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Historical Development

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Definition

Above the critical temperature, the vapour and liquid of a substance have the same density and the fluid cannot be liquefied by increasing the pressure; the supercritical fluid has density and solvating power approaching that of a liquid, viscosity similar to that of a gas, and diffusivity intermediate between those of a gas and a liquid. A typical phase diagram is shown in **Figure 1**. It follows that supercritical fluids have properties which make their use as chromatographic mobile phases very favourable. Thus, gas chromatography (GC) produces narrow peaks and high resolution, but cannot be used for involatile or thermally labile compounds since analytes must be in the vapour phase. High performance liquid chromatography (HPLC) is extensively applied to involatile compounds, but has limited resolution because the high viscosity of the mobile phase limits column length, and the low diffusivity slows the exchange of analyte molecules between liquid streams of different velocity. Supercritical fluid chromatography (SFC) addresses these limitations because of increased solubility (which varies with density and hence applied pressure) for involatile compounds in comparison with GC, and lower viscosity and higher diffusivity in comparison with HPLC.

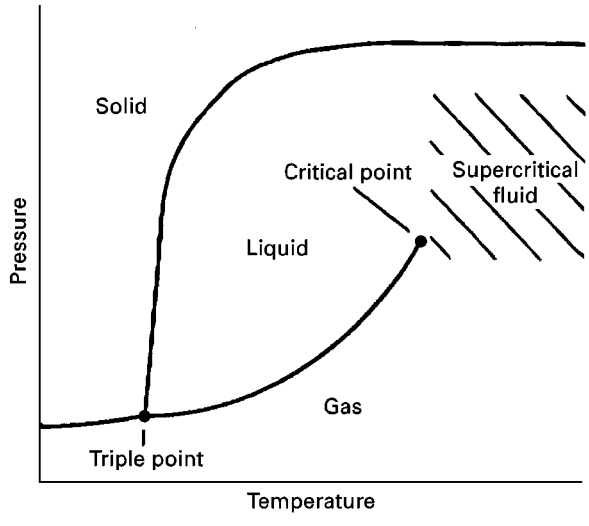

Figure 1 Typical phase diagram of a single substance. (Reproduced with permission from Clifford T(1998) *Fundamentals of Supercritical Fluids*. Oxford: OUP.)

Both capillary and packed columns are used in SFC, and many of the detectors of both GC and HPLC may be employed. Coupling to spectroscopic detectors has proved fruitful. SFC has established a number of niche areas: it extends the range of GC, it can replace many normal phase HPLC methods and it has many advantages over size-exclusion chromatography.

History

Supercritical fluids have been known (**Table 1**) since Baron Cagniard de la Tour observed in 1822 that, above a certain temperature, a liquid can be converted to a gas without the appearance of a meniscus. In 1869, Andrews fully characterized the critical point in terms of critical temperature T_c and critical

Table 1 Advances in supercritical fluid chromatography

Cagniard de la Tour	Discovery of supercritical state	1822
Andrews	Characterization of critical point	1869
Hannay and Hogarth	Increased solubility in supercritical fluids reported	1879
Lovelock	SFC suggested	1958
Klesper	Demonstration of SFC	1962
Giddings, Sie and Rijnders	Development of dense gas GC and SFC	1966–69
Sie, van Beersum and Rijnders	FID used in SFC	1966
Sie and Rijnders	First use of term 'SFC'	1967
	UV/visible detector used in SFC	1967
Jentoft and Gouw	Pressure programming demonstrated	1978
Randall and Wahrhaftig	SFC coupled to mass spectrometry	1978
Novotny and Lee	Capillary column SFC demonstrated	1981
	Commercial packed column SFC	1982
	Commercial capillary column SFC	1986

pressure P_c. Hannay and Hogarth observed in 1879 that metal halides are soluble in ethanol above the critical point, and as early as 1897 Vuillard reviewed solubility in supercritical fluids. Isolated examples of the use of near-critical solvents for separation appeared between 1930 and 1960; more extensive applications, especially to natural products with the pharmacologically acceptable carbon dioxide have since appeared, based on extensive studies of solubility in supercritical solvents and on the properties of solutions. In parallel with SFC, analytical supercritical fluid extraction (SFE) has been extensively developed as a sample preparation technique.

The original idea for SFC was suggested by Lovelock at an international meeting in 1958, before Klesper, Corwin and Turner separated involatile porphyrins in 1962 using supercritical chlorofluorocarbons at pressures up to 136 bar as mobile phase. In 1966, Sie and Rijnders developed both adsorption and partition chromatography using carbon dioxide, *n*-pentane and isopropanol at temperatures up to 245°C and pressures up to 80 bar.

The advantages of carbon dioxide as a mobile phase were quickly realised: it is an inexpensive material with good solvent properties, convenient T_c and P_c, non-combustible, easily delivered by pumping the liquid, and available in high purity. Although other compounds have since been employed as mobile phases (e.g. N_2O, SF_6, NH_3, *n*-butane and *n*-pentane, xenon, and various fluorocarbons and chlorofluorocarbons), CO_2 has remained the most popular and widely used. Because of the variation of solubility with density and hence pressure, Rijnders suggested in 1967 that pressure programming in SFC should have a similar effect to temperature programming in GC.

In 1970, Jentoft and Gouw constructed a versatile high-resolution SFC chromatograph with a pressure-programming facility for packed columns up to 4-m long and *n*-pentane-methanol mobile phase for temperatures up to 215°C and pressures up to 650 bar; detection was by UV absorption. Styrene oligomers up to $n = 32$ could be analysed with this system. The affinities between GC with a dense gas as mobile phase and SFC were discussed and developed by Giddings between 1966 and 1969.

Novotny pointed out in 1971 the serious limitations in SFC of the pressure gradient generated by the column packing, and realized that SFC should be possible on capillary columns with a much smaller pressure drop. In 1981, Novotny and Lee demonstrated capillary column SFC equipment. Commercial equipment for packed column SFC became available in 1982, and for capillary SFC in 1986.

The 1980–90 period was one of rapid growth in the theory, instrumentation and applications of SFC. A multi-authored text edited by Lee and Markides appeared in 1990 summarizing the state of the art, and remains authoritative. Two series of international conferences on SFC and SFE, one based in USA and the other in Europe began in 1988 and continue.

Instrumentation

Figure 2 illustrates the instrumentation for SFC. The mobile phase is pumped as a liquid and the pressurized fluid passes, via an injection valve, into the column maintained in an oven at a temperature above T_c, and then to a detector. A pressure restrictor to maintain supercritical conditions is located either after the detector or at the end of the column.

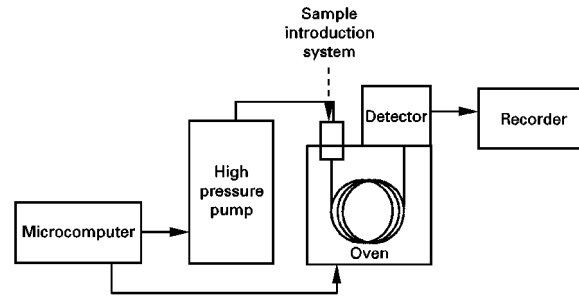

Figure 2 Schematic diagram of an SFC instrument.

Table 2 Columns in SFC

Column	*Internal diameter (mm)*	*Advantages*	*Disadvantages*
Conventional	2.0–4.6	High capacity	Low overall efficiency
Microbore	1–2	Rapid analysis	Highly activity
Packed capillary	0.2–0.8	Highly selective	
Open tubular	0.05–0.1	Low capacity, high resolution inactive	Long analysis times Selectivity only conferred by stationary phase

Conventional HPLC pumps with cooled heads were used from the start for SFC in packed columns. Syringe pumps were initially used in capillary SFC because of their pulseless operation. Small column diameters necessary in capillary SFC make the method of sample introduction important; small volumes and rapid injection are achieved by a pneumatically operated loop valve with electronic timing.

Columns

Although a full range of columns can be used in SFC, the technique is most usually divided into two categories based on column type, with advantages and disadvantages outlined in **Table 2**. Packed columns for SFC have reflected developments in HPLC: (a) 'conventional' packed columns (up to 4.6 mm i.d.); (b) microbore columns; and (c) packed capillaries with smaller i.d. Most of the work has been carried out on 4.6-mm columns, with 3–10 μm particle size packing materials – usually bonded-silica particles (**Table 3**). It was realized early that the unreacted silanol groups on silica particles which have little activity in reversed-phase HPLC led to peak tailing because of the low polarity of the mobile phase. Such effects led to the almost exclusive addition of organic modifiers (alcohols, ethers, etc.) to the carbon dioxide mobile phase in packed column SFC. These modifiers can influence selectivity and increase the solubility of analytes. A number of specially end-capped silica packings were developed, along with polymer-based packings, but most of these have had limited use. The low-pressure drop across packed columns has led to the coupling together of columns, with consequent improvement in selectivity and efficiency.

Table 3 Bonded stationary phases in SFC

Packed SFC	*Capillary SFC*
Octadecyl	Methyl
Octyl	Octyl
Phenyl	Phenyl
Hydroxyl	Biphenyl
Diol	Cyanopropyl
Cyano	Liquid crystal
Amino	Polyether
Chiral	Chiral

Capillary SFC was only made possible by the development from 1979 onwards of fused silica capillary columns for GC. Because of the slower diffusion of analyte in supercritical fluids, open tubular columns for SFC must have internal diameters less than 100 μm to achieve resolution comparable to that on 200 μm i.d. columns for GC. Lee's group undertook an extensive series of developments of columns for both GC and SFC between 1980 and 1990, basing stationary phase structures on a polysiloxane polymer backbone with pendant groups 'designed' for particular applications. As well as the more usual methyl- and phenyl-containing phases (Table 3), polymers with biphenyl, cyanopropyl and ethylene glycol, etc., groups were synthesized; more exotic phases had bonded liquid crystal or chiral groupings. Capillary columns for SFC are highly inert and are usually operated with CO_2 alone as mobile phase.

Detectors

SFC can be carried out with the widest range of detectors of any chromatographic technique: detectors from gas and liquid chromatography are employed, and SFC can be coupled to a number of information-rich spectroscopic detectors (**Table 4**). SFC detection can be carried out in the gas phase after decompression, or in both supercritical and liquid phases. The flame ionization detector (FID) is the most commonly used if unmodified CO_2 is the mobile phase, as is the case for capillary columns, whereas the UV/visible detector is most often used if a mobile phase contains a modifier, e.g. in packed column work. Some limited use has been made of the FID with CO_2 modified by water, formic acid or formamide which give no, or a very small, FID signal.

From 1981 the range of detectors for SFC showed a rapid expansion. The thermionic, electron-capture, photoionization and chemiluminescence detectors from GC, and the fluorimetric and light-scattering

Table 4 Detectors in SFC

Type	*Established*	*Developmental*
GC type	Flame ionization	Chemiluminescence
	Thermionic	Photoionization
	Flame photometric	Ion mobility
	Electron capture	Element-specific plasma
LC type	UV absorption	Fluorescence
		Light scattering
		Electrochemical
Coupled	Diode array UV	Nuclear magnetic resonance
	Fourier transform IR	Inductively coupled plasma
	Mass spectrometric	spectrometry

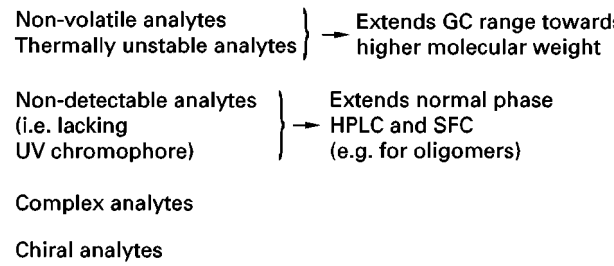

Figure 3 Application areas of SFC.

detectors from HPLC were all demonstrated in SFC. Element-specific plasma emission detectors were demonstrated in SFC from 1987, and since 1990 electrochemical detection has been explored; if the electrodes are sufficiently small, oxidative and reductive detection is possible in CO_2 containing only small concentrations of water or organic modifier.

The detector providing the most structural information in chromatography is the mass spectrometer (MS); a packed-column SFC–MS interface was first reported in 1978, and a practical SFC interface was developed in 1982 for capillary SFC–MS where the low flow rates permit direct introduction into the ion source. A practical packed-column SFC–MS interface was developed in 1987 to cope with the higher flow rates. SFC with both electron impact and chemical ionization has been demonstrated. The interfacing of capillary SFC to Fourier-transform infrared spectroscopy (FTIR) was achieved as early as 1983. Both solvent-elimination (evaporation of CO_2 to deposit separated compounds on a solid substrate or IR transparent plate) and online direct recording of FTIR spectra in a flow cell with IR transparent optics were developed between 1983 and 1990. A major problem with the latter approach is strong IR absorption by CO_2; alternative supercritical mobile phases, especially xenon, have therefore been employed but xenon is vastly more expensive than CO_2. Recently, NMR detection has been explored in SFC.

Applications

After early exponential growth up to 1990, SFC has consolidated to maintain applications complementing GC and HPLC, mainly in the analysis of lipophilic compounds. **Figure 3** illustrates the perceived application areas. Particularly noteworthy have been applications to: fossil fuels and hydrocarbons (where simulated distillation at low-temperature and group-type separations have been particularly important); agrochemicals; explosives; drugs and pharmaceuticals (especially those normally analysed by normal-phase HPLC); lipids and other nonpolar biomolecules; industrial chemicals (especially surfactants and polymer additives); and metal chelates and organometallic compounds. A current growth area is in chiral separation, where the rapid speed of analysis in SFC also permits preparative separations. Current developments are aimed at eliminating the distinction between gaseous and supercritical fluid mobile phases.

See also: **II/Chromatography: Supercritical Fluid:** Instrumentation; Large-Scale Supercritical Fluid Chromatography.

Further Reading

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