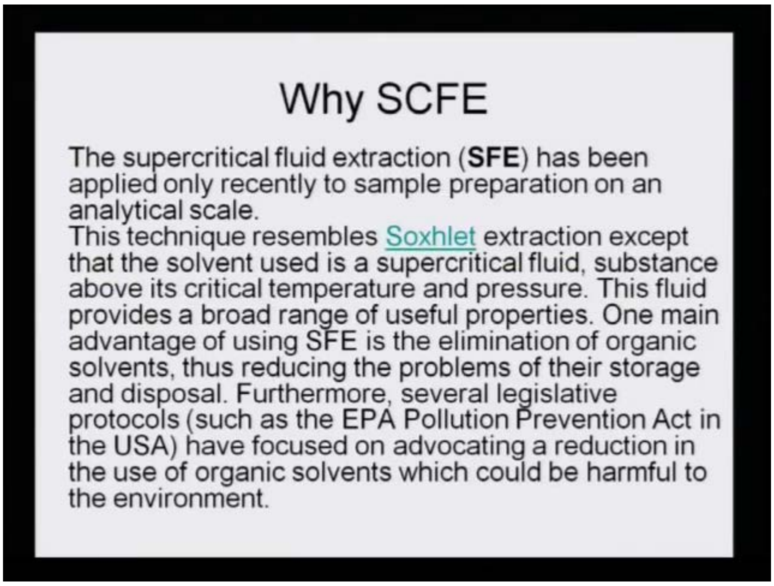

Why SCFE

The supercritical fluid extraction (**SFE**) has been applied only recently to sample preparation on an analytical scale.
This technique resembles Soxhlet extraction except that the solvent used is a supercritical fluid, substance above its critical temperature and pressure. This fluid provides a broad range of useful properties. One main advantage of using SFE is the elimination of organic solvents, thus reducing the problems of their storage and disposal. Furthermore, several legislative protocols (such as the EPA Pollution Prevention Act in the USA) have focused on advocating a reduction in the use of organic solvents which could be harmful to the environment.

Why supercritical fluid extraction? What is so special about it? The supercritical fluid extraction has been applied only recently to sample preparation on an analytical scale. So, as I told you, it is very recent only in 1990s, it was or 1980s, it was discovered and it took some time for people to adapt and to understand its usability.

This technique resembles Soxhlet extraction except that the solvent used is a supercritical fluid, substance above its critical temperature and pressure. This fluid provides a broad range of useful properties. One main advantage of using supercritical fluid extraction is the elimination of organic solvents totally, thus reducing the problem of their storage and disposal. Furthermore, several legislative protocols, such as EPA Pollution Prevention Act in USA, have focused on advocating a reduction in use of organic solvents which could be harmful for the environment.

So, you see why it has an edge over Soxhlet. Soxhlet extraction totally uses only solvent and large volumes of solvent. So, if there is a technique which is using recycling of its own gas by rarefication and contraction of the same gas, the solvating power is increased and decreased, and so, when it is the fluid when it reacts with the plant part, extracts certain compounds and it is transported, and when the gas is rarifying, then it is rejected out or desorbed. So, this is a beautiful example where solvent has been drastically used only as a modifier, only 1 to 10 percent Methanol or Ethyl acetate are used, which are known as entrainers or modifiers with supercritical fluid of Carbon dioxide.

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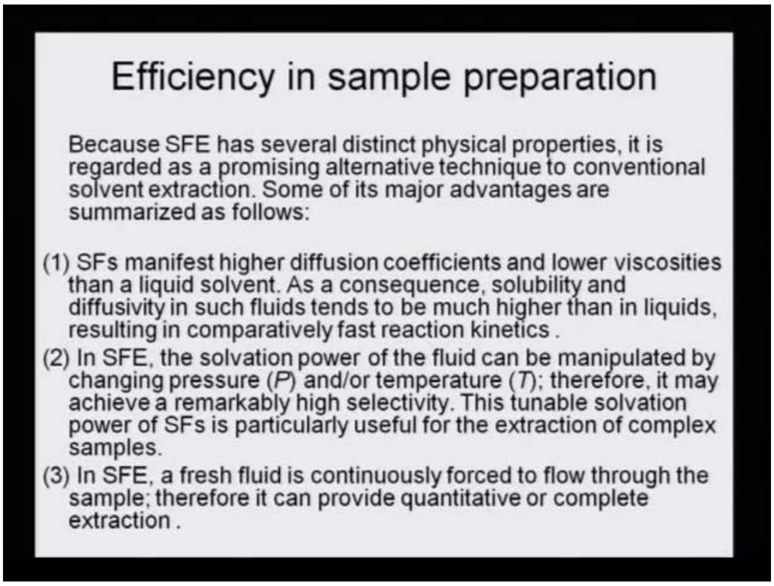

Efficiency in sample preparation

Because SFE has several distinct physical properties, it is regarded as a promising alternative technique to conventional solvent extraction. Some of its major advantages are summarized as follows:

(1) SFs manifest higher diffusion coefficients and lower viscosities than a liquid solvent. As a consequence, solubility and diffusivity in such fluids tends to be much higher than in liquids, resulting in comparatively fast reaction kinetics .

(2) In SFE, the solvation power of the fluid can be manipulated by changing pressure (*P*) and/or temperature (*T*); therefore, it may achieve a remarkably high selectivity. This tunable solvation power of SFs is particularly useful for the extraction of complex samples.

(3) In SFE, a fresh fluid is continuously forced to flow through the sample; therefore it can provide quantitative or complete extraction .

Efficiency in sample preparation - because supercritical fluid extraction has several distinct physical properties, it is regarded as promising alternative technique to conventional solvent extraction. Some of the major advantages summarized are as follows: supercritical manifest higher diffusion co-efficiency and lower viscosities than a liquid sample. As a consequence solubility and diffusivity in such fluids tend to be much higher than in liquids resulting in comparatively fast reaction kinetics.

In SFE, the solvating power of the fluid can be manipulated by changing the pressure and temperature. Therefore, it may achieve a remarkable high selectivity. This tunable solvation power of super critical fluid is particularly very useful for the extraction of complex samples.

I just gave you an example that, how, from one plant material the essential oils and the dyes can be separately extracted without contamination, and there is no need for any further chromatographic purification. Why? By simply manipulating the temperature and the pressure. The solvating power of that supercritical fluid at that point of time is just ideal to extract one type of compound, one nature of compound. And then, when the parameter is changed again, it is more suited for the second type of compound. In SFE, a fresh fluid is continuously forced to flow through the sample. Therefore, it can provide quantitative or complete extraction.

Now, as I told you that, when it is in the fluid state Carbon dioxide, it has a great solvating power; so, it picks up one type of compound, but when the gas is allowed or the fluid is allowed to rarify, then it kind of drops the compound it has solvated or taken along with it. And that is why it can be recycled, and every time a fresh fluid is entering the plant to be extracted.

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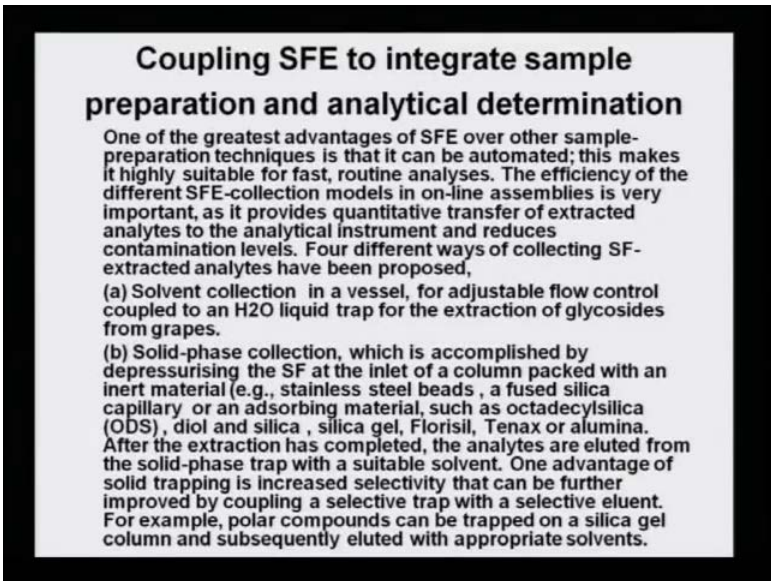

Coupling SFE to integrate sample preparation and analytical determination

One of the greatest advantages of SFE over other sample-preparation techniques is that it can be automated; this makes it highly suitable for fast, routine analyses. The efficiency of the different SFE-collection models in on-line assemblies is very important, as it provides quantitative transfer of extracted analytes to the analytical instrument and reduces contamination levels. Four different ways of collecting SF-extracted analytes have been proposed,

(a) Solvent collection in a vessel, for adjustable flow control coupled to an H2O liquid trap for the extraction of glycosides from grapes.

(b) Solid-phase collection, which is accomplished by depressurising the SF at the inlet of a column packed with an inert material (e.g., stainless steel beads , a fused silica capillary or an adsorbing material, such as octadecylsilica (ODS) , diol and silica , silica gel, Florisil, Tenax or alumina. After the extraction has completed, the analytes are eluted from the solid-phase trap with a suitable solvent. One advantage of solid trapping is increased selectivity that can be further improved by coupling a selective trap with a selective eluent. For example, polar compounds can be trapped on a silica gel column and subsequently eluted with appropriate solvents.

Coupling SFE to integrate sample preparation and analytical determination – so, how can be used this particular technique for the sample preparation and for analytical determination? One of the greatest advantages of SFE over other sample preparation technique is that it can be automated. This makes it highly suitable for fast and routine analysis.

The efficiency of the different SFE-collection models in online assemblies is very important, as it provides quantitative transfer of extracted analytes to the analytical instrument and reduces contamination levels. Four different ways of collecting SF extracted analytes have been proposed. Solvent collection in a vessel, for adjusted flow control coupled with water liquid trap for the extraction of glycosides from grapes. Second method is solid phase collection, which is accomplished by depressurizing the supercritical fluid at the inlet of the column packed with an inert material, that is stainless steel beads, a fused silica capillary, or an adsorbing material, such as octadecylsilica ODS, or diol or silica, or silica gel or Florisil, Tenax or alumina.

After the extraction has completed, the analytes are eluted from the solid phase trap with a suitable solvent. One advantage of solid trapping is increased selectivity. That can be further improved by coupling a selective trap with a selective eluent. For example, polar compounds can be trapped on a silica gel column and subsequently eluted with appropriate solvent.

Now, either it can be collected in a collector or it can be actually taken over on a solid phase and adsorbed on it, and further, with a little amount of solvent, it can be desorbed or eluted from that. So, there are many ways of adapting the supercritical fluid extraction methodology, so that it can serve the purpose.

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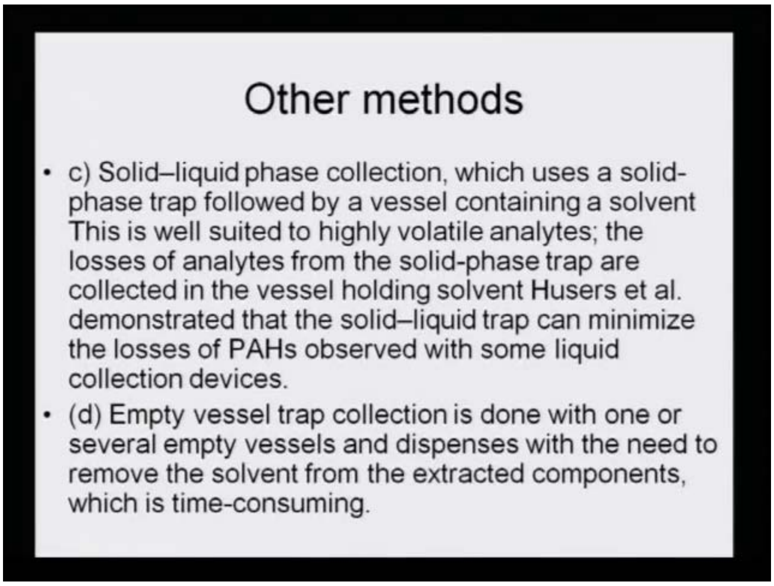

Other methods

- c) Solid–liquid phase collection, which uses a solid-phase trap followed by a vessel containing a solvent This is well suited to highly volatile analytes; the losses of analytes from the solid-phase trap are collected in the vessel holding solvent Husers et al. demonstrated that the solid–liquid trap can minimize the losses of PAHs observed with some liquid collection devices.
- (d) Empty vessel trap collection is done with one or several empty vessels and dispenses with the need to remove the solvent from the extracted components, which is time-consuming.

Other methods – solid-liquid phase collection, which uses a solid phase trap followed by a vessel containing a solvent. This is well suited to highly volatile analytes. The losses of analytes from the solid phase trap are collected in the vessel holding the solvent. Huser et al demonstrated that solid-liquid trap can minimize the losses of polycyclic aromatic hydrocarbon observed with some liquid collection devices.

So, what he did? He found that there were some losses because of this transfer business. So, he tried to use another kind of trapping or collection system where solid liquid collectors were used. Otherwise, empty vessels trap can also be used. With one or more several empty vessels and dispenses can be used, and which need no removal of the solvent, simply the extracted component is collected, but it is time consuming because every cycle, it will come and then it will just desorb, and this goes on for several cycles. So, it may take a little longer; if there are solvent traps, then it is much faster.

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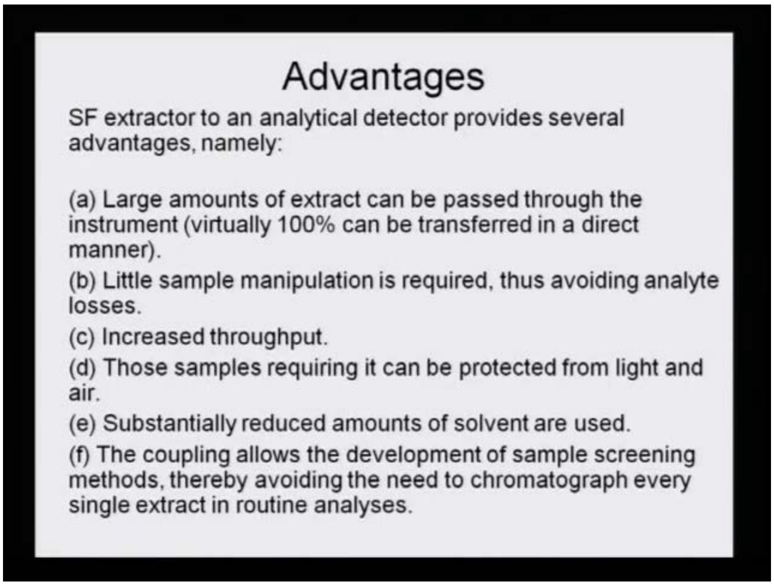

Advantages

SF extractor to an analytical detector provides several advantages, namely:

(a) Large amounts of extract can be passed through the instrument (virtually 100% can be transferred in a direct manner).

(b) Little sample manipulation is required, thus avoiding analyte losses.

(c) Increased throughput.

(d) Those samples requiring it can be protected from light and air.

(e) Substantially reduced amounts of solvent are used.

(f) The coupling allows the development of sample screening methods, thereby avoiding the need to chromatograph every single extract in routine analyses.

Advantages - the SF extractor to an analytical detector provides a several advantages that can be named: large amount of extract can be passed through the instrument; virtually 100 percent can be transferred in a direct manner. Little sample manipulation is required, thus avoiding analyte losses. Increased throughput, that means, the output of the reaction or the extraction is very high. Those samples requiring it can be protected from light and air; some are very light and air sensitive. Now, because this is a pressurized system, it is the closed system; so, air contamination or air oxidation or moisture oxidation or light effect can be avoided.

Substantially reduced amount of solvents are used. As I mentioned, that only under very special condition where simple supercritical Carbon dioxide is not very effective in extraction, 1 to 10 percent of Methanol or Ethyl acetate type of solvents are added as entrainer or modifier. So, you see very small quantities of solvents are required. The coupling allows the development of sample screening methods, thereby avoiding the need to chromatograph every single extract in routine analyses. So, as I mentioned, that these are the methods where sometimes it is so pure, that no further chromatographic technique is required. Therefore, supercritical fluid extraction, as you would also appreciate, has a certain demarcated advantages over the Soxhlet and the conventional extraction methods.

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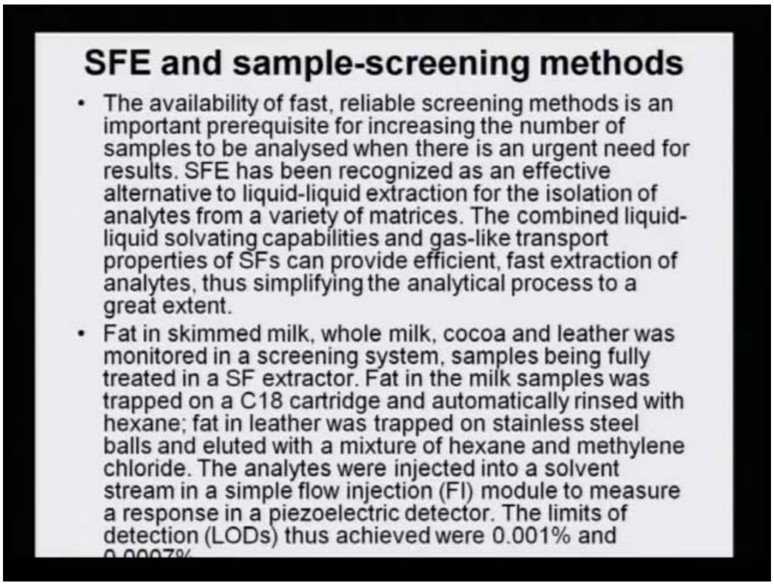

SFE and sample-screening methods

- The availability of fast, reliable screening methods is an important prerequisite for increasing the number of samples to be analysed when there is an urgent need for results. SFE has been recognized as an effective alternative to liquid-liquid extraction for the isolation of analytes from a variety of matrices. The combined liquid-liquid solvating capabilities and gas-like transport properties of SFs can provide efficient, fast extraction of analytes, thus simplifying the analytical process to a great extent.
- Fat in skimmed milk, whole milk, cocoa and leather was monitored in a screening system, samples being fully treated in a SF extractor. Fat in the milk samples was trapped on a C18 cartridge and automatically rinsed with hexane; fat in leather was trapped on stainless steel balls and eluted with a mixture of hexane and methylene chloride. The analytes were injected into a solvent stream in a simple flow injection (FI) module to measure a response in a piezoelectric detector. The limits of detection (LODs) thus achieved were 0.001% and 0.0007%

SFE and sample-screening methods - the availability of fast, reliable screening method is an important prerequisite for screening or increasing the number of samples to be analyzed when there is an urgent need for results. SFE has been recognized as an effective alternative to liquid-liquid extraction for the isolation of analytes from a variety of matrices. The combined liquid-liquid solvating capacities and gas like transport properties of the supercritical fluids can provide efficient fast extraction of analytes, thus simplifying the analytical process to a great extent.

Now, I will give you an example. Fat in skimmed milk, whole milk, cocoa, and leather was monitored in a screening system, samples being fully treated with supercritical fluid extractor. Fat in the milk sample was trapped in C 18 cartridge and automatically rinsed with hexane; fat in leather was trapped in stainless steel balls and eluted with a mixture of hexane and methylene chloride. The analytes were injected into a solvent stream in a simple flow injector module to measure a response in a piezoelectric detector. The limits of detection, thus achieved were 0.001 percent and 0.0007 percent. So, you see, it is so efficient, even in simple sample screening methodology.

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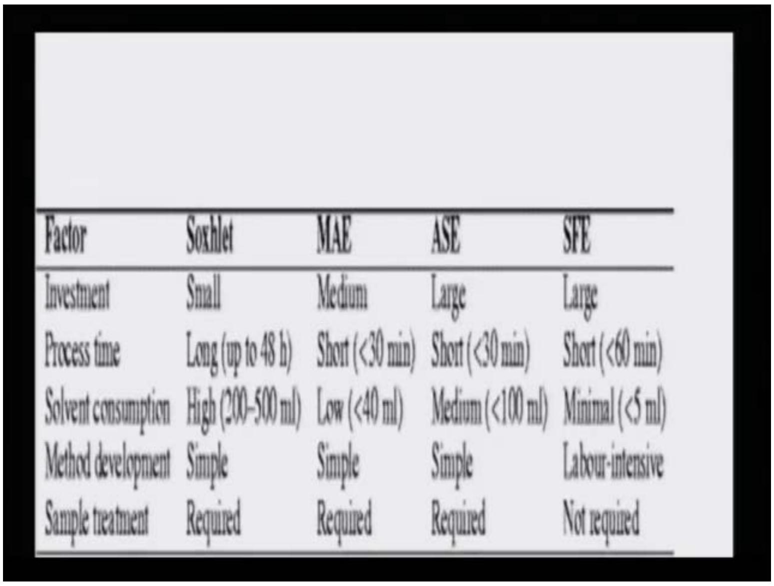

Factor	Soxhlet	MAE	ASE	SFE
Investment	Small	Medium	Large	Large
Process time	Long (up to 48 h)	Short (<30 min)	Short (<30 min)	Short (<60 min)
Solvent consumption	High (200–500 ml)	Low (<40 ml)	Medium (<100 ml)	Minimal (<5 ml)
Method development	Simple	Simple	Simple	Labour-intensive
Sample treatment	Required	Required	Required	Not required

Thus, we can say that, if we look at the investment, process time, solvent consumption, method developments, sample treatment and we make a comparison between a Soxhlet, a microwave assisted accelerated solvent extraction and supercritical extraction, you will see that, in all respect supercritical fluid extraction has an edge over all these three different types of practice methods.

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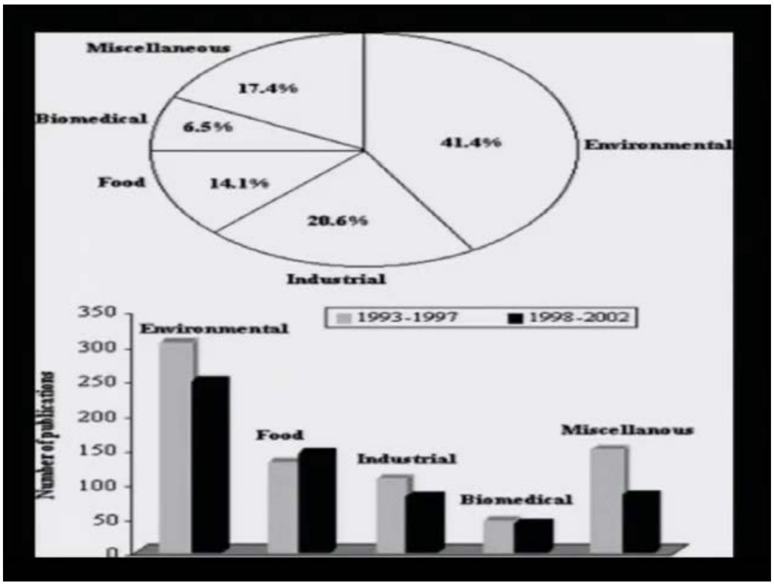

World over industry, and in many other food and industrial and miscellaneous an environmental samples from the year 1993 to 2002, the advantage of using and the propagation and the popularity of supercritical extraction has increased enormously.

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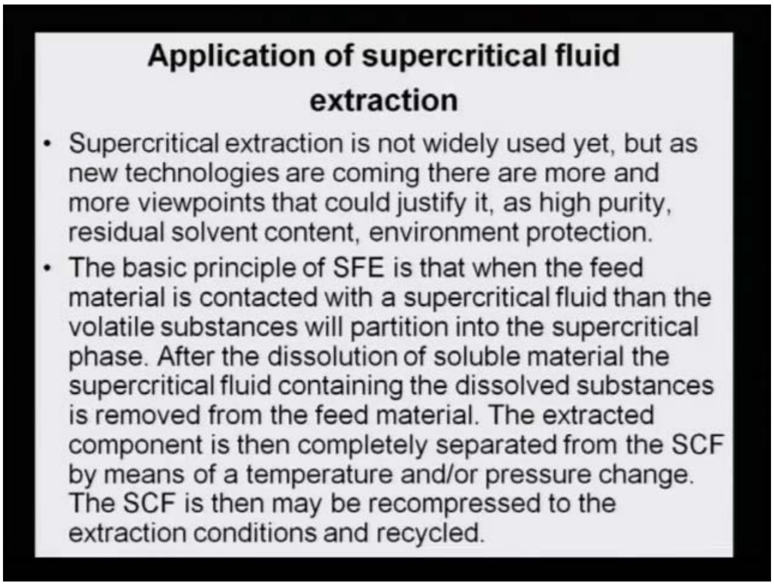

Application of supercritical fluid extraction

- Supercritical extraction is not widely used yet, but as new technologies are coming there are more and more viewpoints that could justify it, as high purity, residual solvent content, environment protection.
- The basic principle of SFE is that when the feed material is contacted with a supercritical fluid than the volatile substances will partition into the supercritical phase. After the dissolution of soluble material the supercritical fluid containing the dissolved substances is removed from the feed material. The extracted component is then completely separated from the SCF by means of a temperature and/or pressure change. The SCF is then may be recompressed to the extraction conditions and recycled.

Application of supercritical fluid extraction - supercritical fluid extraction is not widely used yet still because the machine is very expensive. Although it is a onetime expense, but still it is not taken up as much as what it should have, but as new technologies are coming up, there are more and more view points, and could justify it as high purity, residual solvent content, environmental protection are the basic ideas behind using supercritical fluid extraction.

The basic principle of supercritical fluid extraction is that when the feed material is contacted with a supercritical fluid, then the volatile substances will partition into the supercritical phase. After the dissolution of the soluble material, the supercritical fluid containing the dissolved substances is removed from the feed material. The extracted component is then completely separated from the supercritical fluid, and by means of temperature and pressure change; that means it is rarified, the supercritical fluid. Then, it may be recompressed and reused. So, it can be recycled for the next extraction process. So, it is also economical. Only very small amount of Carbon dioxide is lost in the entire cycle; so, after several cycles, the gas can be replenished.

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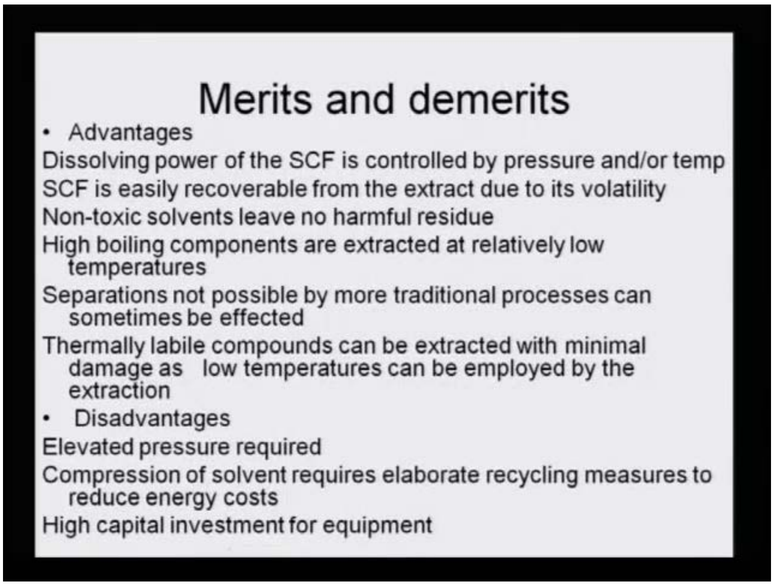

Merits and demerits

- Advantages

Dissolving power of the SCF is controlled by pressure and/or temp

SCF is easily recoverable from the extract due to its volatility

Non-toxic solvents leave no harmful residue

High boiling components are extracted at relatively low temperatures

Separations not possible by more traditional processes can sometimes be effected

Thermally labile compounds can be extracted with minimal damage as low temperatures can be employed by the extraction

- Disadvantages

Elevated pressure required

Compression of solvent requires elaborate recycling measures to reduce energy costs

High capital investment for equipment

Merits and demerits – so, the as usual, there are advantages. These are using sorry advantages, dissolving power of supercritical is controlled by pressure and/or temperature supercritical fluid is easily recoverable from the extract due to its volatility. It is Non-toxic solvent and leaves no harmful residue. High boiling components are extracted at relatively low temperatures. Separations not possible by more traditional processes can sometimes be effected. Thermally liable compounds can be extracted with minimal damage. And there are of course, some disadvantages - elevated pressures are required; compression of the solvent requires elaborates recycling measures to reduce energy costs and high capital investment for the equipment, as what I mentioned.