

A Review on “Super Critical Fluid Chromatography”

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ABSTRACT:

Fluids that are elevated over their critical pressure (Pc) and critical temperature (Tc) are used as mobile phases in supercritical fluid chromatography (SFC). The supercritical fluid most frequently employed as a mobile phase for SFC is carbon dioxide, or CO₂. Most of the analytes that can solvate are non-polar. In order to preserve the bioactive compounds' therapeutic or functional qualities, supercritical CO₂ (SC-CO₂) is inexpensive, easily accessible in bulk, and has a high degree of purity. It also has the ability to solubilize lipophilic molecules and is simple to remove from the finished products. It is a green and generally acknowledged as safe (GRAS) method. After gas chromatography (GC) and high-performance liquid chromatography (HPLC), supercritical fluid chromatography is one of the most significant column chromatography techniques. Supercritical fluids combine advantageous characteristics of the liquid and gas phases. The density, diffusivity, and viscosity of a supercritical fluid are its defining characteristics.

I. INTRODUCTION TO SPECTROSCOPY:^[1]

- The energy that is transferred from one body to another by radiations is known as radiant energy. The term "radiation" refers to electromagnetic vibrations that range from low frequency electric waves, through the visible spectrum, infrared rays, and ultraviolet rays, to high frequency (low wavelength) x-rays and -rays. Maxwell (1864) claimed that an alternating current with a high frequency radiates in the form of waves that move through space at the same speed as light. These waves are referred to as electromagnetic waves or electromagnetic radiations.
- Thus, electromagnetic radiation is a form of energy that travels through space at incredibly fast rates. Energy is wave-like in nature and is

connected to both magnetic and electric forces. Electromagnetic radiations are the name given to these emissions. It should be noted that an oscillating electric charge generates an oscillating magnetic field as well as an oscillating electric field, both of which are referred to as electromagnetic radiation since they travel over space. Electromagnetic radiation includes infrared, ultraviolet, micro waves, radio waves, x-rays, radio waves, and television waves in addition to visible light, which is a subset of the radiation emitted by atoms.

- All electromagnetic radiation travels across space at the same speed, indicated by the letter c (which equals 2.997925×10^8 metres per second) and is known as the speed of light. The wavelength or frequency of a monochromatic (single frequency) wave can be used to describe it. The terms for each form of electromagnetic radiation are already well-known. For instance, ultraviolet rays from the sun produce sunburns, x-rays are now employed in medical, and radio and radar waves are used for communication.

Types of Spectroscopy:-

- Infrared (IR) Spectroscopy
- Ultraviolet-Visible (UV-Vis) Spectroscopy
- Raman Spectroscopy
- Nuclear Magnetic Resonance (NMR) Spectroscopy
- FTIR Spectroscopy
- X-Ray Spectroscopy

Introduction to Supercritical fluid:^[1-5]

- Supercritical fluid extraction (SFE) has a growing number of possible uses in

different parts of the world, as seen by the rise in patents filed in recent years. It is noted that its implementation is already a feature of the current environment, primarily driven by the expanding demand for high-quality products and the globalization of the economy. Additionally, it is distinctive for its application in the trade of pharmaceutical, culinary, chemical, and cosmetic commodities. The selectivity, facility, and separation capacity that the approach provides in order to acquire a large number of organic materials are the major reasons why this technology is being used more and more in the industrial sector.

- Since its introduction, the extraction method most commonly referred to as supercritical fluid extraction (SFE) has been praised for its outstanding performance. Currently, SFE is widely used in a variety of fields, including toxicology, chemistry, the environment, textiles, petrochemicals, and polymers, in addition to the food and medicine industries.
- The extraction of natural plant materials utilizing this method of extraction has been pushed by significant advances in the field of supercritical fluid technology over the past three decades and has been characterized as an environmentally friendly technology. These natural sources could include, among others, plants, algae, and microalgae. Additionally, this method's objectives include excellent selectivity, quick extraction times, increased pollution prevention, and the use of harmless organic solvents. SFE is based on certain fluid characteristics like density, diffusivity, dielectric constant, and viscosity and typically entails changing specific parameters like pressure and temperature to achieve a supercritical fluid. Due to the similar density of an SF to that of a liquid under these circumstances, a fluid exists between a gas and a liquid.
- A dense fluid having intriguing properties intermediate between those of a gas and a liquid is referred to as a supercritical fluid when it is at temperatures and pressures above its critical point. Its viscosity and diffusivity are more like those of a gas, while its density is more like a liquid. Because of this, a supercritical fluid can function as a solvent much like a liquid, but with improved mass transfer kinetics.
- As a separation method, supercritical fluid chromatography (SFC) employs equipment that is nearly equivalent to that of high-performance liquid chromatography

(HPLC). Complex mixtures can be broken apart, and the quantity and occasionally the individual components' identities can be discovered. A high-pressure flow stream that sweeps the sample into a tube or column of small particles is fed with a solution of the sample. As the sample passes through the column, the various parts interact differently with the surface of the particles and are separated in time and space. The components pass through a detector as Gaussian or pseudo-Gaussian peaks that emerge from the column at various moments.

- The biggest departure from HPLC is the majority of the liquid mobile phase being replaced by a thick compressed gas, nearly always carbon dioxide (CO₂). CO₂ functions as a solvent under high pressures, such as those greater than 80 bar. A backpressure regulator is necessary on the system output since the mobile phase is a compressed gas, ensuring that it stays as a single density phase throughout the chromatograph. In turn, this necessitates operating some detectors at high pressures, such as a ultraviolet (UV) detector.
- Although it belongs to a different solvent family than hydrocarbons, CO₂ is a strongly nonpolar solvent. As a result, an organic modifier, commonly referred to as a cosolvent and most frequently an alcohol, is added to the mobile phase for more polar solutes. The typical elution pattern is a gradient from low to high modifier concentration. Peaks elute in order of increasing polarity.
- Considering that composition is coded from low to high polarity, SFC is often an normal phase approach. SFC, however, has a number of benefits over HPLC's normal phase. It is possible to infuse even aqueous-based substances, and equilibration happens incredibly quickly.
- Polar stationary phases are utilized with polar solutes. The traditional polar phases comprised amino, bare silica, cyano, and diol. New stationary phases have been created recently that are especially suited for SFC. Several ethylpyridines and a number of exclusive phases are among these phases. Reversed phase columns like C18, C8, C4, and methylare sometimes utilized for low polarity solutes.
- SFC can also be used to separate substances that are far less polar, such as a variety of natural products like lipids, carotenoids, and fat-soluble vitamins. The stationary phase in these sam

- plis often C18.

□ A phase diagram displays the ranges of temperature and pressure at which the material can exist as a solid, liquid, or gas. The borders of the phase areas are defined by curves where two phases (solid-gas, solid-liquid, and liquid-gas) coexist. For the solid-

gas, solid-liquid, and liquid-gas boundaries, respectively, these curves comprise sublimation, melting, and vaporisation. In addition to these binary existence curves, the triple point is a location where all three phases are simultaneously present and in equilibrium (TP).

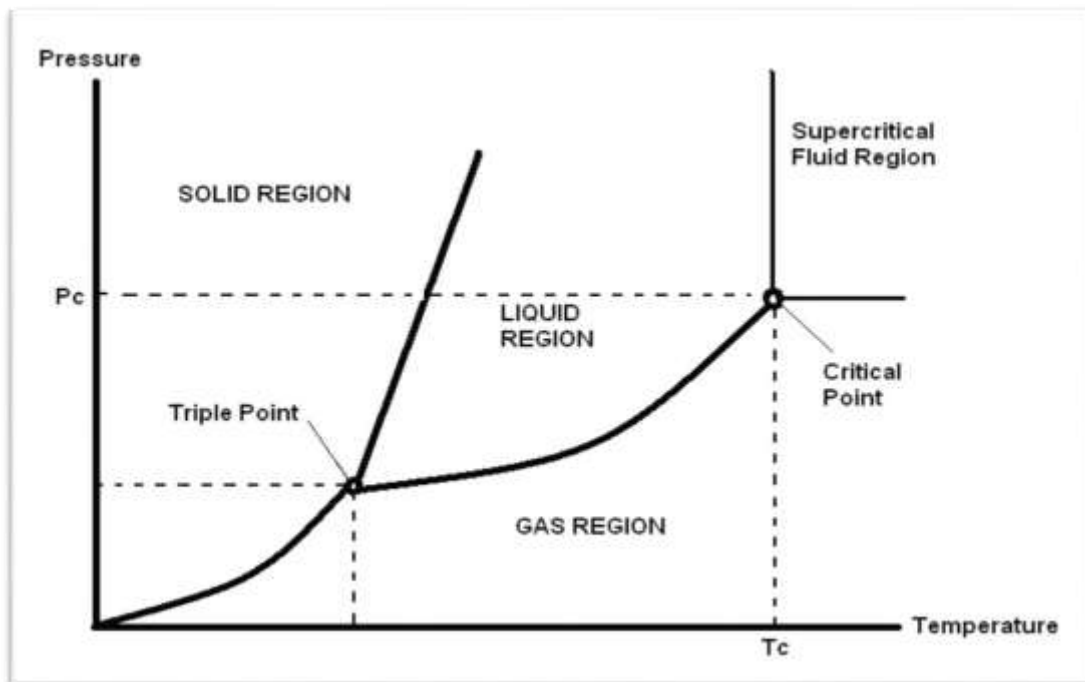


figure1: Schematic Representation of an Idealized Phase Diagram

- The critical point, another distinguishing feature in the phase diagram (CP). At critical pressure (P_c) and critical temperature (T_c), this point is reached (P_c). The material cannot change from the gas to the liquid or from the liquid to the gas phase after the CP, regardless of how much pressure or temperature is raised. The supercritical fluid form is this one. At this point, increasing pressure or temperature cannot cause a substance to convert into a gas or a liquid. The supercritical region is identified in the phase diagram as the region above the T_c and P_c values.

PRINCIPLE:^[2]

The pasteurization of bioactive compounds in food and medicine, which compounds would be destroyed by conventional thermal processes, is a process known as supercritical carbon dioxide technology (SC-CO₂ technology), which uses pressure along with carbon dioxide to kill microorganisms without affecting the nutritional value or

organoleptic attributes. The solubility of the target compound in the chosen solvent, which depends on how the solvent and solute interact, is the driving factor in any extraction process. Because it shortens the extraction process, uses fewer organic solvents, is suitable for substances that are sensitive to heat, produces cleaner extracts, and is less harmful to the environment than other alternative techniques, supercritical fluid extraction (SFE) has emerged as a superior method for extracting bioactive species from natural products. The solvating properties of are the foundation for SFE. The extractability of supercritical fluid can also be changed by properly manipulating SFE parameters, allowing this technology to find applications in a variety of fields, including food and pesticide research. Despite the fact that CO₂ is the preferred extraction solvent (for extracting non-polar chemicals), supercritical SC-CO₂ can have its polarity increased by adding a miscible polar modifier, such as ethanol. The SFE process involves selectivity, therefore the extracts produced using

this method have low concentration of undesirable chemicals. In addition, after depressurization, SC-CO₂ turns gaseous and is simple to remove from a flow system.

Deploy of SFC:^[5]

The van Deemter (Knox) equation defines how a chromatographic column behaves kinetically. This equation, in its most basic version, consists of three terms, each of which describes a different type of diffusion, see Equation:

$$H = A^{0.33} + \frac{B D_{1,2}}{\mu} + \frac{C d_p^2}{D_{1,2}}$$

Equation.1: The van Deemter (Knox) equation

[The terms B and C denote the contributions of axial and radial diffusion, respectively, and comprise the relationship between the linear velocity of the mobile phase, and the solute binary diffusion coefficient, D_{1,2}. Higher diffusion coefficients lead to higher optimum linear velocities, according to the term B. Higher diffusion coefficients in term C lead to less efficiency (lower flow rates)].

Pure CO₂ has diffusion coefficients that are 10 to 15 times quicker than those of water or other aqueous mixes. For instance, at 20 °C, benzoic acid has a diffusion coefficient in water of 1.0 x 10⁻⁵ cm² s⁻¹. At 100 pressure and 40 °C, benzoic acid has a diffusion coefficient of 16 x 10⁻⁵ cm² s⁻¹ in 100% CO₂, which decreases to roughly 9.5 x 10⁻⁵ cm² s⁻¹ at 300 bar. Similar results were obtained with a few slightly bigger dimethyl anilines, which showed diffusion coefficients between 7 and 12.5 x 10⁻⁵ cm² s⁻¹ between 150 and 350 pressure and 40 to 60 °C.

Physical properties of Supercritical Fluid:^[3]

- Density
- Diffusivity
- Viscosity

➤ Density:

A supercritical fluid has a density that falls between a liquid and a gas, but leans more toward a liquid. Pressure and temperature conditions play a major role in it: The density and solvating power of a supercritical fluid increase as pressure rises at constant temperature. On the other hand, a supercritical fluid's density and solvent strength decrease as temperature increases while maintaining a constant pressure. As a result, this is the primary quality and underlying cause of the

fluid's superior dissolving characteristics in the presence of a powerful solute molecule. Due to their greater density, supercritical fluids are also better transporters than gases. Density is therefore a crucial variable for analytical methods that use supercritical fluids as solvents.

➤ Diffusivity:

SCF viscosity is more sensitive to temperature than liquid viscosity. A supercritical fluid normally has a diffusivity that is higher than that of a liquid and lower than that of a gas. As a result, the solute may exhibit more diffusivity in a SCF as opposed to a liquid. Additionally, the effects of temperature and pressure are different: as the temperature rises, the diffusivity in a SCF increases and vice versa as the pressure rises. This characteristic increases SCF's suitability as a solvent for analytical purposes. Diffusivity is inversely correlated with pressure and parallel with temperature. Supercritical fluids have the potential to be faster carriers for analytical applications because of their higher diffusivity.

➤ Viscosity:

One of the thermophysical characteristics of a supercritical fluid is its viscosity. It is generally acknowledged to be lower, almost equal to that of a gas, and lower than that of a liquid. Due to the lower resistance than that of a liquid to a solute, this low viscosity increases the SCF's ability to penetrate materials. SCF viscosity is more sensitive to temperature than liquids. A supercritical fluid's viscosity is roughly 1/10 of that of a liquid, making it essentially identical to that of a gas. Supercritical fluids are hence less resistive to components moving through than liquids. The viscosity of supercritical fluids differs from that of liquids in that temperature has a much greater impact on the viscosity of supercritical fluids than on liquids.

Supercritical fluid chromatography:^[3]

Similar to how supercritical fluids combine the advantages of liquids and gases, SFC combines the benefits and key features of HPLC and GC. When analysing substances that break down at high temperatures with GC and lack functional groups that can be recognized by HPLC detection systems, SFC may be more useful than HPLC and GC. There are three major qualities for column chromatographies:

- Selectivity

- Efficiency
 - Sensitivity
- Due to variable mobile phases (particularly within a specific experimental run) and a variety of stationary phases, HPLC typically provides superior selectivity than

SFC. SFC has good quality in terms of sensitivity and efficiency even if it lacks the selectivity of HPLC. During the chromatographic process, SFC makes it possible to adjust a few characteristics. The analysis can be optimized thanks to this adjusting capability. SFC also offers a wider selection.

Instrumentation of SFC:^[4]

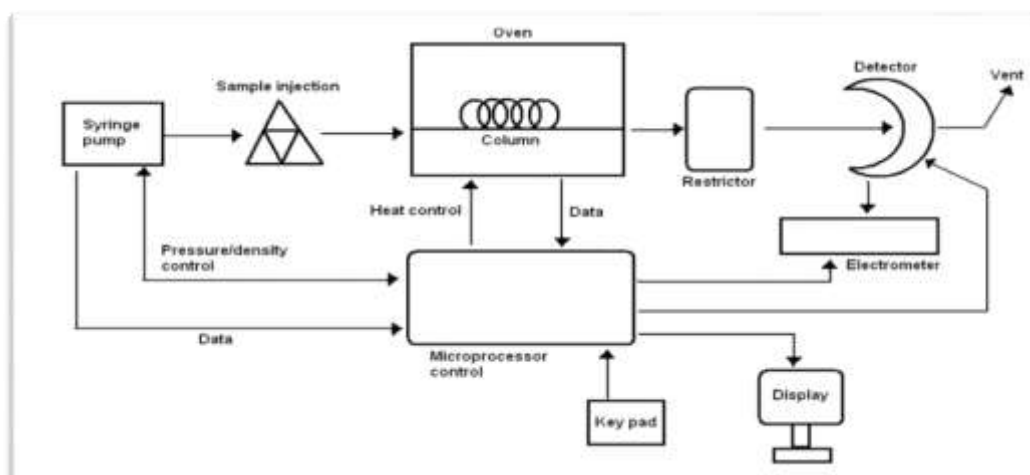


figure.2: Scheme of a supercritical fluid chromatography instrument^[3]

➤ It consists of the following components:

- A) Pumps
- B) Oven
- C) Injectors
 - Loop injector
 - Inline injector
 - In column injector
- D) Column
- E) Back pressure restrictor
- F) Detector

A) PUMPS:

The type of column used in SFC determines the high-pressure pump used. Syringe pumps are typically utilized for capillary SFC while reciprocating pumps are typically used for packed columns. Reciprocating pumps make it simpler to mix the mobile phase or add modifying fluids. Consistent pressure is provided by syringe pumps for a clean mobile phase.

B) OVEN:

The mobile phase needs to be precisely controlled for temperature, which calls for a thermostated column oven. Usually, standard GC or LC ovens are employed.

C) INJECTORS:

In SFC, injection is typically accomplished by opening an appropriate valve at the column entrance and transferring the contents of a sample loop into the carrier fluid. A typical HPLC injection system works well for packed column SFC, however for capillary column SFC, where sample volume is dependent on column diameter and fast injection of small sample quantities into the column is required, pneumatically driven valves are employed.

D) COLUMN:

The careful selection of stationary phases is essential because to the high solvating properties of the mobile phase in SFC. In SFC, packed and capillary analytical columns are essentially employed. Alumina, silica, polystyrene, and other absorbents as well as stationary phases insoluble in SC-CO₂ were used in earlier research. In more recent packed column studies, bonded nonextractable stationary phases such as octadecylsilyl (C18) or aminopropyl bound silica have been used as stationary phases. In terms of coating materials, SFC columns and HPLC columns are fairly comparable. In SFC, two different sorts of

columns are employed.

E) BACKPRESSURE RESTRICTOR:

In order to maintain the same column outlet pressure regardless of the mobile phase pump flow rate, this device uses a pressure-adjustable diaphragm or controlled nozzle to maintain the desired pressure in the column. It maintains the supercritical state of the mobile phase throughout the separation and frequently needs to be heated to avoid clogging. Either after the detector or at the end of the column is where the pressure restrictor is put. A 2-10 cm length of 5-19 capillary tubing attached to the column serves as the standard restrictor for a 50 or 100 m open tubular column. Alternately, the restriction can be an essential component of the column created by bringing the column's tip into the flame.

F) DETECTOR:

Mobile phases, either liquid or gas-like, are used in SFC. As a result, it works with GC and HPLC detectors alike. Traditional liquid-phase detectors like refractive index detectors, ultraviolet-visible spectrophotometric detectors, flame ionization detectors, and light scattering detectors have also been used for SFC. SFC can also be utilized well with mass spectrometry and Fourier transform infrared spectroscopy. The selection of detectors will be influenced by the mobile phase's composition, column type, flow rate, and resistance to the high SFC pressures. Regarding detectors, SFC has a significant advantage over HPLC. It is possible to use the flame ionization detector, which is typically part of a GC system, with SFC. The effectiveness of this detector can be improved.

Applications of Supercritical fluid chromatography:

- The use of SFE is based on the experimental finding that many gases have a tendency to dissolve more readily when compressed beyond a critical point. Different supercritical applications, often utilizing CO₂ gas, have been documented in some investigations. S. F. Liza and others (2010) investigated the viability of using the SFE method to extract lipid components from sorghum, such as tocopherols, phytosterols, and free fatty acids, and the potential health benefits of these substances. On the other hand, research has suggested that numerous spices, including oregano, rosemary, sage, and thyme, among others, may have antioxidant properties. Due to this, Cavero and

colleagues

(2006) investigated whether oregano leaves may serve as a source of antioxidants under a variety of extraction settings (various pressures, temperatures, and extraction times).

- SFE is now widely utilized in a variety of commercial processes, including coffee decaffeination, fatty acid refinement, and the extraction of flavours and essential oils from natural sources for use in nutraceuticals and functional foods. This technique is a significant improvement over the widely used organic solvent-based extraction techniques for biologically active substances. However, in order to create a good SFE, it is necessary to take into account a number of variables, such as SFs, raw materials, co-solvents, and extraction conditions for a specific molecule of interest in order to maximize the extraction. Additionally, it has been successfully demonstrated that SFE may be utilized to create stable and active compounds in microparticles and dry powders.
- Currently, supercritical extraction is regarded as a green alternative to conventional phase separation technology that avoids the majority of its drawbacks. Additionally, the use of organic modifiers such as methanol, isopropanol, and acetonitrile helps separate moderately polar molecules. But the use of this technology has progressively grown over the past few years, even though it is still at a reasonable level. This pattern clearly demonstrates current technical developments as well as the overall advantages of this separation method. Other natural substances than lipids have also been studied in foods of botanical origin, including polyphenols⁶¹, biogenic amines, and polyacetylenes. In addition, utilizing supercritical technology, many lipid classes have been examined simultaneously in bilberry⁶⁵ and a complex vegetable extract.
- SFC has a variety of uses in the food, environmental, and pharmaceutical industries. In this way, classes of substances such as pesticides, herbicides, polymers, explosives, and fossil fuels can all be studied. Antibiotics, prostaglandins, steroids, taxol, vitamins, barbiturates, non-steroidal anti-inflammatory drugs, and many more pharmacological components can all be analyzed using SFC. Numerous medicinal substances can be separated chiroselectively. Because carbon

dioxide, the most popular mobile phase in supercritical fluids, is ineffective at dissolving polar solutes, SF is mostly employed for non-polar molecules. SFC is employed in the petroleum sector for other hydrocarbon separations as well as the determination of total aromatic content analysis.

ADVANTAGES OF SUPERCRITICAL FLUID CHROMATOGRAPHY:

- Due to the decreased viscosity of supercritical fluids, which make SFC a faster procedure than HPLC, the SFC technique can combine the best features of GC and HPLC.
- This is made possible by the physical qualities of supercritical fluids, which exist between liquids and gases.
- Low viscosity promotes rapid flow of the mobile phase.
- SFC can be used to investigate some delicate materials that are susceptible to high temperatures because of the critical pressure of supercritical fluids.
- These substances could be molecules that break down at high temperatures or substances with low vapour pressure or volatility, including polymers and big biological molecules.
- High pressure situations give the opportunity to work at lower temperatures than are often required. Therefore, SFC analysis of the temperature-sensitive components is possible.

INTRODUCTION TO SFE:^[3]

Due to their special physical characteristics, which include densities, diffusivities, and viscosities that are different from those of liquids and gases, supercritical fluids can be used for extraction processes that liquids cannot carry out due to their high density,

and low diffusivity, and gases cannot carry out due to their insufficient density.

Complex mixtures with numerous components should undergo an extraction procedure before being chromatographically separated. The optimal extraction process should be quick, easy, and affordable. At the conclusion of the extraction, sample loss or decomposition shouldn't occur. There should be a quantifiable collection of each component after extraction. The trash produced from the extraction should ideally be kept to a minimum, be simple to dispose of, and not negatively impact the environment.

Unfortunately, conventional extraction techniques frequently fall short of these standards. SFE has a number of advantages over conventional methods in this regard.

The viscosity and diffusivity of the mobile phase affect the extraction speed. The component that needs to be extracted can easily pass through the mobile phase if it has a low viscosity and a high diffusivity. The components can be removed more quickly with supercritical fluids than with other methods due to their increased diffusivity and low viscosity when compared to regular extraction solutions. With SFE, an extraction procedure can be completed in about 10 to 60 minutes as opposed to hours or even days using traditional techniques.

INSTRUMENTATION OF SFE:^[3]

The equipment required for an SFE setup is straightforward.

The fundamental components of an SFE instrument are shown in Figure and consist of a reservoir of supercritical fluid, a pressure tuning injection unit, two pumps (to bring the components in the mobile phase into the extraction cell and send them out), and a collection chamber.

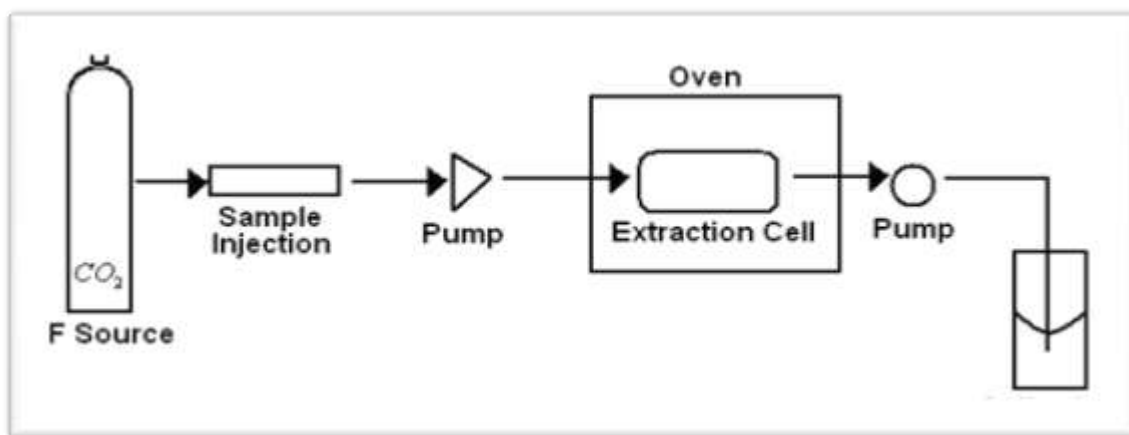


Figure 3: Scheme of an idealized supercritical fluid extraction instrument

The instrument can be operated in one of two main modes :

- Extracting static
- Extraction in motion

During the extraction process in dynamic extraction, the second pump that sends the material to the collection chamber is always open. Consequently, the mobile phase enters the extraction cell and extracts the constituents to remove them consistently.

There are two main steps in the static extraction experiment:

1. The extraction cell is filled with the mobile phase, which interacts with the sample.
2. The second pump is turned on, and the extracted materials are immediately removed. The polarity and solubility of the materials in the mobile phase are two factors taken into account while selecting the mobile phase for SFE.

The polarity and solubility of the materials in the mobile phase are two factors taken into account while selecting the mobile phase for SFE. For SFE, carbon dioxide is the most prevalent mobile phase. Alkanes and other non-polar compounds can be dissolved by it. Carbon dioxide may be employed as a single component mobile phase for semi-polar substances such as polycyclic aromatic hydrocarbons, aldehydes, esters, alcohols, and other similar substances. However, polar solvents like methanol must be added to supercritical carbon dioxide in order to modify it for substances that have polar properties (CH₃OH). These additional solvents may be added to the system using a different injection pump.

EXTRACTION MODES:^[3]

In terms of gathering and detecting the components, there are two modes:

1. Extraction done offline
2. A web-based extraction

Offline extraction is carried out by removing the mobile phase and directing it toward the collection chamber with the extracted components. The components are now either captured in a solution or a handy adsorption surface as the supercritical fluid phase evaporates and is discharged to the atmosphere. The retrieved pieces are then cleaned up and made ready for a separation technique. Errors may result from the additional manipulation step between the extractor and the chromatography device.

The online approach is more accurate because it transfers all extracted elements without removing them from the mobile phase to a separation unit, often a chromatography device. There is no further sample preparation required for this separation procedure in this extraction/detection type. By doing this, manipulation-related errors are reduced to a minimum. Additionally, there is no sample loss, and sensitivity is raised.

APPLICATIONS OF SFE:^[3]

- Polymers, oils and lipids, carbohydrates, pesticides, organic pollutants, volatile toxins, polyaromatic hydrocarbons, biomolecules, foods, tastes, pharmaceutical metabolites, explosives, and organometallics are just a few examples of the wide variety of materials that can be treated using SFE.
- The pharmaceutical and biochemical industries, the polymer industry, industrial synthesis and extraction, natural product chemistry, and the food industry are a few examples of common industrial applications.
- Oils and fats, pesticides, alkanes, organic pollutants, volatile toxins, herbicides, nicotine, phenanthrene, fatty acids, and aromatic surfactants have all been found in samples ranging from clay to petroleum waste, from soil to river sediments, as examples of materials that have been analyzed in environmental applications.
- In food analyses, substances such as caffeine, peroxides, oils, acids, cholesterol, and samples of coffee, lemon, olive oil, cereals, wheat, potatoes, and dog food are removed.
- The materials that are recovered through industrial use range from stabilizers to petroleum fractions to additives, various oligomers, and additives.
- Samples such as plastic, PVC, paper, and wood are evaluated.

II. CONCLUSION:

This method is unique and sensitive. SFC is still an underutilized resource for chromatography. The benefits of utilizing mobile phases made of supercritical fluids show how resolution can be raised without compromising time or lengthening the column. Supercritical fluid chromatography uses supercritical fluids and their distinct physical characteristics to outperform other related techniques in the chromatography and extraction fields.

REFERENCE:

- [1] Dr. B. K. Sharma, "Introduction to

- Spectroscopy”, instrumental method of chemical analysis, **1972**,3-67
- [2] Tariq Ahmad, et.al, “Supercritical Fluid Extraction: A Review”, *J. Biol.Chem. Chron*, **2019**, 5(1),114-122
- [3] Pavan.M.V.Raja. Raja, Andrew R. Barron, “Basic Principle of Supercritical Fluid Chromatography and Supercritical Fluid Extraction”, *Libretexts chemistry*, **2022**, 3.3.1-3.3.6
- [4] Preeti Gopaliya, Priyadarshani R Kamble, “A Review Article on Supercritical Fluid Chromatography”, *International Journal of Pharma Research & Review*, **2014**, 3(5), 59-66
- [5] Terry A. Berger, “Supercritical Fluid Chromatography”, *Agilent Technologies*, **2015**, 1-170
- [6] Raffaele Romano, Alessandra Aiello et.al, “Bioactive Compounds Extracted by Liquid and Supercritical Carbon Dioxide from Citrus Peels”, *International Journal of Food Science and Technology*, **2022**, 57, 3826-3837
- [7] Shuisheng He, Andrea Pucciarelli et.al, “Study of Fluid-To-Fluid Scaling for Upward Pipe Flows of Supercritical Fluids Using Direct Numerical Simulation”, *International Journal of Heat and Mass Transfer*, **2022**, 189, 1-17
- [8] Michela Antonelli, Michal Holcapek et.al, “Ultrahigh-Performance Supercritical Fluid Chromatography- Mass Spectroscopy for The Qualitative Analysis of Metabolites Covering a Large Polarity Range”, *Journal of Chromatography A*, **2022**, 1665, 1-12
- [9] Jinwen Shi, Hui Jin et.al, “Numerical Simulation on Natural Convection and Temperature Distribution of Supercritical Water in A Side-Wall Heated Cavity”, *the journal of supercritical fluids*, **2022**, 181, 1-13
- [10] Maria Victoria Alvarez-Henao, Liliana Cardona, “Supercritical Fluid Extraction of Phytosterols from Sugarcane Bagasse: Evaluation of Extraction Parameters”, *the journal of supercritical fluids*, **2022**, 179, 1-10
- [11] Carlos Garcia-carnelli, Ines Carrera, “A Comparative Study of Supercritical Fluids and Ethanol Extracts of Cannabis Inflorescences: Chemical Profile and Biological Activity”, *the journal of supercritical fluids*, **2022**, 179, 1-10
- [12] Chaowalit Monton, Natawat Chankana et.al, “Optimization of Supercritical Carbon Dioxide Fluid Extraction of Seized Cannabis and Self-Emulsifying Drugs Delivery System for Enhancing the Dissolution of Cannabis Extract”, *the journal of supercritical fluids*, **2022**, 179, 1-10