The Past, Present, and Future of Analytical Supercritical Fluid Chromatography

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The history of supercritical fluid chromatography has been fraught with controversies that largely held back the development of the technique for decades. The origin and resolution of some of these controversies is briefly discussed. The development of adequate hardware has also been an important issue, culminating in the last few years with true 3rd generation instruments. The present state of affairs is also briefly outlined, and some predictions about the future made.

The Pioneers

Since the late 1800's it has been known that some heavy, non-volatile organic compounds were surprisingly soluble in some inorganic gases above their critical point ('super'-critical). These gases include SO^2, CO^2, ammonia, etc. At an early GC conference in 1957, Jim Lovelock suggested using such inorganic gases, above their critical points as a chromatographic mobile phase, in order to separate much more polar compounds compared to normal GC. Acting as a solvent is a critical characteristic that differentiates the compressible mobile phases used in supercritical fluid chromatography (SFC) from gas chromatography (GC), where the mobile phase is considered to be an inert carrier. Lovelock suggested the name "critical state chromatography".

Ernst Klesper was the first to actually use a fluid above its critical point to separate thermally labile metal porphyrins. He used dichlorodifluoromethane, and chlorodifluoromethane at 800-2300 psi and 115°C which were pre-heated in a copper coil, before passing through the 30 inch long column with a 60-80 mesh (180-250 µm) diatomaceous earth GC packing(!). The first publication we can call SFC appeared in 1962, as a three page 'Communications to the Editor' in the Journal of Organic Chemistry [1]. The elution strength was found to be proportional to pressure (density). Flow rate was not 'controlled', but was basically adjusted within a range, by replacing fixed restrictors, or using a metering valve.

From this beginning, there was, and still is, significant difficulty in coming up with an appropriate name that captures the essence of the technique in a short, simple phrase. Klesper called the technique "high pressure GC above critical temperatures". Clearly this was a poor name since the mobile phase acted as a solvent and wasn't inert. Thus, it isn't GC.

Giddings, probably the most influential chromatographic theorist in the 1960's used many names, including: turbulent flow chromatography [2], ultra high pressure gas chromatography (to 2000 atmospheres) [3], and dense gas chromatography [4]. None of these names captured the solvating nature of the technique. Sie and Rijnders [5] first used the name supercritical fluid chromatography, but this wasn't much better since this name implies the fluid must ALWAYS be 'super' (meaning above) the critical point to display the desirable characteristics, which also isn't true. Caude [6] was apparently the first to use the term 'subcritical' to denote the fact that some modified CO_2 based fluids were of high density, while still highly compressible, but acted as a solvent even below their critical temperature.

Early Controversies Retarded the Growth of SFC

In the late 1960's Giddings estimated Hildebrand solubility parameters [7] which suggested that dense CO_2 could be as polar as isopropyl alcohol (IPA). It was thought that programming pressure (or density) could adjust the elution strength of CO_2 from that of a hydrocarbon to that of an alcohol! This was before the invention of high performance liquid chromatography (HPLC), and there was much that was poorly understood. If this were true, SFC would today be as big, or bigger, than HPLC. Unfortunately, it isn't true.

Another controversy began in the late 1960's when Sie and Rijnders [5], Milos Novotny [8], Gouw and Jentoft [9], and later, L.B. (Buck) Rogers [10] all suggested that the efficiency of their very crude packed columns (up to 120µm packings) seemed to degrade with higher pressure drops. Gouw and Jentoft [9] suggested that decreasing density along the axis acted like a decreasing temperature gradient in GC, causing a loss in efficiency. By the mid to late 1980's, it was widely thought that pressure drops of as little as 20 bar seriously degraded efficiency. There were actually several competing theories [11,12] trying to explain why this should occur. It was claimed that SFC packed columns could never produce more than ≈20,000 plates [13], and virtually ruled out any use of particles smaller than 5µm. These theories were unrelated to, but contemporary with, the commercialisation of capillary SFC (see next section). This problem stemmed directly from inadequate home-made equipment, related to back pressure control and the use of fixed restrictors instead of a back pressure regulator. In many cases the column outlet pressure was not even monitored, allowing operation in inappropriate regions.

One additional controversy further retarded the use of packed columns through the 1980's. Polar solutes often did not elute, or eluted with poor peak shapes even at high modifier concentrations. This was blamed on 'active sites' on the silica based stationary phases. Widespread consensus was to make the phases more non-polar and increase end-capping to cover such sites. However, it was typical to have minimal retention and poor peak shapes with such columns. The problem was largely solved when it was shown [14-19] that the introduction of polar additives, such as a strong acid or base, in the modifier, dramatically improved peak shapes. Many papers about the use of additives have followed. This discovery dramatically expanded the application areas amenable to SFC. Today SFC brackets all the area amenable to both normal phase and reversed phase HPLC and further includes parts of ion chromatography, HILIC etc.

Capillary or Open Tubular SFC

Due largely to the perceived limitations discussed in the previous sections, a major detour occurred starting around 1981 when Milton Lee and Milos Novotny first described capillary SFC [20], made possible by the then recent, nearly concurrent inventions of fused silica capillary columns and bonded stationary phases. By simply using a syringe pump to change the pressure of pure CO_2, the solvent strength of the mobile phase could be programmed from weaker to stronger conditions with the universal flame ionisation detector (FID). Such an approach seemed to solve all the problems associated with packed columns.

The simplicity of such an approach was, for a time, overwhelming, and almost completely replaced packed column usage. At one point there were six or seven companies manufacturing and selling capillary SFC's, and only one (Jasco) selling packed column SFC's. Unfortunately, the technique was oversold as being appropriate for more polar solutes, such as small drug-like molecules, due in large part by the unfortunate mis-step by Giddings.

The Return to Packed Columns

When density measurements for methanol/ CO_2 mixtures were finally made in 1990 [21,22], it was shown that, at constant density, modifiers significantly increased the solvent strength of the mobile phase. Later, solvatochromic dye studies [23] unequivocally showed that CO_2 was never much more polar than hexane, and modifiers dramatically increased solvent strength. Never the less, decades of belief are slow to be changed.

The idea that large pressure drops cause significant losses in efficiency and limited total possible efficiency was largely put to rest in the early 1990's when it was demonstrated [24] that columns could be connected in series, producing at least 220,000 plates with a pressure drop of up to 300 bar.

By the early 1990's, the concentration of polar modifiers was shown to be the primary retention control variable when added to the CO_2, while pressure/density became a secondary control variable. The use of additives allowed the elution, with high efficiency, of polar solutes, such as primary aliphatic amines. Thus, modern SFC was born, while capillary SFC almost completely died out.

Availability of Instrumentation

Until the early 1980's all instrumentation was home-made, using components from various other fields. Many of the misconceptions of the 1960 through the1980's can be attributable to the poor instrumental controls then prevalent, particularly the poor control of outlet pressures. In many ways, reproducible SFC started with the introduction of a hardware kit that converted a Model 1084 HPLC into an SFC, introduced by Hewlett Packard (now Agilent) in 1982. This instrument was crude by today's standards. Its main contribution was a heated mechanical back pressure regulator (BPR) controlling the column outlet pressure, with large mechanical gauges unequivocally showing both the inlet and outlet pressures. Retention control rested entirely on the mobile phase composition. This instrument was withdrawn around 1984-5, when the Model 1090 HPLC was introduced which was incompatible with SFC. Jasco introduced a SFC-SFE combined instrument in 1985. This instrument included the first electronic BPR, but did not perform dynamic compressibility compensation.

By the early 1990's capillary SFC had been grossly oversold, particularly for the separation of much more polar solutes. This poisoned the well for about ten years where many regarded SFC as "science fiction chromatography". Fortunately the clear superiority of packed column SFC over HPLC for chiral analysis was obvious and, just sustained the small start-up manufacturers through this period. It is something of a miracle that SFC exists at all today. The development of reliable semi-preparative SFC, particularly for chiral separations, probably is the reason we now have two of the largest instrument companies involved, as most chromatographers who have tried semi-preparative SFC are easily convinced it is far superior to HPLC.

In 1992 several second generation SFC's capable of packed column operation were introduced by Hewlett Packard (HP), and Gilson, although only one (HP) used dynamic compressibility compensation. Proper compressibility compensation means that both the flow rate and composition are much more accurate than with previous instruments, making transferring methods from machine to machine much easier. This also marked the first Peltier cooled pump head, a return to pressure, density and temperature programming as well as composition programming. Electronic back pressure regulators became the norm on virtually all subsequent SFC's. Diode array detectors significantly extended the utility of UV detectors. Many GC detectors, such as the FID, ECD, NPD, etc were also commercially available. GC like ovens allowed for much higher column temperatures.

Changing Applications

In the very early days most SFC separations involved polymers, surfactants, lipids, silicone oils, etc. However, packed column SFC really started to gain traction in the early 1990's with the separation of enantiomers. The first use of a chiral stationary phase was by Caude and co-workers in 1985 [6]. The first SFC use of a chiral additive in the mobile phase was by Erni [25] in 1988. Today, far and away, the 'killer app' for SFC has been in chiral chromatography at both the analytical and semi-preparative scale. SFC is easily 3-5 times faster than normal phase HPLC, is less expensive to operate, generates much less toxic and flammable waste and is much easier to dry down fractions. Consequently, in some major pharmaceutical companies SFC has become the technique of choice for these applications, whereas HPLC has been relegated to a few difficult cases [26], or has been eliminated from use altogether [27].

The Present

The present probably started with the

introduction in 2009 of the Aurora SFC conversion module that converted an Agilent Model 1100 or Model 1200 HPLC into a world class SFC. Waters introduced its Ultra Performance Convergence Chromatography (UPC^2) a year later. Agilent bought Aurora in 2012 and better integrated the module. These instruments are true third generation SFC's, with superior performance, although neither has the versatility of some earlier instruments More importantly, it means that for the first time two of the largest instrument companies are committed to selling and supporting SFC, worldwide. This is a major change from the past. From 1995 until ~2007 the majority of SFC's sold were produced by small start-up companies with limited resources. It worked, in large part, due to the stunning superiority of SFC over HPLC in chiral analysis and purification.

Agilent uses the conversion module mounted upstream of a nearly standard HPLC pump to pre-compress the CO_2 to just below column head pressure. The compressibility compensation for the HPLC pump delivering CO_2 is then set to zero. Thus, this pump merely meters the CO_2 flow, virtually eliminating any flow/pressure fluctuations from the CO_2 pump, a major source of UV noise in previous SFC's. This, in combination with an ultra low noise back pressure regulator (BPR), and careful control of mobile phase temperature entering the detector cell, resulted in as much as a 50 fold decrease in UV detector noise compared to earlier instruments.

Waters uses a slightly different approach to deal with compressibility compensation related noise. Each piston of a two piston pump (delivering a single fluid) is driven by a separate motor, much like the old Rainin SD-1 pumps. While the first piston is delivering flow, the other refills its pump head, then pre-compresses the fluid to just below column head pressure, and then waits. When the first piston reaches the end of its delivery stroke, it slows down and passes off delivery to the already refilled and pre-compressed pump head. Since the compression stroke is completely independent of the delivery stroke there is minimal flow/pressure/composition noise, similar to the Agilent approach. Both use two motors to pre-compress the CO_2, just differently.

The dramatic improvement in UV noise means that, for the first time, SFC is appropriate for trace analysis where it can easily quantify peaks representing 0.1% (area/area) of a major component, with a signal to noise ratio >10. This opens up the possibility of using SFC in quality assurance (QA), and quality control (QC). In the past, the poor sensitivity relegated SFC to major minor component analysis, mostly in drug discovery. The possibility of also performing routine and trace analysis will dramatically expand the use of SFC.

Although not mentioned previously, SFC has been widely used with mass spectrometric detectors (MS) in all its forms. Virtually every MS instrument and interface has been used with SFC over the years, including APCI, ES, QTOF, etc. Today the interfaces have become trivial, sometimes no more that a specifically sized stainless steel tube.

The Future

Instrumentation. It is likely that all SFC's will eventually use pumping systems that separate the compression stroke from the delivery stroke, since this can dramatically decrease UV detector noise, and reduce or largely eliminate gradient delays. Such pumping systems are common in HPLC and will probably be the norm in SFC soon.

Today there is little need for significantly higher pumping pressure since even 1.8µm particles seldom create more than 250 bar pressure drops, even at high modifier concentrations. Thus, a 600 bar, or to a lesser extent, a 400 bar pumping system is adequate for most work. There is much less incentive to use porous shell particles in SFC since the totally porous particles generate such low pressure drops, and there is some concern about sample capacity with the porous shell particles. Sub-2 µm particles are now more than a decade old, but have only recently been used in SFC. However, there are scattered reports of the use of sub-1 µm particles, at least in HPLC, which will require substantially higher pressures. Since pressure drop is inversely proportional to the square of the particle diameter, halving particle diameter would quadruple pressure drop, requiring pumping systems capable of operating at >>1000 bar (like Giddings in 1968!).

Today, it is relatively straightforward to separate up to 15-20 components in a few minutes. Using particles half the diameter will result in a further four fold decrease in run times, causing analysis times to approach real time. At this point it will be hard to imagine a large number of applications where the sample load justifies such capability, other than as in process monitoring or library validation, since sample prep will become an overwhelming issue.

The current layout of the most modern instruments directs fluid through a rather long length of relatively large ID tubing, including necessary heat exchangers in the column oven. Even today, this layout is inadequate to produce true 'ultra' high performance SFC (UHPSFC) using sub-2µm particles. 'Ultra' performance is defined here as producing >90% of theoretical efficiency with a k of ≥2 with sub-2 µm particles. The standard high pressure UV detector cells are 8 to 13 µL, much too large for UHPSFC where cells ≈1-2 µL are required. It is hoped that any subsequent instrument designs include much shorter lengths of smaller ID connector tubing with smaller volume, but hopefully long pathlength UV detector cells.

Autosamplers need to be able to 'pre-position' the 'next' sample during each run since the run time can be less than the autosampler access time. 'Solventless' (in-line solid phase extraction) injection is also likely to become important for trace analysis.

Future Applications

There is no crystal ball enabling us to peer into the future, but this author will attempt to project what ought to happen based on the physical chemistry of the fluids involved.

It is obvious that SFC will continue to dominate chiral separations in major pharma. Amazingly, most academics and smaller organisations continue to ignore SFC, possibly due to the higher entry cost to buy the equipment. Such ignorance is difficult to explain. It is hoped that the greater marketing capabilities of the major instrument companies now involved can get the message across that much better, faster results are possible. One of the biggest obstacles for SFC growth in the past has been the very limited teaching of the technique in universities. This remains an obstacle.

SFC is now 10-50 times more sensitive than previous instruments and ought to be capable of validation for the quantification of 0.1% area/area components in complex mixtures with a S/N >10, with the same sensitivity as HPLC. The mobile phase used in SFC is generally cheaper, and 'greener' than HPLC. Since SFC is 3-5 times faster than either normal or reversed phase HPLC, SFC ought to be the preferred choice for routine analysis. Will this happen? There is huge momentum behind the standard methods of the past that will only change gradually as new methods are developed using SFC instead of HPLC.

SFC is orthogonal to HPLC. It is usually necessary to have two methods for trace analysis to prove that a trace component was NOT eluting under a major component in the first method. In HPLC, normal phase is so slow and difficult that two reversed phase methods, with minimal differences, have often been used. SFC is normal phase but actually faster than reversed phase HPLC. Thus, there is no compromise when using a normal phase (SFC) technique, based on polar-polar interactions, and a reversed phase technique based on hydrophobic interactions. In fact Agilent sells a commercial hybrid system that can switch back and forth between SFC and reversed phase HPLC in only a few minutes.

Somewhat less likely, is the possibility that pressure (or better density) programming will return as a significant application area. It is unlikely to be capillary based but there are probably adequate applications to justify packed column operation. It is likely that the temperature range of current SFC ovens will be found inadequate, particularly on the upper end. The FID (pure CO_2 only) should become important again but only if the inertness and robustness of the fixed restrictor is shown to be adequate. It is unlikely that many of the GC detectors used in the past will be significantly revived. As in GC and HPLC, MS detectors are likely to be the detector of choice.

SFC is likely to be used to a much greater extent than in the past in food, fuels, and natural products in both research, and in routine analysis. Fields other than pharma, including pesticides, are likely to perform many more chiral analyses. The 'green' aspects of SFC are likely to become more widely appreciated.

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