Quantitative Methods in Chemistry
Prof. Dr. Aasheesh Srivastava, Dr. Bharathwaj Sathyamoorthy
Department of Chemistry
Indian Institute of Science Education and Research-Bhopal

Lecture-45
Supercritical Fluid Chromatography

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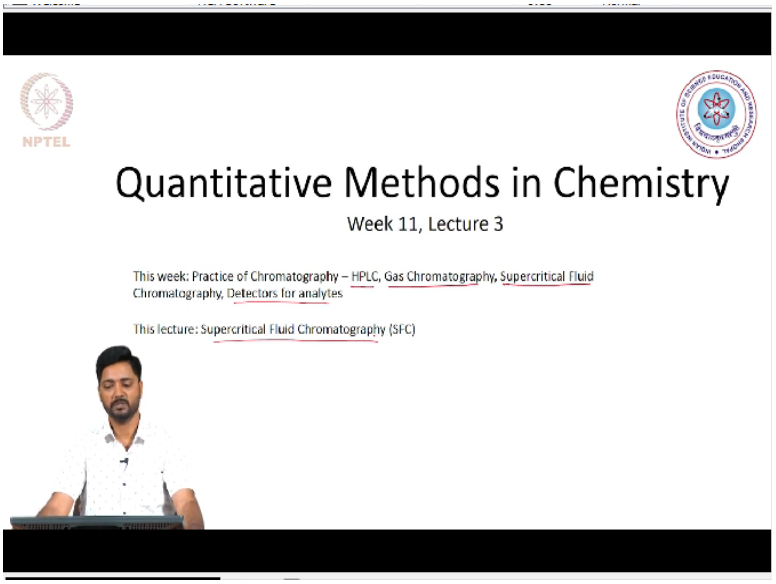

Quantitative Methods in Chemistry

Week 11, Lecture 3

This week: Practice of Chromatography – HPLC, Gas Chromatography, Supercritical Fluid Chromatography, Detectors for analytes

This lecture: Supercritical Fluid Chromatography (SFC)

Hello and welcome back to this course quantitative methods in chemistry. We are in week 11 of this course and this will be lecture 3 for week 11. So, this week our focus has been on developing clear understanding of how practice of chromatography is undertaken. We have already got to know about the practical considerations with regards to high performance liquid chromatography.

We also got introduced to gas liquid partition chromatography, which is colloquially abbreviated as gas chromatography. So, this lecture, our focus will be on the supercritical fluids that are used in chromatographic separations, and the final lecture of this week will focus on the various detectors that are employed the chromatographic systems and the detectors that are commercially available. So, this lecture is specifically dedicated to the supercritical fluid chromatography.

And let us try to understand what this concept is and what are its unique advantages and disadvantages with a base the traditional techniques such as high performance liquid chromatography and gas chromatography.

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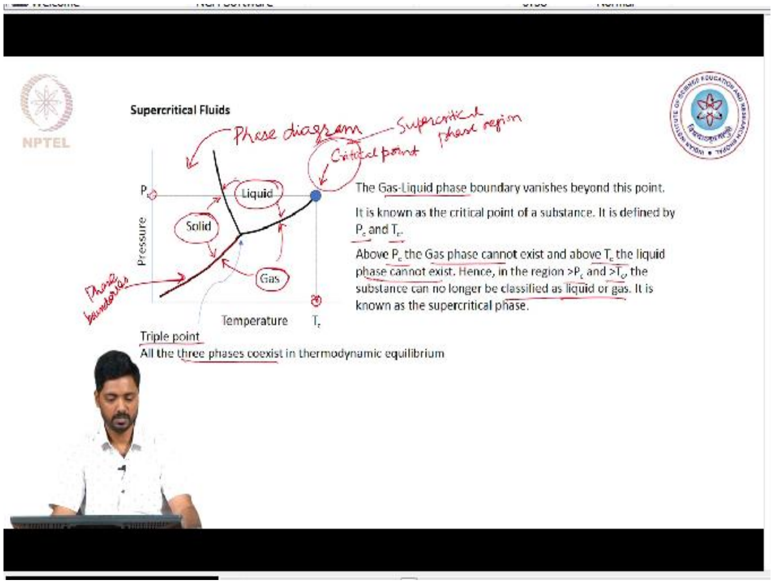

Supercritical Fluids

The Gas-Liquid phase boundary vanishes beyond this point.

It is known as the critical point of a substance. It is defined by P_c and T_c.

Above P_c the Gas phase cannot exist and above T_c the liquid phase cannot exist. Hence, in the region $>P_c$ and $>T_c$, the substance can no longer be classified as liquid or gas. It is known as the supercritical phase.

Triple point

All the three phases coexist in thermodynamic equilibrium

So, let us first get ourselves introduced to what we mean by supercritical fluids. This is a very interesting concept in that, when we make a phase diagram, which is shown here we can segregate any substance into the phases that it will take for example, it will take a solid phase in a certain regime of pressure and temperature, it will be in its gaseous state at a different temperature and pressure regime.

And intermediate region will have the substance present as liquid. So we can create what are known as the phase boundaries, where which are denoted by these black lines. So, these black lines are our phase boundaries where both phases do co-exist. So, for example, this line here indicates the regime of pressures and temperatures at which the solid phase and the gaseous phase coexist.

Similarly, we can have region where the solid and the liquid coexist and finally, we can find regions where the liquid and the gas phases coexist in thermodynamic equilibrium. All of this often brings us to the concept of triple point, which is unique temperature and pressure value at which all the 3 phases can coexist in thermodynamic equilibrium, I have highlighted this blue point here, which is called as the critical point.

Because beyond this point, the gas liquid phase boundary vanishes, and we will not be able to discriminate or distinguish whether the substance that we are dealing with is existing in a gaseous phase or a liquid phase. Its properties will be intermediate of the 2. So, we can define this critical point in terms of the critical temperature, which is highlighted here, as well as critical pressure, which is indicated here.

So, we require to know both the critical temperature and the critical pressure beyond which the substance will no longer be in a unique phase either liquid or a gas phase. So, the critical pressure PC and the critical temperature TC sort of tell us the values beyond which our substance will be undergoing a supercritical phase transition and above the critical pressure, because of the higher pressure the gas phase cannot exist.

And above the critical temperature, the liquid phase cannot exist. So, obviously, the phase that results in this region of pressure greater than critical pressure and temperature is greater than critical temperatures cannot be classified as either liquid or gas regimes. And this phase, which will be the region here will be known as the supercritical phase region. So, in this region, our substance cannot be classified as either liquid or gas.

It will have properties that will be intermediate of both gases and liquids and that becomes very advantageous for us in the chromatographic separations.

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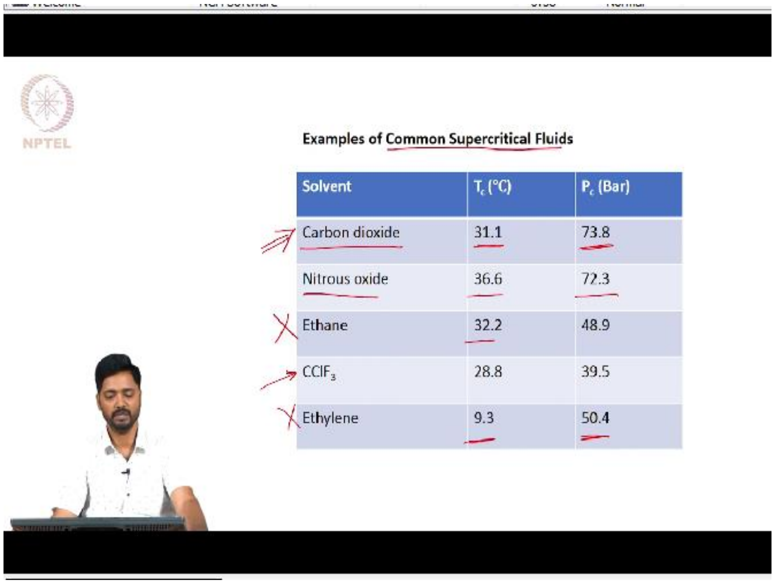

Examples of Common Supercritical Fluids

Solvent	T_c (°C)	P_c (Bar)
Carbon dioxide	31.1	73.8
Nitrous oxide	36.6	72.3
Ethane	32.2	48.9
$CClF_3$	28.8	39.5
Ethylene	9.3	50.4

So, before that, let us look at some examples of what are considered as common supercritical fluids or compounds or substances that can be readily converted into a supercritical phase. So, the most common example in this case is that of carbon dioxide, which has a critical temperature of 31.1 degrees Celsius and a critical pressure of 73.8 bars. So, obviously, at temperatures and pressures greater than these 2 numbers, the carbon dioxide will exhibit supercritical fluid properties.

And as we can see that it is not very difficult to achieve the conditions in which carbon dioxide will exist as a supercritical fluid or will exhibit a supercritical phase. Other common gas is the nitrous oxide, which has a critical temperature of 36.6 degrees Celsius and a critical pressure of 72.3 bars of pressure. So, conditions where the temperature and the pressure exceed these values will result in nitrous oxide going into a supercritical phase.

Now, ethane is also a gas or a substance which can be readily converted into a supercritical phase at temperatures of about 32.2 degrees Celsius and pressures greater than 48.9 bars. Now, chlorofluorocarbons are other examples of gases that can be converted readily into supercritical fluid phase. And finally, amongst the various examples that are available to us, I have enlisted ethylene, which has a quite low critical temperature and a decently low pressure, which is the critical pressure.

What we need to realize and keep in mind when we are utilizing these supercritical fluids for practical applications are the other properties that these gases or these substances have. For example, ethane and ethylene both, despite their very favorable temperatures and pressures cannot be used in practical chromatographic separations because of their high flammability. So, using these gases will entail a lot of safety considerations which inhibit their use in supercritical fluid chromatography or supercritical fluid extractions that are otherwise commonly undertaken.

So, usually and most often the gas that is the most appropriate for use in supercritical fluid applications is the carbon dioxide gas. Now, this gas obviously is inherently inert to a decent extent and is also having the appropriate temperatures and pressures at which it will undergo a supercritical phase transition. So carbon dioxide is going to be the most used gas for undertaking supercritical fluid chromatography.

And indeed, most of the commercial systems utilize the carbon dioxide as the mobile phase or the gas in its supercritical fluid form. Chlorofluorocarbons obviously again despite their favorable critical temperature and critical pressure values are having environmental drawbacks which inhibit their use as supercritical fluids now.

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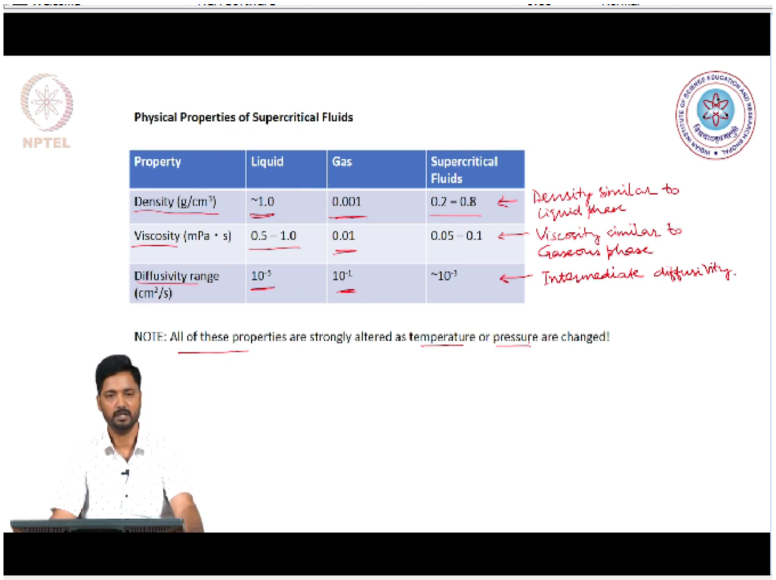

Physical Properties of Supercritical Fluids

Property	Liquid	Gas	Supercritical Fluids
Density (g/cm^3)	~1.0	0.001	0.2 – 0.8
Viscosity (mPa · s)	0.5 – 1.0	0.01	0.05 – 0.1
Diffusivity range (cm^2/s)	10^{-5}	10^{-1}	~10^{-3}

Density similar to Liquid phase

Viscosity similar to Gaseous phase

Intermediate diffusivity.

NOTE: All of these properties are strongly altered as temperature or pressure are changed!

So, what are the properties of supercritical fluids and how do they contrast with the traditional liquid and gaseous phases of matter that we are dealing with in our day to day lives. So, if I consider the density of the material, then in its liquid phase the material has very high density. So, for example, liquid water will have the density of 1 gram per ml or 1 gram per centimeter cube, on a clear contrast is the density of the gaseous phase.

And the density is in fact almost thousand times slower in gaseous phase when compared to the density in the liquid phase and that is very clearly demonstrated here that the density of the gaseous phase is of the order of point 001 gram per ml or gram per centimeter cube. Now, the supercritical fluids are decently dense. So, they have density, which is similar to that of liquids. So, the density of supercritical fluids is similar to the liquid phase of material and this is an important point to be kept in mind.

The other parameter that is of interest to us is the viscosity of the medium or the fluid phase. And as we have already seen that the viscosity clearly influences the various diffusion terms or the resistance to the mass transfer terms. So, we would want viscosity to be intermediate. Because another challenge that viscosity brings is that it reduces the flow of the mobile phase through the column and creates greater back pressures.

So the liquids that we deal in our day to day lives have the viscosity of 0.5 to 1 milliPa per second, and gases have inherently much lower viscosities or in other words they can easily travel through the chromatographic columns. Now, the viscosity of supercritical fluids is similar to the gaseous phase. So, while the density of the supercritical fluids was closer to that of the liquid state, the viscosity is closer to the gaseous state.

And finally, if we talk about the diffusivity range or the diffusion coefficient of materials in there 3 different states, in the liquid state, the diffusivity is quite less that is again because the material is quite dense and the molecules are having interactions with each other, in the gaseous phase, the diffusivity is much higher and again that is because molecules are almost non interacting with each other. In supercritical fluids, they have intermediate diffusivity.

So, they are diffusivities are in between that of the liquid and the gaseous phase. So, as we see that the physical properties of supercritical fluids are pretty unique and very different from that of either the liquid phase or the gaseous phase and they are more intermediate a combination of the 2 states that result in a supercritical fluid phase. So, an important point to be kept in mind here is that all of these properties whether we are talking about the density, viscosity or the diffusivity of the supercritical fluids are very strongly altered by change of temperature or pressure.

So, changing any of these 2 parameters will result in its phase from a supercritical phase to either a gaseous phase or a liquid phase with concomitant changes in the physical properties of density, viscosity and diffusivity. So, we are required to maintain the temperature and the pressure very carefully to keep our supercritical fluid in its optimum performance regime.

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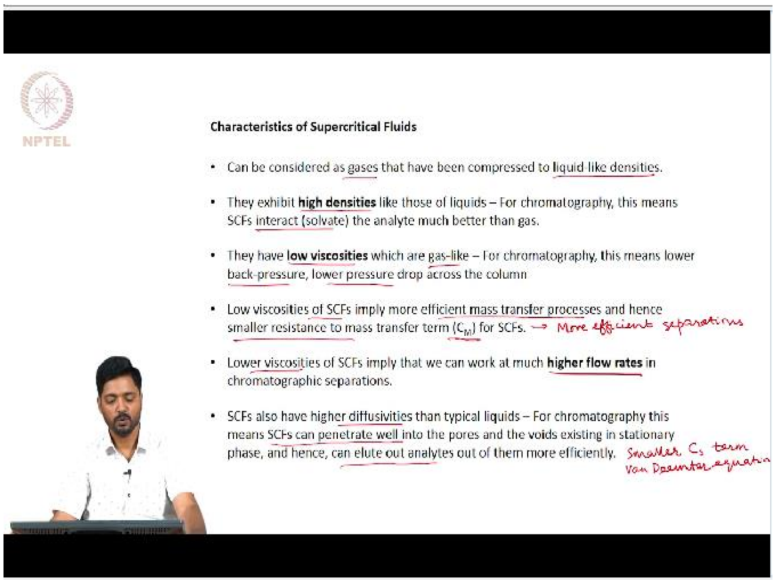

Characteristics of Supercritical Fluids

- Can be considered as gases that have been compressed to liquid-like densities.
- They exhibit **high densities** like those of liquids – For chromatography, this means SCFs interact (solvate) the analyte much better than gas.
- They have **low viscosities** which are gas-like – For chromatography, this means lower back-pressure, lower pressure drop across the column
- Low viscosities of SCFs imply more efficient mass transfer processes and hence smaller resistance to mass transfer term (C_M) for SCFs. → More efficient separations
- Lower viscosities of SCFs imply that we can work at much **higher flow rates** in chromatographic separations.
- SCFs also have higher diffusivities than typical liquids – For chromatography this means SCFs can penetrate well into the pores and the voids existing in stationary phase, and hence, can elute out analytes out of them more efficiently. Smaller C_s term
Van Deemter equation

So, what are the characteristics of the supercritical fluids. So, they can be considered as gases, but gases that have been compressed to liquid like densities, we already saw that supercritical fluids have very high densities similar to that of liquids. And this is also mentioned here that they have high densities and this for chromatographic purposes implies that the supercritical fluids can interact or solvate the analyte of our interest much better than the gas as the mobile phase.

So, we can achieve enhanced solvation of our analyte when we are using supercritical fluids. Another aspect that is of use to us is that the supercritical fluids have low viscosity and these viscosities are more like those of the gases and for chromatographic utility, this is an important aspect because low viscosity is directly related to low back pressures being generated, because their resistance to the flow of this medium is much less.

They also reserved in lower pressure drops across the column. So, indeed, in supercritical fluid chromatography, we can utilize really long columns, which will not be possible in the case of liquid chromatography. So, as we can see that we have a clear advantage of having high densities that of liquid and low viscosity is that of gas in case of supercritical fluids. Now, the low viscosities of the supercritical fluids also imply more efficient mass transfer processes.

And as we have seen in the C term of the Van Deemter equation, this relates to the resistance of mass transfer and efficient mass transfer processes in supercritical fluids implies smaller resistance to mass transport term and a lower C M values will result in more efficient separations compared to that of liquids. And of course, because of the low viscosities of supercritical fluids, we can work at much higher flow rates.

So, the solvent or the mobile phase can be passed through the column at much faster speeds resulting in faster separations in the chromatography, the diffusivities of supercritical fluids are much higher and that implies for chromatographic separations that the supercritical fluids can penetrate well into the pores and the voids that are present in the stationary phase and hence, they will be able to elute out the analytes out of them more efficiently. And this again relates to a smaller C s term in the Van Deemter equation.

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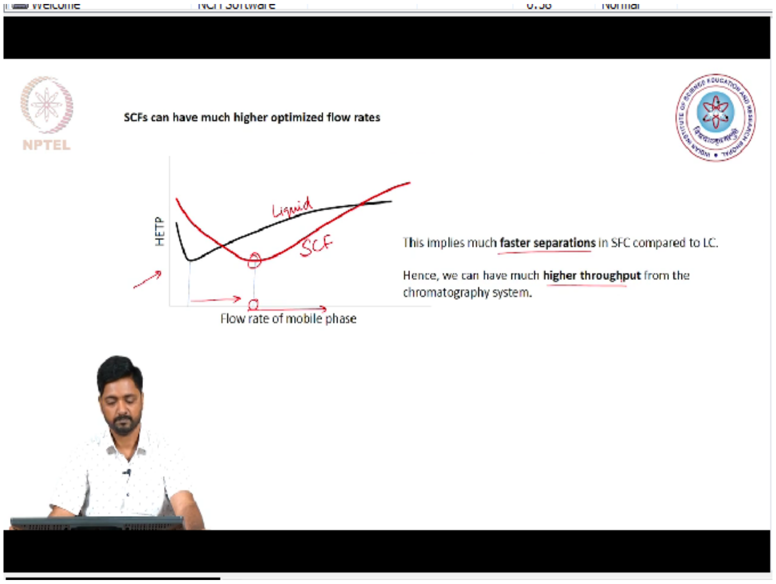

SCFs can have much higher optimized flow rates

This implies much **faster separations** in SFC compared to LC.

Hence, we can have much **higher throughput** from the chromatography system.

So, let us move forward and compare how the improved flow rate of supercritical fluids is advantages to us. So, here I have plotted the change in the plate height with the flow rate of the mobile phase for the liquid chromatography and for supercritical fluid chromatography. So, the liquid chromatography profile is shown as the black curve, while the supercritical fluid chromatography is shown as the red curve.

And what we observe is that we can achieve similar plate height values both the conditions, but in the supercritical fluid chromatography, the most optimum flow rate with the lowest value of the plate height comes much ahead than that for the liquid chromatography state. So, compared to liquid chromatography, supercritical fluid chromatography can be having a much higher optimized flow rates.

And this implies that the separations that we undertake in supercritical fluid chromatography can be undertaken much faster because of the high flow rates that are resulting in the lowest height equivalent of the theoretical plate value. And that means that the times required for chromatographic separations will be much shorter. And as a result, the throughput from our equipment will be much higher or we will be able to analyze larger number of samples within a given time of instrument use.

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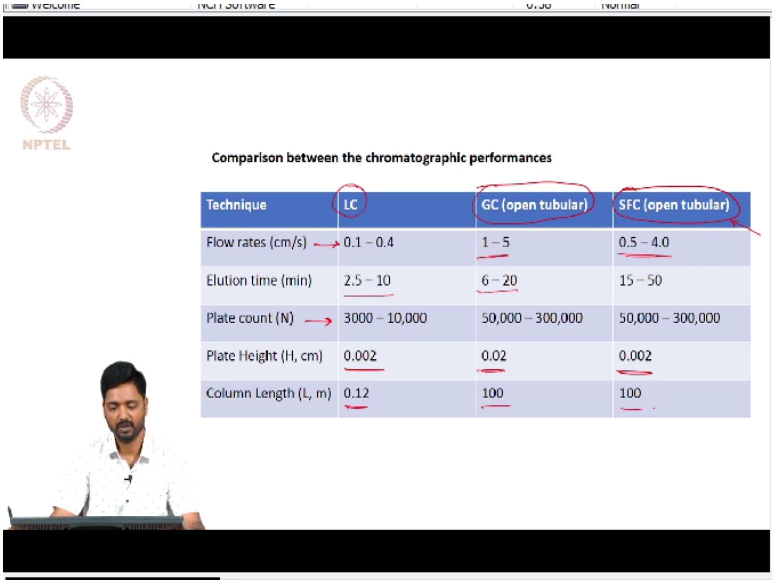

Comparison between the chromatographic performances

Technique	LC	GC (open tubular)	SFC (open tubular)
Flow rates (cm/s)	0.1 – 0.4	1 – 5	0.5 – 4.0
Elution time (min)	2.5 – 10	6 – 20	15 – 50
Plate count (N)	3000 – 10,000	50,000 – 300,000	50,000 – 300,000
Plate Height (H, cm)	0.002	0.02	0.002
Column Length (L, m)	0.12	100	100

Now, here I have compared the chromatographic performances of the 3 systems, the liquid chromatography systems, the gas chromatography being operated in the open tabular form and the supercritical fluid chromatography again being operated in the open tubular form. So, if I compare the flow rates, the flow rates of course, are the lowest for liquid chromatography systems and they are in the range of 0.1 to 0.4 centimeters per second.

In gas chromatography, the flow rates are definitely much higher. And in supercritical fluid chromatography, these numbers are more intermediate, but definitely much higher than that for the liquid chromatography. As a result, the illusion time of analytes are also different. In case of liquid chromatography the chromatography can be completed in the range of 2 to 10 minutes, the gas chromatography because the column length is really long, takes much longer time.

And the supercritical fluid chromatography being operated with the same open tabular column will require still longer time, because now we are dealing with the material which is more dense and have higher viscosity compared to gases. The plate count values are of course, lower in the liquid chromatographic separations because of the shorter column lengths. And in gas chromatography and supercritical fluid chromatography, these numbers are very comparable.

The plate height, which indicates how efficient is our chromatography is the lowest for liquid chromatography systems and this point we have discussed multiple times. So, I will not be discussing why this happens for gas chromatography, the plate height is much higher about 10 times higher than that for the liquid chromatography that is because of the low density of the mobile phase.

And finally in the supercritical fluid chromatography, enhanced solvation of the analyte results in much lower plate heights and hence very efficient separations. So, the column length again is listed here, it is the smallest for the liquid chromatography system and the columns can be really long in case of both gas chromatography and supercritical fluid chromatography. Now, it is important also to make note that properties of supercritical fluids are very intimately dependent upon the pressures and the temperatures that are operating.

So, under one temperature and pressure condition, the supercritical fluid will behave more like gas while at different temperature and pressure, it will behave more like a liquid and hence supercritical fluid chromatography is amenable to use of both gas chromatography columns as well as the liquid chromatography columns.

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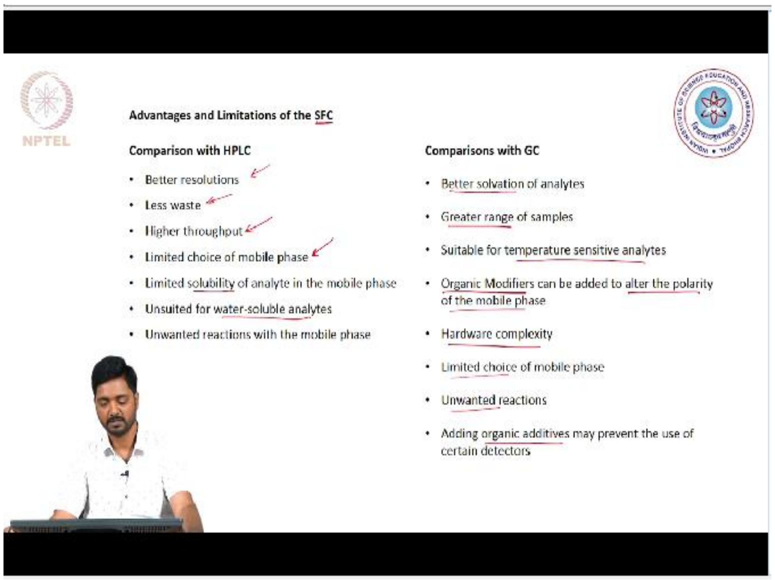

Advantages and Limitations of the SFC

Comparison with HPLC

- Better resolutions
- Less waste
- Higher throughput
- Limited choice of mobile phase
- Limited solubility of analyte in the mobile phase
- Unsuited for water-soluble analytes
- Unwanted reactions with the mobile phase

Comparisons with GC

- Better solvation of analytes
- Greater range of samples
- Suitable for temperature sensitive analytes
- Organic Modifiers can be added to alter the polarity of the mobile phase
- Hardware complexity
- Limited choice of mobile phase
- Unwanted reactions
- Adding organic additives may prevent the use of certain detectors

Now, let us look at what are the advantages and limitations of supercritical fluid chromatography in comparison to the traditional techniques like high performance liquid chromatography and gas chromatography. So, compared to HPLC the supercritical fluid chromatography which is abbreviated as SFC results in better resolutions, less waste, because another advantage with regards to the supercritical fluids is that one can convert them from the supercritical phase to a gaseous phase by change of temperature and pressure.

They also result in higher throughputs because the flow rates are much higher. However, there are clear limitations that have inhibited this technique from gaining widespread acceptance. And first and foremost limitation is the choice of mobile phases. So, typically carbon dioxide is in many of the practical senses our only choice of the mobile phase, carbon dioxide being inherently non polar results in the limited solubility of the analyte in it.

And, again because of its non polar nature, carbon dioxide based supercritical fluid chromatography systems are not suitable for analytes that are soluble in water. So, this again is a clear major limitation of supercritical fluid chromatography over high performance liquid chromatography, the latter that is the high performance liquid chromatography in this sense is more versatile.

Now, another challenge with regards to the supercritical fluid chromatography is that sometimes the carbon dioxide can undergo spurious reactions or unwanted reactions with the analyte especially if the analyte is having amine residues, it can result in the formation of carbon mates. And that, of course, is an undesirable side reaction that needs to be avoided, if I compare the supercritical fluid chromatography with gas chromatography.

Of course, we see that we again compared to gas chromatography, because of the higher density of the supercritical fluids, we achieve much better solvation of the analytes and as a result, we will be able to handle a greater range of samples, that also becomes possible because, with supercritical fluids, we will be able to analyze temperature sensitive analytes because the operating temperatures are much smaller or much lower in the case of supercritical fluids compared to that of gas chromatography.

Operating temperatures of 300 degrees Celsius are often routine. Now one more important point here is that we can use certain organic modifiers to alter the polarity of the mobile phase when using carbon dioxide as the supercritical fluid. However, the challenges that needs to be overcome when using supercritical fluid chromatography is the hardware complexity. And this aspect needs to be explained in detail.

Now, we have realized that all the properties of supercritical fluids are very sensitive to changes in temperatures and pressures. So, we need to maintain or alter them in a very precise manner, so that we are able to change the properties of our supercritical fluids appropriately. This brings about hardware requirement to be more complex when compared to gas chromatography and that adds to the cost of the supercritical fluid chromatographic systems.

Of course, the common challenges here are the limited choice of the mobile phase and the unwanted reactions that have already been discussed. And finally, adding of organic additives to alter the polarity of the mobile phase may prevent the use of certain detectors.

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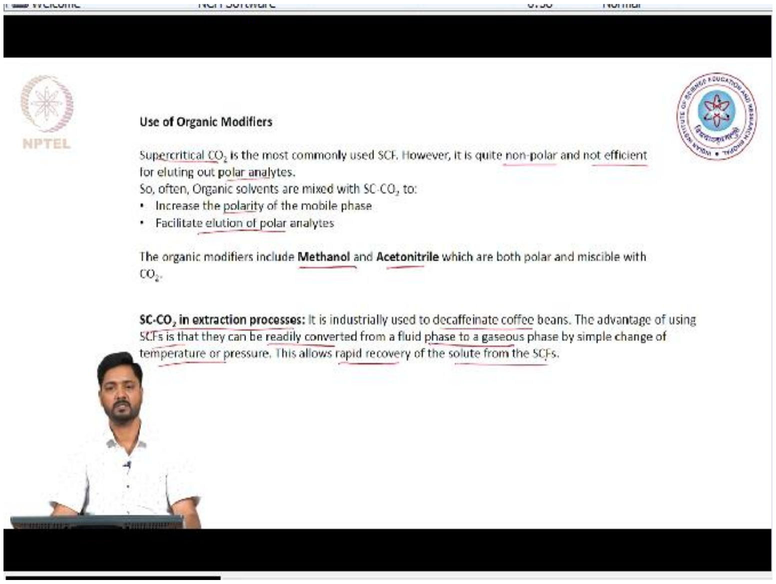

Use of Organic Modifiers

Supercritical CO_2 is the most commonly used SCF. However, it is quite non-polar and not efficient for eluting out polar analytes.
So, often, Organic solvents are mixed with SC-CO_2 to:

- Increase the polarity of the mobile phase
- Facilitate elution of polar analytes

The organic modifiers include **Methanol** and **Acetonitrile** which are both polar and miscible with CO_2.

SC-CO_2 in extraction processes: It is industrially used to decaffeinate coffee beans. The advantage of using SCFs is that they can be readily converted from a fluid phase to a gaseous phase by simple change of temperature or pressure. This allows rapid recovery of the solute from the SCFs.

So, let me delve a little bit on why organic modifiers are added to supercritical fluids. When we use supercritical carbon dioxide it has inherently low polarity, it is almost non polar, and hence is not efficient for eluting out polar analytes. So, we add organic solvents to make the supercritical carbon dioxide a little bit more polar, that is increase its polarity and that will facilitate better elution or faster elution of polar analytes.

Now the most common organic modifiers or organic additives being used with supercritical carbon dioxide are methanol and acetone nitride, which are both polar and miscible in carbon dioxide, because of its unique nature and easy converting from a supercritical phase to a gaseous phase, supercritical carbon dioxide is also used in extraction processes in industry, for example, it is used to decaffeinated coffee beans.

And one clear advantage with regards to using the supercritical fluids is that we do not generate waste organic solvent, because the supercritical fluids can be readily converted from the fluid phase to gaseous phase by change of temperature or pressure. And this is advantages in terms of allowing rapid recovery of the solids from the supercritical fluids. So, I hope that this lecture has given you good understanding of what supercritical fluids are.

And when are their advantages properties, these fluids in chromatographic separations. This brings us to the end of this lecture. In the fourth and final lecture of this week, we will be discussing about different types of detectors that are implied in chromatographic separations, we will understand the principles, the sensitivity and the limitations of these detectors. Thank you.