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Supercritical Fluid Chromatography: A- Review

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Abstract

A version of the normal phase chromatography technique, supercritical fluid chromatography (SFC) has been in use since 1962. Since carbon dioxide is usually used as the mobile phase in SFC, it is necessary to pressurise the whole chromatographic flow route. The supercritical phase is a condition where the characteristics of liquids and gases converge, which is why supercritical fluid chromatography is also known as "convergence chromatography." One of the most significant column chromatography techniques, after gas chromatography (GC) and high-performance liquid chromatography (HPLC), is supercritical fluid chromatography. The desirable qualities of both gas and liquid states are brought together in supercritical fluids. Density, diffusivity, and viscosity are the defining characteristics of a supercritical fluid. In supercritical fluid chromatography (SFC), a supercritical fluid carries the sample down a separating column, where it is separated into distinct bands according to the degree of interaction between the analytes and the stationary phase. Identities and amounts of these bands are determined by a detector when they exit the column. Because the mobile phase behaves as a liquid when it is below its critical temperature and above its critical pressure, the technique is called liquid chromatography (LC), and when it is above its critical temperature and below its critical pressure, the mobile phase acts as a gas, making SFC a hybrid of gas and liquid chromatography. The equipment needed for supercritical fluid chromatography is compatible with several detectors, making it very adaptable. The list of items that have found a use for SFC is long and varied, and includes things like natural goods, medications, foods, herbicides, surfactants, polymers, polymer additives, petroleum, explosives, and propellants.

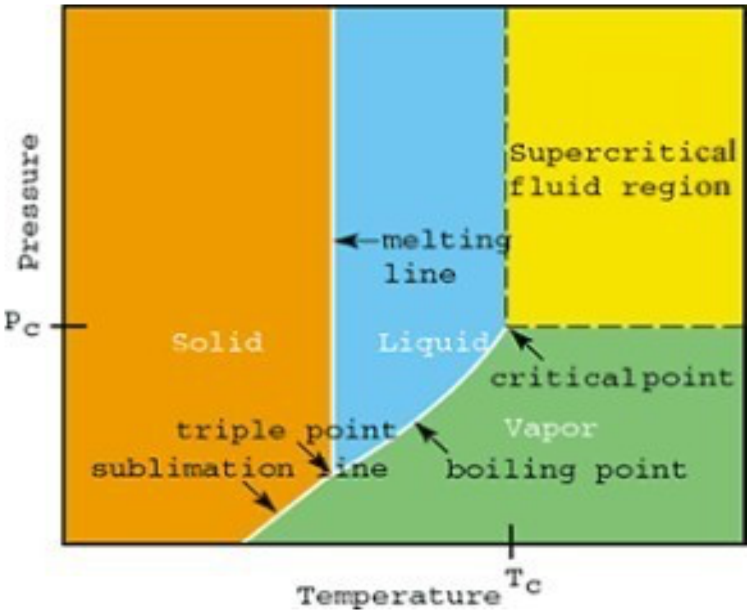
Keywords: Critical pressure, critical temperature, supercritical fluid, diffusivity

Introduction

For the purpose of analysing and purifying molecules with low to intermediate molecular weights that are thermally labile, a technique known as supercritical fluid chromatography (SFC) was first used in 1962. Additionally, chiral chemical separation is a possible use. Although the principles are similar to those of high performance liquid chromatography (HPLC), the pressurisation of the whole chromatographic flow route is necessary for SFC since carbon dioxide is usually used as the mobile phase. The supercritical phase is a condition where the characteristics of liquids and gases converge, which is why supercritical fluid chromatography is also known as "convergence chromatography." Supercritical fluids (SF) are a kind of fluid with improved diffusion characteristics, reduced viscosities, and a dissolving capacity comparable to certain liquids. So, similar to mobile phases in gas chromatography (GC) and solvents in liquid chromatography (HPLC), SF used as mobile phases in chromatography should transport compounds and dissolve them. Supercritical fluid chromatography (SFC) describes this variation of the chromatographic process.

In 1962, the process of isolating porphyrin derivatives that are sensitive to temperature changes was detailed utilising supercritical chlorofluoromethanes at pressures of up to 140 bar and temperatures ranging from 150 to 170◦C. In the 1960s, several researchers expanded on this approach, refining it conceptually and empirically. While HPLC was experiencing its tumultuous expansion at the same time, the development of SFC during this era was disappointingly not similar. Therefore, SFC's first significant boom era was in the 1980s, some 20 years later.

The state of a material when its critical temperature and pressure have been reached is known as its supercritical fluid phase. The desirable qualities of both gas and liquid states are brought together in supercritical fluids. From one angle, their actions are similar to those of a gas, while from another, they are more like those of a liquid. As it fills a container and conforms to its shape, a supercritical fluid imparts a gas-like quality. A dynamic equilibrium causes a supercritical fluid to develop. A dynamic equilibrium is created when a material is heated to its critical temperature in a closed system, meaning that the pressure remains constant. The number of molecules entering the gas phase from the liquid phase and the number of molecules leaving the gas phase from the liquid phase are equal in this equilibrium. Here, supercritical material takes shape, and the phase curve that separated the gas and liquid phases vanishes.A phase diagram is used in order to define SF.



At various temperatures and pressures, a phase diagram displays the material's many states as a solid, liquid, or gas. The borders of the phase regions are defined by certain curves that include two phases simultaneously, such as solid-gas, solid-liquid, or liquid-gas. For instance, the solid-gas boundary curve includes sublimation, the solid-liquid boundary curve includes melting, and the liquid-gas boundary curve includes vaporisation. Beyond these two-state existence curves, there exists a third state, Triple Point (TP), where equilibrium exists among all three.

Table 1: Comparison of densities, viscosities and diffusivities for liquid, supercritical fluid and gas

State	Density (g/ml)	Viscosity (poise×10)	Diffusivity (cm2/s×103)
Liquid	0.8-1	3-24	0.005-0.02
Sup.fluid	0.2-0.9	0.2- 1	0.01-0.3
Gas	0.001	0.05-0.35	10-1000

Physical Properties of Supercritical Fluids

Density

The density of a supercritical fluid lies between that of a gas and a liquid, closer to that of a liquid. Within the supercritical zone, the density of a supercritical fluid exhibits an increase as the pressure rises while maintaining a constant temperature. Under constant pressure conditions, the density of a material drops as the temperature increases.

The dissolving ability of a supercritical fluid is contingent upon its density. Supercritical fluids are superior transporters compared to gases due to their greater density. Hence, density plays a crucial role in analytical procedures that use supercritical fluids as solvents.

Diffusivity

The diffusivity of a supercritical fluid may be 100 times greater than that of a liquid and 1,000-10,000 times lower than that of a gas. Supercritical fluids have higher diffusivity compared to liquids, resulting in improved solute diffusivity in supercritical fluids compared to liquids. Diffusivity is directly proportional to temperature and inversely proportional to pressure. Elevated pressure causes supercritical fluid molecules to undergo more intermolecular interactions, resulting in reduced diffusivity within the substance. Supercritical fluids, due to their higher diffusivity, have the ability to transport substances more quickly, making them advantageous for analytical purposes. Supercritical fluids are crucial for chromatography and extraction techniques.

Viscosity

The viscosity of a supercritical fluid is comparable to that of a gas and about one-tenth that of a liquid. Supercritical fluids exhibit lower resistance compared to liquids when it comes to the flow of components through them. The viscosity of supercritical fluids differs from that of liquids in that temperature has a little impact on liquid viscosity, but it may significantly affect the viscosity of supercritical fluids. These three significant qualities are interconnected. Variances in temperature and pressure may impact each of them in many permutations. Increasing pressure leads to an increase in viscosity, whereas increasing viscosity leads to a decrease in diffusivity.

Supercritical Fluid Chromatography (SFC)

SFC is a chromatography technology that follows HPLC and GC in terms of column separation methods. Supercritical fluid chromatography (SFC) offers greater benefits compared to high-performance liquid chromatography (HPLC) and gas chromatography (GC) in the analysis of chemicals that undergo decomposition at high temperatures with GC and lack functional groups that can be recognised by HPLC detection systems. SFC allows for the modification of some characteristics while the chromatographic process is taking place. This capability to adjust the tuning gives a benefit in order to optimise the analysis. In addition, SFC has a wider array of detectors compared to HPLC. Column chromatography has three primary characteristics:

- Selectivity
- Efficiency
- Sensitivity

GC has superior efficiency and sensitivity. HPLC has superior selectivity due to its ability to use interchangeable mobile phases and a wide range of stationary phases. While SFC may not possess the same level of selectivity as HPLC, it does exhibit commendable sensitivity and efficiency.

The mobile phases used in chromatography, namely in gas chromatography (GC) and liquid chromatography (HPLC), should serve as transporters for chemicals and also have the ability to dissolve these molecules.

The specific kind of chromatography being referred to is called supercritical fluid chromatography (SFC).In 1962, they explained the process of separating thermo-labile porphyrin derivatives using supercritical chlorofluoromethanes at pressures ranging from 0 to 140 bar and temperatures between 150 and 170◦C. Subsequently, in the 1960s, other researchers made more advancements to this strategy, both in terms of theory and experimentation.Regrettably, the progress of SFC during this era did not match the rapid expansion of HPLC, which took place at the same time. The significant phase of expansion for SFC happened about 20 years later, namely in the 1980s.

Instrumentation for SFC

The configuration of SFC instruments closely resembles that of HPLC devices in terms of equipment. Specifically, they use comparable stationary phases with analogous column types. There are some disparities. Supercritical fluids are very sensitive to temperature, hence it is essential to include a heat management mechanism in the system, similar to what is found in a gas chromatography (GC) system. Additionally, it is crucial to include a pressure control device, such as a restrictor, since maintaining the desired amount of pressure is a vital factor for handling supercritical fluid materials.

Pumps

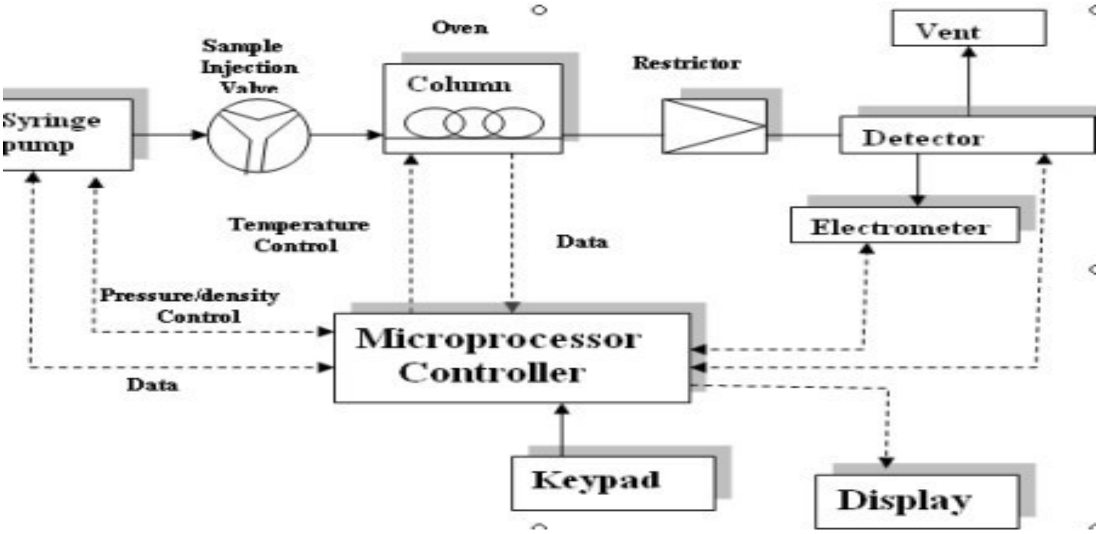
The selection of the high-pressure pump utilised in SFC is dependent on the kind of column being used. Reciprocating pumps are often used for packed columns, whilst syringe pumps are usually applied for capillary SFC. Reciprocating pumps facilitate the blending of the mobile phase or the addition of modifying fluids. Syringe pumps provide a steady pressure to maintain a tidy mobile phase.

Injector

Injection in Supercritical Fluid Chromatography (SFC) is typically accomplished by diverting the contents of a sample loop into the carrier fluid at the entry of the column using an appropriate valve. For a packed column in Supercritical Fluid Chromatography (SFC), a typical High Performance Liquid Chromatography (HPLC) injection system is sufficient. However, for a capillary column in SFC, the sample volume is determined by the diameter of the column, and tiny sample quantities need to be injected rapidly into the column. Therefore, pneumatically driven valves are used.

Oven

A thermo stated column oven is required for precise temperature control of the mobile phase. Conventional GC or LC ovens are generally used.



Columns

The high solvating capacity of the mobile phase in SFC necessitates the meticulous choice of stationary phases. There are two primary kinds of analytical columns often used in Supercritical Fluid Chromatography (SFC): packed columns and capillary columns. Prior research used absorbents such as alumina, silica, or polystyrene, or stationary phases that are not soluble in supercritical carbon dioxide (SC-CO2). Recent research on packed columns has focused on using non-extractable stationary phases, such as octadecylsilyl (C18) or aminopropyl bound silica.

SFC columns closely resemble HPLC columns in terms of their coating ingredients. There are two distinct sorts of column that are used in SFC.

- Open tubular columns
- packed columns

Open tubular

Ones are more favoured and they have commonalities with HPLC fused-silica columns. These columns are equipped with an interior covering of cross-linked siloxane substance, which serves as the stationary phase. The thickness of the coatings might range from 0.05 to 1 μm. The length of such columns might vary between 10 and 20 metres.

Packed Columns

Similar to HPLC columns (10, 5, or 3 □m porous particles) Silica based chemically bonded phases Typically 10 cm long X 4.6 mm.

Restrictor or Back-Pressure Device

This device is used to regulate the pressure in a column by means of a pressure-adjustable diaphragm or controlled nozzle. Its purpose is to maintain a consistent outlet pressure in the column regardless of the flow rate of the mobile phase pump. The mobile phase is maintained in a supercritical state throughout the separation process and may need to be heated to avoid blockages. The pressure restrictor is positioned either downstream of the detector or at the outlet of the column.

A common kind of restrictor used for a 50 or 100 μm open tubular column is a 2-10 cm section of 5-19 capillary tubing that is connected to the column. Alternatively, the constraint may be an inherent component of the column created by pulling down the end of the column in the flame.

Microprocessor

Typically, commercial equipment for Supercritical Fluid Chromatography (SFC) are fitted with one or more microprocessors to regulate factors such as pumping pressures, oven temperature, and detector performance.

Detector

SFC employs mobile phases that may be either liquid or gaseous. As a result, it is compatible with both HPLC and GC detectors. Traditional gas-phase detectors, such as flame ionisation detectors and flame photometric detectors, as well as liquid-phase detectors including refractive index detectors, ultraviolet-visible spectrophotometric detectors, and light scattering detectors, have been used for SFC. SFC can successfully use mass spectrometry and Fourier transform infrared spectroscopy. The selection of detectors will be contingent upon the composition of the mobile phase, the kind of column, the flow rate, and the capacity to endure the elevated pressures of SFC. One major benefit of SFC compared to HPLC is in the area of detectors. The flame ionisation detector often used in gas chromatography setups may also be used in supercritical fluid chromatography. This detector can enhance the accuracy and effectiveness of SFC analyses due to its exceptional characteristics. The FID is an extremely sensitive detector. It is non-disruptive and this is crucial for analytical operations. Superfluid chromatography (SFC) may be more readily integrated with mass spectrometry, ultraviolet spectroscopy, and infrared spectroscopy compared to high-performance liquid chromatography (HPLC). Additional detectors that may be used with HPLC include fluorescence emission spectrometers and thermionic detectors, which can also be connected to SFC.

Mobile phases

There is a diverse range of materials available for use as the mobile phase in SFC. The mobile phase may be chosen from many classes of solvents, including inorganic solvents, hydrocarbons, alcohols, ethers, and halides. Alternatively, it might consist of specific solvents such as acetone, acetonitrile, or pyridine.

Carbon dioxide is the most often used supercritical fluid in SFC due to its easily attainable critical temperature and pressure. Additionally, carbon dioxide is inexpensive, readily available, resistant to ultraviolet radiation, non-toxic, and an effective solvent for non-polar compounds. In addition to carbon dioxide, other substances such as ethane, n-butane, N2O, dichlorodifluoromethane, diethyl ether, ammonia, and tetrahydrofuran may be used.

Table 2: Properties of some solvents as mobile phase at the critical point

Critical Temperature (°C)	Critical Pressure (bar)
Carbon dioxide	31.1 72
N2O	36.5 70.6
Ammonia	132.5 109.8
Ethane	32.3 47.6
n-Butane	152 70.6
Diethyl ether	193.6 63.8
Tetrahydrofuran	267 50.5

Effect of Pressure

The principle of separation in SFC relies on the density of the supercritical fluid, which is directly related to its solvating ability. As the pressure in the system rises, the density of the supercritical fluid also increases, resulting in an increase in its solvating power. As pressure varies in SFC, the retention of analytes is significantly affected, resulting in a shorter elution time for the eluent. This phenomenon is universal and comparable to the controlled temperature in gas chromatography (GC) or the gradual change in eluent composition in high-performance liquid chromatography (HPLC).

Comparison of SFC with Other Types of Chromatography

Supercritical fluid chromatography (SFC) incorporates some attributes from both gas and liquid chromatography, since the physical properties of supercritical fluids (SCFs) lie between those of gases and liquids. Similar to gas chromatography (GC), supercritical fluid chromatography (SFC) is naturally quicker than liquid chromatography (LC) due to the decreased viscosity, which allows for larger flow rates. The diffusion rates of supercritical fluids (SCFs) are moderate, falling between those of gases and liquids. Band broadening is more pronounced in supercritical fluids (SCFs) compared to gases, but less than in gases. Therefore, the moderate rates of diffusion and viscosity of supercritical fluids (SCFs) lead to quicker separation compared to liquid chromatography (LC), while also resulting in less spreading of zones compared to gas chromatography (GC). The mobile phases serve distinct functions in gas chromatography (GC), liquid chromatography (LC), and supercritical fluid chromatography (SCF). In gas chromatography (GC), the mobile phase is responsible for the mobility of the analyte zone. In liquid chromatography (LC), the mobile phase carries the solute molecule and also interacts with it, so affecting the selectivity. When a molecule is dissolved in a supercritical media, the process is similar to volatilisation but occurs at a far lower temperature compared to that of gas chromatography (GC). Therefore, at a certain temperature, the vapour pressure of a big molecule in a supercritical fluid (SCF) may be 10^10 times higher compared to when that fluid is not present. Therefore, it is possible to separate and remove high molecular weight chemicals, thermally unstable species, polymers, and big biological molecules from a column at a very low temperature. SFC has a significant advantage over GC in its capability to isolate thermally unstable chemicals. This is highly valued in the pharmaceutical industry since around 20% of all medication candidates belong to this group. In contrast to gas chromatography (GC), the selectivity in supercritical fluid chromatography (SFC) may be altered by modifying the mobile phase.

SFC in chromatographic techniques

SFC has the potential to be beneficial for a range of applications.The technique's primary distinction is in its capacity to change selectivity by adjusting the parameters of pressure (P) and temperature (T), rather than altering the chemical makeup of the eluent. The mobile phase's low viscosity allows for the assembly of many HPLC-type columns in a series. SFC can analyse a variety of chemicals, such as lipids and oils, emulsifiers, oligomers, and polymers. These molecules have a molecular mass larger than 1000, making them unsuitable for study with GC. SFC has enhanced velocity and effectiveness in comparison to HPLC. Supercritical carbon dioxide may be used as a mobile phase to easily connect with a mass spectrometer, infrared spectrophotometer, and NMR spectrometer.

Advantages of Working with SFC

- The unique physical characteristics of supercritical fluids, which lie between those of liquids and gases, allow the SFC method to effectively integrate the advantageous features of both HPLC and GC. The reduced viscosity of supercritical fluids allows SFC a much quicker technique compared to HPLC. Reduced viscosity results in increased flow velocity for the mobile phase.
- Supercritical fluids, which are capable of reaching high pressures, may be analysed using SFC to determine the critical pressure. This method is particularly useful for studying delicate materials that are sensitive to high temperatures. These materials may include chemicals that undergo decomposition at high temperatures, substances with low vapour pressure or volatility, polymers, and big biological molecules. Under high pressure settings, it is possible to operate with lower temperatures than what is typically required. As a result, temperature-sensitive components may be analysed using Supercritical Fluid Chromatography (SFC).
- The diffusion of components in a supercritical fluid is greater than in HPLC due to the increased diffusivity of supercritical fluids compared to liquids. This enhances the dispersion in the mobile phase and improves the separation at the end.

Drawbacks

There have been a few technical issues that have limited adoption of SFC technology,

- The first factor to consider is the high pressure working circumstances. High-pressure vessels are costly and large in size, requiring the use of specific materials to prevent the deterioration of gaskets and O-rings when exposed to supercritical fluids.
- Another disadvantage is the challenge of sustaining pressure, especially in terms of backpressure control. While liquids have a constant density regardless of pressure because they are almost incompressible, supercritical fluids are extremely compressible and their physical characteristics, such as the pressure drop across a packed-bed column, vary with pressure.At present, automatic backpressure regulators are capable of ensuring a consistent pressure in the column, regardless of any fluctuations in the flow rate, therefore reducing this issue.
- Another disadvantage is the challenge of separating gas and liquid during the process of collecting the product. When the pressure decreases, the CO2 quickly changes into a gas and causes any dissolved analyte to convert into aerosol particles. Cyclone separators have mitigated challenges in the separation of gases and liquids.

Applications of SFC

Applications exist for food, environmental, and medicinal items. Additionally, insecticides, herbicides, polymers, explosives, and fossil fuels are other categories that may be used. This approach allows for the analysis of a diverse range of pharmacological molecules, including antibiotics, prostaglandins, steroids, taxol, vitamins, barbiturates, and non-steroidal anti-inflammatory drugs. Chiral separations may be performed on a wide range of medicinal substances. Supercritical fluid chromatography (SFC) is mostly used for non-polar chemicals because to the limited ability of carbon dioxide, the most often utilised supercritical fluid mobile phase, to efficiently dissolve polar solutes. SFC may be used in the petroleum sector for tasks like as analysing the total aromatic content or separating different hydrocarbons.

This technique is used for the examination and refinement of molecules with low to intermediate molecular weight that are susceptible to heat degradation. Additionally, it may be used for the isolation of chiral substances. SFC is largely used in industry for the separation of chiral compounds and utilises the same columns as normal HPLC systems. SFC is now a prevalent method used for the separation and purification of substances that lack chirality in the pharmaceutical sector.

Applications in the material and polymer industry

Supercritical fluids are widely used in the material and polymer sector. Commercially, the process of precipitating solids is achieved by rapidly expanding supercritical solutions via an aperture or nozzle. This approach involves the quick depressurisation of a solute that is dissolved in a supercritical fluid. By meticulously managing the operational parameters, one may get the desired shape of the precipitate. The gas anti-solvent procedure involves the fast addition of a supercritical fluid to a solution containing a dissolved crystalline solid in an organic solvent. Due to the solute's restricted solubility in the fluid, the supercritical fluid functions as an anti-solvent, causing solid crystals to precipitate. The particle size distribution of final crystals may be precisely manipulated by adjusting the density of the fluid. Another method involves the precipitation of a substance using a compressed fluid anti-solvent. This technique involves the spraying of a solution via a nozzle into a compressed fluid. The solvent quickly diffuses into the supercritical fluid, causing the solution to expand and the solute to precipitate. This technique has been used in commercial applications to produce nanoscale, uniformly sized microspheres made of polymers. Supercritical fluid carbon dioxide is now being used commercially to create foamed products. Supercritical fluids lower the glass transition temperature of the polymer, allowing polymer foams to be created at ambient temperature by immediately introducing the supercritical fluid into the extruder.

Food applications

Carbon dioxide is the predominant supercritical fluid used in the food business. Because of its non-toxic nature and low critical temperature, it may be used for extracting thermally unstable food components without contaminating the final product with any leftover solvents. Moreover, the colour, content, odour, and texture of the extracts may be controlled, and the use of supercritical fluid carbon dioxide for extraction preserves the scent of the product. Supercritical carbon dioxide extraction is used as a substitute for hexane in the process of obtaining soybean oil. It has also been examined for its efficacy in extracting oil from maize, sunflower, and peanuts. Supercritical fluid extraction offers a clear benefit by not only replacing oils but also extracting oils with reduced levels of iron and free fatty acids. Ongoing research is being conducted to meet market demand for "lighter" meals by using supercritical extraction to remove oils from potato chips and other snack foods. Furthermore, supercritical carbon dioxide has been used for the extraction of lilac, essential oils, black pepper, nutmeg, vanilla, basil, ginger, camomile, and cholesterol. Extensive research has focused on using supercritical carbon dioxide to decaffeinate coffee. Therefore, it is unsurprising to see that this was the first procedure to be brought into commercial use (in 1978), with its main stage being supercritical extraction. Dehydrated carbon dioxide is not efficient in extracting caffeine from dehydrated coffee. Therefore, it is necessary to moisten the coffee beans with water beforehand. An immersion period of around 2 hours is required for optimal extraction of caffeine from coffee beans using supercritical carbon dioxide. The decaffeination of coffee is carried out by many commercial procedures, such as the Kraft General Foods method in the USA and numerous methods in Germany.

Pharmaceutical applications

Supercritical fluid carbon dioxide has been widely used in the pharmaceutical sector due to its critical role in removing residual solvents from recovered materials. Extensive research has been conducted on the process of extracting vitamin E from soybean oil and developing a purification technique for this vitamin. The latter procedure circumvents the need for vacuum distillation, which often leads to the thermal decomposition of the product. The solubilities and recrystallization of several pharmaceuticals have been proven in supercritical fluids.

Environmental applications

Supercritical fluids are used as substitutes for dangerous compounds like hexane due to stringent environmental laws. Supercritical fluid extraction has been suggested as a viable method for soil remediation and the regeneration of activated carbon. More than 99% of the bulk of organic substances may be eliminated from polluted soil. Extracted organic compounds that have been effectively obtained include polycyclic aromatic hydrocarbons (PAHs), polychlorinated biphenyls (PCBs), dichlorodiphenyltrichloroethane (DDT), and toxophene. Carbon dioxide has been used in conjunction with entrainers to extract highly polar chemicals. CF Systems Corporation, USA, has developed a commercial method for extracting oils from refinery waste and polluted land. Chelating agents that can dissolve in carbon dioxide have been created for the purpose of extracting heavy metals from soil.

Conclusion

A supercritical fluid is a substance that is at a temperature and pressure above its critical point, where it exhibits unique properties that are intermediate between those of a gas and a liquid. Chromatography is a set of procedures that use supercritical fluids and their distinct physical characteristics to outperform other methods in the disciplines of chromatography and extraction. Occasionally, these approaches are used as alternate instrumental analytical methods, while in other instances, they serve as complementing counterparts in binary systems. The efficacy of an analytical approach in addressing practical issues is indicative of its utility that may be derived.

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