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Feature

From supercritical carbon dioxide to gas expanded liquids in extraction and chromatography of lipids

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Summary

Analytical-scale extraction and chromatography of oils, fats and other liposoluble compounds can be achieved by using supercritical carbon dioxide as a solvent. Since the 90's when supercritical fluid chromatography (SFC) was a hot topic, this technology has developed into a robust, modern analytical technique that uses any proportions of compressed CO_2 mixed with an organic solvent. Supercritical fluid extraction (SFE) on the other hand is only recently starting to reform in a similar way, towards the use of more robust extraction system and enabling mixing of compressed CO_2 with larger proportions of organic solvents. In this Feature article, the development of SFC and SFE into what options we have today is described, including the latest trend of using CO_2-expanded liquid (CXL) as extraction solvent for lipids.

Introduction

Chemistry with lipids comes with the disadvantage of having to dissolve the sample in an organic solvent, for instance hexane, acetone, chloroform or diethyl ether. These solvents are toxic, environmentally polluting, and energy-demanding to produce and to purify. In chemical analysis of lipids, extraction and chromatography methods are rather wasteful in terms of consuming organic solvents. Take for example Soxhlet or any other classical extraction technique. It is not unusual with several hundreds of milliliters of solvent used per sample. In chromatography, it is often necessary to use normal phase HPLC, in which large volumes of solvents such as hexane and ethers are employed as mobile phase. An exception is when gas chromatography is used for analysis – then the challenge is mainly in the need for derivatization, and the fact that not all lipids can be made volatile enough for this separation technique.

During the 90's, extraction and chromatography using supercritical carbon dioxide ($scCO_2$) as a solvent developed into a hot topic in lipid applications. Many neutral, low-polarity lipids show excellent solubility in $scCO_2$ at high, liquid-like density. However, polar lipids such as phospholipids require the addition of a co-solvent such as ethanol to make the supercritical fluid polar enough and a viable alternative to conventional solvent mixtures. This is because neat $scCO_2$ has solubilizing properties similar to that of hexane and heptane, with a dipole moment close to zero. Moreover, since $scCO_2$ is a relatively inert fluid that does not interact with hydrogen bonding, a co-solvent may be needed in order to break the intermolecular interaction between the target-lipids and the sample matrix (or the stationary phase in chromatography).

For many years during the 90's, lots of studies on supercritical fluid extraction (SFE) and chromatography (SFC) were published in the area of lipid analysis, including oils, waxes, fats, fat-soluble vitamins, essential oils, polychlorinated biphenyls, etc. in food and environmental applications. It was clearly demonstrated that SFE and SFC enable faster processes as compared to conventional methodologies, due to the uniquely low viscosity and high diffusivity of $scCO_2$, in combination with zero surface tension. In SFE, a clear advantage pointed out was that an evaporation step is usually not necessary after the extraction. In SFC, thanks to the low viscosity of $scCO_2$, several (different) packed columns could be serially connected, improving both the efficiency and the selectivity of the chromatographic separation step.

However, the use of SFE and SFC in the 90's was not without challenges, especially when homebuilt equipment was employed. Pressure restrictors easily got blocked due to the density drop and the cooling of CO_2 upon expansion to gas, which then caused an irregular flow of $scCO_2$ through the vessel or column. Unfulfilled expectations in the following decade resulted in that commercial equipment for both SFC and SFE almost completely disappeared from the market and analytical chemistry papers in the field became fewer.

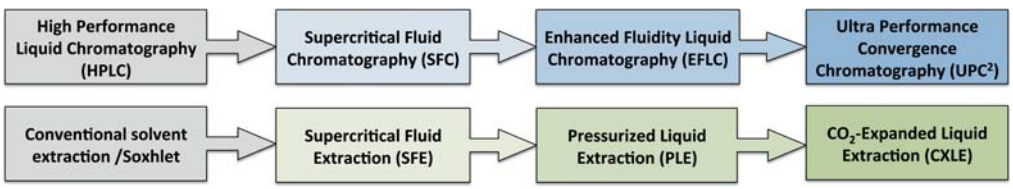


Figure 1. Development of CO_2-based chromatography and extraction since the 90's and until 2015.

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At the same time in the mid 90's, Richter et al. [1], came up with the idea of using SFE with 100% co-solvent, i.e. pressurized liquid extraction (PLE), trade named by Dionex as accelerated solvent extraction (ASE™). PLE enabled extraction of lipids using conventional organic solvents at elevated temperature and pressure. The elevated temperature, over the atmospheric boiling point of the solvent, resulted in lower viscosity, higher diffusivity and improved solubilization of the lipids. Hence, PLE offered fast and robust extraction of lipids with a relatively small consumption of organic solvents. It was perhaps the launch of PLE in the mid 90's that was the deathblow for the continuation of analytical-scale SFE.

In summary, SFC and SFE have developed from being rather conservative supercritical fluid technologies in the 90's to more open-minded flexible solutions as further described below and schematically shown in **Figure 1**.

CO_2-based chromatography – from SFC to UPC²

High-diffusion CO_2-based chromatography in the 90's used either neat $scCO_2$ as a mobile phase, or mixtures of $scCO_2$ and a polar co-solvent such as methanol in low volume percentage. There were two factions of SFC – one side exploring open capillary "GC-style" SFC with primarily neat $scCO_2$ as carrier fluid, and the other side developing packed column "HPLC-style" SFC using $scCO_2$ containing small volume percentage of for instance methanol or ethanol as mobile phase. Gradient elution in both types of chromatography set-ups was done by a gradual change in pressure and/or temperature. Since retention in SFC depends on both partitioning between the mobile phase (the supercritical fluid) and the stationary phase, as well as on the selective interaction with the stationary phase, strange phenomena of changing elution order of the peaks could happen when varying the pressure and/or the temperature. This is because at constant pressure, there is a solubility minimum at a certain temperature, which is referred to as the "cross-over" point. At this point, the retention factor in SFC reaches a maximum.

The unpredictability of elution order was a great challenge in method development in SFC. Other challenges for SFC in the 90's were issues with the relatively large dead-volumes of the equipment, which resulted in unnecessary losses in efficiency. Furthermore, techniques for injection of small volumes of sample into a supercritical fluid were not fully developed, and there were also a lack of robust stationary phases for packed column SFC. These aspects together with the unfulfilled expectation that SFC was supposed to replace both GC and HPLC at the time resulted in the "death of SFC".

In parallel to SFC research activities, Olesik et al. explored during the 90's the use of compressed CO_2 as an "additive" to organic solvents in packed column LC in what was called "enhanced fluidity liquid chromatography" (EFLC), see a recent review [2]. This idea aroused from the fact that mass transfer properties in a conventional liquid can be improved by adding a compressed gas such as liquid CO_2. For instance, at 40 °C the viscosity of methanol decreases from around 0.46 to 0.19 cP when increasing the mole fraction of liquid CO_2 from zero to 0,5 (see **Figure 2**).

The idea of enhancing the mass transfer properties by adding compressed CO_2 to an organic solvent comes from the field of gas expanded liquids (GXLs), for CO_2 called CXLs. In chemical engineering, GXLs are used in chemical synthesis and material processing as a rapid way to change the solvent and mass transfer properties of a liquid solvent [3]. What happens when the solvent is pressurized with CO_2 is that it volumetrically expands, but not necessarily leading to decreased density. Diffusivity increases, and the solvent properties change. For instance, when ethanol or methanol is pressurized with CO_2, the polarisability (dielectric constant) decreases. In chromatography, it is the drastic increase in diffusivity, which is attractive and is inherently leading to more efficient separations.

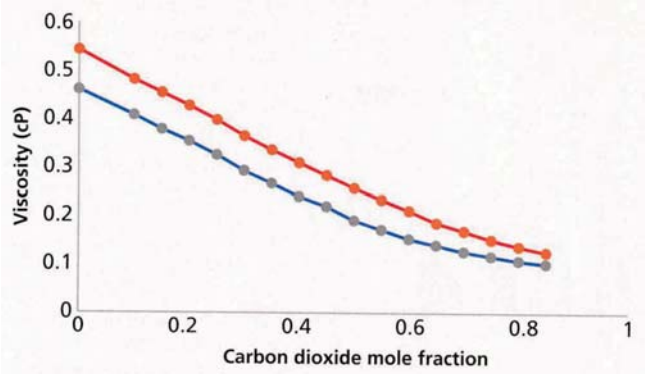


Figure 2. Variation of viscosity of a methanol/CO_2 mixture as a function of added amount of CO_2 at 25 °C (red curve) and 40 °C (blue curve). Printed with permission from LCGC North America [2].

A few years ago, chromatography with mixtures of compressed CO_2 and organic solvent as mobile phase in packed column LC has been developed even further through the launch of Waters Ultra Performance Convergence Chromatography (UPC²) system, along with Agilent and Jasco's similar SFC systems. What has changed is the view on gradient elution, in which an increase in molar fraction of co-solvent is applied rather than a change in pressure and/or temperature. This concept is in fact similar to what has been proposed by Olesik et al., but instead of adding compressed CO_2 to an organic solvent, the organic solvent is added to the compressed CO_2. A separation starts with a mobile phase consisting of $scCO_2$ containing a small volume fraction of co-solvent, for instance 1 vol% of methanol, and then the methanol content is gradually increased during the chromatography run, see **Figure 3**. As high volume fractions of co-solvent as 30% is often used in a separation method. For instance, in our own work, lipid classes from bilberry were se-

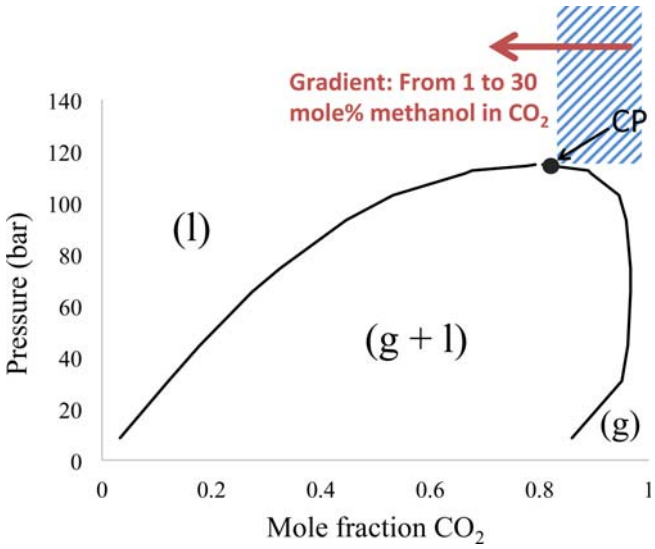


Figure 3. Phase equilibrium of methanol/CO_2 showing the isotherm for 60 °C.

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parated using a C18 column and a gradient of 2 to 50 vol% methanol added to $scCO_2$, [4].

In addition, since the general performance of equipment in LC, for instance the higher pressure and smaller dead volumes and inner diameters in ultra high performance liquid chromatography (UHPLC) equipment, also the new UPC^2 instrumentation has improved similarly. Furthermore, back pressure regulators (BPR) in combination with continuous rinsing with a make-up solvent through the BPR are used instead of capillary restrictors that kept on getting blocked. In addition, novel stationary phases suitable for UPC^2 have been developed, consisting of higher-strength silica with bonded C_8, C_{18}, 2-ethylpyridine, fluoro-phenyl, 2-picolylamine, diethylamine, high density diol, 1-aminoanthracene as well as bare silica columns. Several different types of retention mechanisms can be used, including reversed phase, normal phase, hydrophilic-interaction and size-exclusion chromatography. This recent development of SFC until today as well as basic principles of operation has been well described in a tutorial article by Nováková et al. [5].

Some points to remark are firstly the importance of assuring a one-phase mobile phase mixture, i.e. to make sure that a high enough pressure is maintained to stay within the one-phase liquid mixture, see **Figure 3**. Secondly, a major question is how the density, viscosity, diffusivity, compressibility and surface tension change while increasing the molar fraction of the co-solvent during the gradient run, and how this affects the chromatographic performance in terms of efficiency and selectivity.

More research is definitely needed in this area – most of the chemical engineering research conducted on CXLs has been done at vapor-liquid equilibrium (VLE), within "the envelope", see Figure 3. For sure, it is easier to produce and to study the properties of a CXL at two-phase VLE, since then the exact molar ratios in both the liquid phase and the vapor phase is known. However, in UPC^2 and similar techniques, this is not the case, and the temperature and pressure control is inadequate for knowing the exact molar fraction of compressed CO_2 respectively co-solvent. This is one of the challenges for fundamental research in CO_2-based chromatography.

Current and future trends in CO_2-based extraction

SFE has been through a slightly different development since the 90's. Back then, SFE was commonly conducted with either neat $scCO_2$, or $scCO_2$ mixtures containing 5–15 vol% of a co-solvent such as ethanol, and typically a pressure of 200–400 bar and a temperature of 40–60 °C. There was commercial analytical-scale equipment available from e.g. Agilent (then HP), Isco, Jasco and Applied Separations. What has happened since then, when the technology was new and it was eventually realized that the robustness and repeatability were unacceptable, is that the multi-vessel carousel-type of equipment for routine analysis were discontinued on the market. Currently, there are a few vendors that have equipment for analytical-scale SFE, also with a multi-vessel approach but with manual loading of the sample vessels rather than the carousel-type. BPRs with make-up solvent are used instead of capillary restrictors or nozzles.

The trend in lipid extraction in large-scale systems is to use pressures of 700 bars and above. It has since long been known that the solubility of lipids is directly proportional to the density of $scCO_2$ (and less dependent on the temperature). Many lipids are miscible with $scCO_2$ at pressures over 700 bar. However, analytical-scale SFE systems are not commercially available for pressures over 500 to 600 bar. This is a clear disadvantage in lipid applications.

Another aspect is the conservative view on the use of $scCO_2$ as a fluid with a relatively small addition of co-solvent to increase the polarity of the fluid. In SFE, there is still a large gap to PLE. Just like the development in chromatography, from SFC to EFLC and UPC^2, a similar approach should be applicable to SFE. Why not enable the use of any proportion of co-solvent to compressed CO_2 with the existing systems? There are only a few publications describing CO_2-expanded liquid extraction (CXLE), for instance the extraction of γ-linolenic acid from Arthrospira platensis (Spirulina) algae by CO_2-expanded ethanol [6]. Obviously, much research is remaining, considering for instance the lack of knowledge on solubility of lipids in CXLs, also including CXLs at one-phase liquid condition, as well as knowledge on mass transfer limitations in CXLE. Just as described above for EFLC and UPC^2, what is the actual density, viscosity, diffusivity, compressibility and surface tension, in addition to solvent properties like polarisability, acidity and basicity, of different CXLs? How can a certain molar ratio of CO_2/solvent at a certain pressure and temperature be accurately produced and used in analytical chemistry applications?

Conclusions

High-diffusion CO_2-based chromatography has developed into methodologies where there are no borders for the proportions of co-solvent to compressed CO_2. It is now possible to run SFC at high pressure and density with minimal or zero co-solvent addition, or EFLC with smaller proportions of compressed CO_2 added to a liquid organic solvent, or anything in-between.

High-diffusion CO_2-based extraction should experience a similar development, also in terms of available commercial equipment. In CXLE, lipids can be extracted with any appropriate liquid solvent, just like in PLE, and mass transfer rate is increased in the presence of compressed CO_2 rather than by increasing the temperature. This ought to be an attractive methodology for lipids sensitive to heat, such as certain fat-soluble vitamins and carotenoid pigments, as well as for lipids of high vapor pressure like certain essential oils.

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