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Supercritical Fluid Extraction: Applications to Biological System.

Vidya Pradhan[1]*, Mohd Moaviyah Moghal[2], Vishal Ladniya[2] and Samreen Fatema[2].

[1]Dr. Rafiq Zakaria College for Women, Navkhanda, Aurangabad, Maharashtra, India.
[2]Post Graduate and Research Center, Maulana Azad College, Aurangabad, Maharashtra, India.

ABSTRACT

Supercritical fluid extraction is the most successful and well-organized way to extract precious component. Supercritical Fluid Extraction is a separation technique in which one component (the extractant) is separated from another component (the matrix) using supercritical fluids. In supercritical fluid extraction technique carbon dioxide is commonly used as supercritical fluid. Carbon dioxide is non toxic, eco friendly, inexpensive and its critical temperature is also low, all these things make it the best extracting solvent. Supercritical fluids are highly compressed gases, which have combined properties of gases and liquids in an intriguing manner. Supercritical fluids can show the way to reactions, which are not easy or even impractical to achieve in conventional solvents. Supercritical fluid extraction is a rapid process compared to conventional extraction methods. It can be completed in 10 to 60 minutes. After extraction supercritical fluid can be easily separated from analyte by simply releasing pressure, leaving almost no trace and provides a pure extract. Supercritical fluid extraction is one of the best analytical tools which have many biological applications. In this review paper information about Supercritical fluid and Supercritical fluid extraction with its biological applications is provided.

Keywords: SCF, SFE, Applications

Corresponding author

INTRODUCTION

Supercritical Fluid

A fluid is called supercritical, when its temperature and pressure go over their respective critical value (critical temperature and critical pressure). It exhibits good solvent power where distinct liquid and gas phases do not exist [1]. The critical temperature of a gas is the temperature at and above which it cannot be liquefied by pressure alone. All substances have a critical temperature. The critical pressure is the pressure of a gas or vapor in its critical state or the critical pressure of a gas or vapor is the pressure required to liquefy a gas at its critical temperature. Supercritical fluid(SCF) dyeing is an attention-grabbing alternative to the conventional aqueous process due to its environmental benefits [2]. SCF are able to Pass through solids like a gas and dissolve materials like a liquid. Furthermore, close to the critical point, small change in pressure or temperature result in large changes in density, allowing many properties of a supercritical fluid to be "fine-tuned". SCF are suitable alternative choice for organic solvents [3]. A SCF is a condition where substance is compressible and acts like a gas (i.e. it fills and occupies the shape of its container), It is not possible when it is in a liquid state (an incompressible fluid that occupies the bottom of its container). SCF has the typical density of a liquid and its characteristic dissolving power. That is why we cannot define the SCF as a liquid or as a gas. This is a new state of matter in principle [4].

History of Supercritical Fluid

It was Baron Charles Cagniard de la Tour who discovered SCF in 1822. He conducted an experiment to prove that there is a critical temperature above which a single substance can only exist as a single fluid and not as either liquid or gas. During experiments, he heated various solvents sealed within metal cannons to prove the existence of SCF. He was able to identify the existence of a new single phase, rather than the two separate liquid and gas phases by observing the changes in solvent. He observed that the liquid and gas phases of sample became undistinguishable after crossing a “critical” temperature. After the discovery of SCF in I869 Dr Thomas Andrews reported the critical parameters of carbon dioxide and showed the phases by making the first supercritical phase diagram [5]. In 1879 at a Royal Society meeting, Hcmnay and Hogarth described the capability of certain compressed gases to dissolve salts such as cobalt chloride, potassium iodide, and potassium bromide, which was until that time thought to be impractical.

Properties of SCF

A SCF acts as a dense gas phase and has neither a surface tension nor an enthalpy (heat) of vaporization. The characteristics of a SCF can be diverse with small changes in temperature or pressure. SCF has higher diffusion coefficient, lower viscosity, higher compressibility factor, lower dielectric constant and lower solubility parameter than Liquid has. If we compare SCF has with gas, we see that SCF has higher thermal conductivity, higher heat capacity and higher thermal diffusivity than Gas has. SCF provides efficient heat and mass transfer compared with that in the liquid state or gas state because of its favorable characteristics [6].

SCF technology has been broadly used in extraction and purification processes in the food and pharmaceutical industry and for techniques such as supercritical fluid chromatography(SFE). In recent times, there has been a considerable increase in interest of the use of supercritical carbon dioxide as a substitute for chlorofluorocarbons for a range of specific and specialized applications. Carbon dioxide is one of the most suitable and widely used SCF. It has many qualities and these qualities make it a very attractive SCF.

Important qualities of Carbon dioxide are as follows:

There are many useful benefits associated with the use of supercritical carbon dioxide as a solvent. Carbon dioxide is often endorsed as a sustainable solvent, as CO_2 is non-flammable, shows a relatively low toxicity and is very inexpensive and easily available in pure form worldwide. It is environment-friendly [7]. Critical temperature of carbon dioxide is 31°C, which allows operations at near-ambient temperature. Critical temperature of carbon dioxide neither affects the product nor changes the properties of the product [8-10].

In supercritical state carbon dioxide has a both gas-like and liquid-like property. This dual property of supercritical carbon dioxide provides the best conditions for extracting compounds with a high degree of recovery in a short period of time [11]. By regulating pressure and temperature, the density or dissolving power of supercritical carbon dioxide can be changed. This dissolving power can be used to purify, extract, fractionate, infuse, and recrystallize a broad range of substances. Though, carbon dioxide always acts as a "non-polar" solvent that selectively dissolves the lipids that are water-insoluble compounds like seed oils, animal fats, hydrocarbons etc. Carbon dioxide does not dissolve the hydrophilic compounds like sugars and mineral species like salts etc. Because carbon dioxide is non-polar solvent, a polar organic co-solvent (or modifier) can be mixed with the supercritical carbon dioxide for processing polar compounds. By regulating the level of pressure or temperature or modifier, supercritical carbon dioxide can dissolve a wide range of polar and non-polar compounds [12].

Advantages of SFE

SFE method is increasingly being used and endorsed at a laboratory and pilot scale to make high value, natural bioactives from biologically based raw stuffs [13]. SFE technique is better than the traditional techniques, like steam distillation and organic solvent extraction, in the recovery of edible and essential oils, as the use of a non-toxic and volatile solvent, such as carbon dioxide, guards extracts from thermal degradation and solvent contamination[14-16]. SFE has a number of benefits such as small extraction time, probability of selective extraction, and no residual solvent in the final extracts [17]. The advantages are as follows

- The viscosity of SCF is relatively low and the diffusivity of SCF is relatively high. For that reason, they can penetrate into porous solid substances more efficiently than liquid solvents, and provide much faster mass transfer resulting in faster extractions [18].
- In SFE; SCF is continuously allowed to flow through the materials; therefore, it can provide quantitative or complete extraction.
- SCF exhibit tunable dissolving power. The dissolving power of the SCF can be regulated by changing pressure and temperature; for that reason, it can attain extremely high selectivity. This tunable dissolving power of SCF is particularly useful for the extraction of complex samples such as animal tissues, volatile oils can be extracted from a plant with low pressures (100 bar).
- Extract can be easily separated from SCF by simply releasing pressure, leaving almost no trace and yields a pure residue.
- SFE process can be performed at low temperatures; therefore it is a best technique to extract thermally labile compounds and may lead to the discovery of new natural compounds. For instance, SFE method can effectively be used to extract valuable compounds from ginger and many undesirable reactions such as hydrolysis, oxidation, degradation and rearrangement which occur during extraction can be avoided.
- Conventional extraction methods such as soxhlet extraction method require 20–100 g of sample but SFE method requires only 0.5–1.5 g of sample. It has been reported that from only 1.5 g of fresh plant samples, more than 100 volatile and semi-volatile compounds were extracted and detected by gas chromatography –mass spectroscopy (GC-MS), of which more than 80 compounds were in sufficient quantity for accurate quantifications
- Conventional extraction methods use toxic and harmful solvents; on the other hand SFE method uses nontoxic or significantly less environmentally hostile organic solvents.
- SFE can be easily paired with a chromatographic method, which is a creative way to extract and directly quantify highly volatile compounds.
- In large scale SFE processes, generally carbon dioxide is used as SCF. It is recycled and reused in order to reduce extraction cost and minimize waste production [19-20].

Application of SFE

One of the eye-catching characteristics of SFE is that it is comparatively simple to couple the extraction technique directly with chromatographic techniques [3]; SFE has been fruitfully coupled to TLC, LC, GC, packed column SFC and capillary SFC. An on-line combination of SFE and capillary SFC can successfully be used to avoid Potential loss of volatile material where extracts are collected on a comparatively large amount of sorbent before they are re-eluted directly into the capillary column. The most important benefits of this dual technique are that sensitivity is very high since 100% of the extract is moved into the chromatograph, and only extremely small samples are needed for analysis [21], class-selective extractions and fractionation of the extract can be obtained by changing the pressure, temperature and flow rate [22]. Analysis of drugs from hair samples is one of the most important applications of SFE; hair can be analyzed rapidly without the need for lengthy preparation procedures. The method is very helpful for hair because of its sensitivity and specificity that permit for the determination of the very low concentrations of drugs found in hair [23].

Extraction of Oil and Polymers

SFE is one of the best methods to extract low vapor pressure oils and polymers. It is difficult to extract oil and polymers using distillation method since the impurities have about the same volatility as the primary components reducing the overall selectivity. A commercial process for the separation of heavy components of crude oil has been developed by Kerr-McGee Inc. Extraction with respect to chemical composition is possible and has been studied to produce polymer fractions of low polydispersity starting from a parent material of high polydispersity.

Pharmaceutical Applications

The pharmaceutical sector is experiencing many challenges in relation to the fast and never-ending growth and aging of the world population, and a rising gap between health care systems in industrialized and developing countries. At the same time as authorities and consumers require higher quality levels and extremely large low-cost supply of drugs is strongly required by developing countries. It looks more complicated to introduce novel and new drugs and to enhance the therapeutic efficacy against numerous pathologies. In addition, the drug making companies must also make nonstop efforts to shift to environmentally friendly processes. Concerning the environmental protection, one of the first requirements of the pharmaceutical sector is to move to "green chemistry" and to stay away from potentially unsafe and injurious solvents. The use of SCF solvents is a favorable way for lower pollutant release and enhancing the final drug standard and effectiveness through novel processes for active substance preparation and drug formulation. In SFE generally carbon dioxide gas is used as SCF and methanol or ethanol is used as co-solvent. Carbon dioxide is non-toxic, non-flammable and is generally regarded as safe for use in food products. It is "tunable"; the solvency power of carbon dioxide can be regulated by increasing or decreasing pressures and/or temperatures. It is environmentally friendly. Industrial Carbon dioxide comes from byproducts. Carbon dioxide used for extractions does not contribute to the overall atmospheric Carbon dioxide levels.

Sterilization

The supercritical Carbon dioxide can be used to kill bacteria or to inhibit bacterial growth. It penetrates bacterial cells, blocks the bacterial metabolism and kills them. For that reason supercritical carbon dioxide cleaning method has been used to clean satellites and landing craft and other components, to ensure no body was taken to outer space life.

There are many sterilization techniques such as gamma radiation, steam sterilization, dry heat sterilization, moist heat sterilization etc. Each of these sterilization techniques has serious limitations for the sterilization of some materials used in medicine, especially thermally and hydrolytically sensitive polymers by themselves and in combination with proteins. SFE is a potential new method of sterilization; it usually uses carbon dioxide as SCF. Dillow et al [24] used SFE method to inactivate a wide variety of bacterial organisms at moderate temperatures. When they added biodegradable polymers poly(lactic-co-glycolic) acid and polylactic acid in the sterilization process, they observed that there was no effect on the inactivation efficiency, however they did not find any physical or chemical damage to these thermally and hydrolytically labile materials

Extraction of plants

There are many important and useful applications of SFE such as the extraction of active ingredients, including various flavors and medicinal constituents from plants and animals advanced unsaturated fatty acids and fatty esters, fat-soluble vitamins etc. Other applications include the removal of unnecessary constituents, such as decaffeination and desolvation in pharmaceutical tablets. It can also be applied to the pre-processing of samples prior to separation using HPLC or GC.

Essential Oils are highly concentrated and potent oils extracted from plants, leaves, flowers, roots, buds, twigs, rhizomes, heartwood, bark, resin, seeds and fruits. The aroma of certain natural product extracts is due to the presence of essential oil. Essential oil is generally a complex mixture of a number of heat-labile monoterpenes and sesquiterpenes and other aromatic compounds. Right reproduction of the natural aroma in a concentrated extract is a difficult job because of the heat-labile nature of the compounds, the possibility of hydrolysis and hydrosolubilization. Being Suitable for heat-labile compounds, SFE has been one of the best methods for the extraction of natural essential oils over the past few decades. The extraction of essential oil components using SFE has received significant attention over the past several years, particularly in food, pharmaceutical, and cosmetic industries, because it provides an environment-friendly and cost-effective substitute to conventional extraction processes.

Scientists are interested in the potential health benefits of flavonoids associated with fruit and vegetable-rich diets. Flavonoids are becoming very popular because they have many health promoting effects. Some of the activities attributed to flavonoids include: anti-allergic, anti-cancer, antioxidant, anti-inflammatory and anti-viral. Liza et al [25] used SFE technique to extract flavonoid from Strobilanthes crispus (Pecah Kaca). They used carbon dioxide as a SCF. Since carbon dioxide is a non-polar solvent. The most favorable extraction condition occurred at 200 bar, 50^{o} C and 60 min

Yuefei et al [26] used SFE method to extract flavonoids from Ampelopsis grossedentata Stems. They used carbon dioxide as a supercritical fluid and methanol and ethanol (1:1) a modifier. They performed extractions at different pressures and temperatures. According to them, the most excellent conditions obtained for SC-CO_2 extraction of flavonoids was 250 bar, 40 °C, 50 min, and with a modifier of methanol/ethanol (1:3, v/v).

Mandant et al [27] employed SFE technique to extra Bioactive Flavonoid Compounds from Spearmint (Mentha spicata L.) Leaves. They used Ethanol (99.9% purity) as the co-solvent to increase the polarity of solvent and improve the efficiency of SC-CO2 extraction of bioactive compounds from spearmint leaves. The most favorable extraction condition occurred at 209.39 bar, 50^{0} C

Wu et al [27] used SFE method to take out Flavonoids from Dandelion. They crushed dried dandelion (baked at 45°C for 12 h) in a high -speed mixer -grinder and sieved the Powder over a 60 - mesh screen. They used carbon dioxide as a SCF and performed extractions at different pressures and temperatures. They extracted flavonoids from the raw material after the less polar material essential oil was extracted by CO_2 in the system. The most suitable extraction condition occurred at 35 MP, 50^{o} C and 80 min

Supercritical Water Oxidation

The most important objective of wastewater treatment is to allow human and industrial effluents to be disposed of without threat to human health or unbearable damage to the natural environment. Conventional wastewater treatment comprises a combination of physical, chemical, and biological methods and operations to take out solids, organic matter and, sometimes, nutrients from wastewater. Replacing conventional wastewater treatment methods with SFE is a major improvement in today's pollution prevention programs. SFE technology is fast, simple and effective technique for wastewater treatment. Other advantages of SFE include high efficiency, high extraction rates and more selectivity. Supercritical Water Oxidation is also known as Hydrothermal Oxidation, is a high-efficiency, thermal oxidation process capable of treating a wide variety of hazardous and non-hazardous wastes. Supercritical Water Oxidation reaction occurs at raised temperatures and pressures above the critical point of water (P_c= 220.55 bar, T_c=373.976^{o}C). Supercritical Water Oxidation is perfectly suitable for treating waste streams containing high concentrations of water. Supercritical Water Oxidation processing units are completely enclosed and do not generate hazardous air pollutants. Kianoush and Mohammad[29] used SFE technology to treat waste water. They used water with hydrogen peroxide as a SCF to treat a model municipal solid waste containing proteins, fats, vitamins, fiber, and inorganic minerals. They investigated the effects of temperature, oxidant concentration, and reaction time on the decomposition of solid waste

Food Industry Applications

The food and beverage sector was the first to make commercial use of supercritical carbon dioxide extraction. As early as 1974, European companies were extracting caffeine using SCF-CO_2.another example is fat extraction from processed snack foods and extraction of flavor oils froms hops for the brewing industry.

The methylene chloride extraction method is used to extract the caffeine from the coffee beans. The drawback of the methylene chloride extraction method is that it is toxic and residue is often left on the beans[30]. It does not extract any of the flavor material in the coffee, unlike methylene chloride. This method uses liquid carbon dioxide. The coffee to be decaffeinated is placed in a large extraction vessel which can withstand relatively high pressures. Liquid carbon dioxide is pumped into the extraction vessel where it solubilizes the caffeine. After the extraction process, the liquid carbon dioxide is pumped to a second column, where it is depressurized; the liquid carbon dioxide vaporizes to form a gas. The gaseous carbon dioxide is then recompressed to go back into its liquid form and is used to decaffeinate a fresh batch of coffee. In the meantime, the caffeine extracted from the coffee is recovered and used as an ingredient in many pharmaceutical preparations. The decaffeinated coffee is safe and contains no harmful chemicals. It is packaged and sold without any risk. Fred j. Eller and jerry W. King [31] extracted fat from ground beef by using SFE. Natalia Mezzomo et al[33] used SFE to extract valuable products from pink shrimp residue.

Sarker et al[34] applied SFE for extracting oil from viscera of African catfish. Gianpiero Boatta [36] used SFE method to extract the toxins from the lyophilized using carbon dioxide as a SCF. Ki-Souk[22] to detected pesticide in meat product. John W. pensabene [37] used SFE to separate sulfonamides from chicken eggs. J. W. King [38] used SFE equipment to extract fat and cholesterol from beef patties. Janet M. Snvder and Jerry W. King[39] used SFE technique to extract fat from poultry tissue so as to determine pesticides. Nooreen din[40] used SFE to separate sulphamethazine from meat tissue. Isameldin B. Hashim, et al [41] used SFE process to extract fat from lamb meat. Nuria Rubio-Rodríguez et al[42], used SFE to extract Omega 3 rich oil from fish. Jerry W. King et al[43] used extracted fat from meat products. Ningping Tao et al[44] extracted oil from yellow fin tuna. Ram Chandrasekar[45] extracted crude fat from the meat Yih-Dih Cheng et al[46] extracted components from black ant. A. Bustamanteet al[47] extracted astaxanthin from H. pluvialis. Scott L. Taylob et al[48] used SFE to take out Aflatoxin M1 from beef liver. Joo-Hee Lee et al[48]extracted out oil from yellow croaker muscle. Ji et al[49] extracted out protein from mackerel viscera. Byung-Soo Chun et al[50] used SFE to remove fat from viscera of mackerel. They prepared defatted powder of mackerel viscera. Kai Chang et al[51]extracted oil from animal tissue and plant seeds. Saadat et al[52] employed SFE to extract oil from black cumin seeds. V. Micic et al[53] extracted oil from Salvia officinalis L

CONCLUSION

SFE is a perfect, speedy, trouble-free and economical method. It produces quantitative recovery of the target analytes without loss or degradation; provides a sample that is immediately ready for analysis without additional concentration or class fractionation steps; and generates no additional laboratory wastes. Concisely it can be said that SFE technique is better than conventional extraction techniques. SCF can be used as the mobile phase to separate analytes with chromatographic columns. In SFE process solvating power of supercritical fluid is similar to organic solvents, but with higher diffusivities, lowers viscosity, and lower surface tension. The lower viscosity permits higher flow rates compared to liquid chromatography, and the solvating power can be regulated by changing the pressure.

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