Advantages and disadvantages of supercritical fluid chromatography engineering es...

Even though high performance liquid chromatography is a widely used technique for extractions of analytes in many classes, SFC has clear advantages over it. In HPLC a substantial amount of organic solvent is generated with each extraction, which then needs to be disposed. However, the disposal of the organic solvents is expensive at $5 – $10 per gallon, whereas SFC uses considerably less or no organic solvent which leads to a decrease in analysis costs [1]. In replacement of organic solvents an inert environmentally friendly mobile phase is used, often carbon dioxide which can be collected from the atmosphere, as it is energy efficient in the isolation of the desired products [2]. Also without the use organic solvents the product is more concentrated compared to HPLC where the solvent must be evaporated, without the need to evaporate any solvent there is a reduction in energy and labour costs [2].

SFC is similar to gas chromatography (GC) in that it has a lower viscosity and higher diffusion coefficient than HPLC which allows for quicker, more efficient separations as it more effective at entering porous solid materials than liquid solvents. The separation time can be cut down from hours or days to a few tens of minutes [3]. As seen in Table 1, supercritical fluids lie between liquids and gases, which allows for SFC to use features of both HPLC and GC.

Due to supercritical fluids having gas like and liquid like density it has a greater solvating power so SFC has a larger molecular range which includes non-volatile molecules which methods like GC do not include [1, 4]. Also, unlike GC which does not analyse thermally unstable compounds, SFC is able to due to the low critical temperatures of supercritical fluids such as carbon dioxide (31oC) [1] ; an advantage of supercritical fluid carbon dioxide is that

it has a varied solvating strength that allows for selective extractions [5]. Along with this by altering the temperature and/or pressure it is possible to achieve higher selectivity.

The range of detectors is also wider for SFC compared to GC or HPLC this is because in SFC the mobile phase can be liquid or gas like, so GC and HPLC detectors can be used [1]. For example SFC with flame ionization detection (FID) can provide quantification of resolved materials with a sensitivity of 0. 1 ng [4]. Due to the range of detectors available for SFC and the low critical temperature of the carbon dioxide mobile phase, the detection and analysis of thermally labile compounds has been successful [3, 5].

Another advantage SFC has over HPLC is separation of chiral compounds, in HPLC the process is very time consuming, in SFC however, due to the lower viscosity of the supercritical fluids, the chiral separation can be run at a flow rate of up to 5 times faster than that of the HPLC all while avoiding pressure build up. The higher flow rate of SFC consequently means that the productivity is higher than HPLC methods [2].

When used in large scale extractions, fluid carbon dioxide can be recycled and then reused this minimize the amount of waste generated [3].

Property

Gas

Supercritical Fluid

Liquid

Density g/cm3

(0. 6-2) x 10-3

0. 2-0. 5

0. 6-1. 6

Diffusion Coefficient cm2/s

(1-4) x 10-1

10-3-10-4

(0. 2-2) x 10-5

Viscosity g cm-1 s-1

(1-3) x 10-4

(1-3) x 10-4

(0. 2-3) x 10-2Table 1: Comparison of Properties of Supercritical Fluids, Liquids and Gases [1]

Due to the fact that SFC has features of both GC and HPLC, SFC has diversity in the columns that can be used which are either open tubular (GC) or packed (HPLC). In packed column SFC by choosing suitable column dimensions and particle size [6], this can cause an increase in the number of theoretical plates (over 100, 000) [2, 6].

Further advantage is SFC is very clean; mobile phase contaminants are usually trace quantities of other gases. The mobile phase is very free of

dissolved oxygen and is not particularly reactive and the mobile phase is easily and rapidly removed [2].

A disadvantage of using carbon dioxide as the mobile phase is it does not elute very polar or ionic compounds; this is overcome by using an organic modifier.

However, there are some disadvantages of SFC these include that if molecules are highly polar they are not soluble in the mobile phase.

Usually SFC only moves a small amount of a large specimen onto the column

Limited availability

However, these limitations have been overcome through instrumental modifications that more appropriately address purifications of micro-scale and nano-scale quantities of physiological molecules. More sophisticated 2D systems (2D-SFC) allow for the interfacing of 2 SFC columns having different column coatings or packing and thus provide for orthogonal separation capabilities [2].

Instrumentation used in SFC

Originally, SFC instruments were based on HPLC designs with some modifications, however now the design includes a pumping system, modifier module, post-column nozzle and a separator detector [2].

The mobile phase in SFC is pumped as a liquid and then heated up past supercritical temperature until it reaches the supercritical region. The mobile phase passes through the injection valve before the sample is introduced,

which carries the sample into the analytical column. To ensure the mobile phase stays supercritical, pressure restrictors are placed at the sides of the detector or at the end of the column. The pressure restrictors are heated as too avoid clogging [7].

As SFC uses a supercritical fluid as mobile phase, there are two possible types of column setup; one setup is HPLC like which consist of two reciprocating pumps these allow the mobile phase to mix and the introduction of a modifier, a packed column which are placed in an oven the detector used is an optical detector and the pressure and flow rates can be controlled separately [7]. Packed column SFC has recently become popular again over the past decade due to drug discovery and the pharmaceutical industry, as it offers the use of an environmentally friendly mobile phase, carbon dioxide, decrease in waste generation and provides purified materials even on a large scale, when used for drug discovery packed SFC is usually coupled with a mass spectrometer detector [2, 8]. In SFC there are lower eluent viscosity and higher diffusion coefficient which as a result lead to an increase in efficiency and a shorter separation time, the low viscosity causes only slight pressure drops which in turn allows for the flow rate to be quicker (3-5 mL min-1) compared to that of HPLC (typically ~1 mL min-1) [1, 8].

The other column setup is capillary SFC which is an extension of GC that includes a syringe pump and a capillary column inside a GC oven with a restrictor with a flame ionisation detector (FID), however, in capillary SFC the flow rate of the pump controls the pressure of the system [6, 7]. Other detection methods are also used for capillary SFC one method is Fourier transform infrared (FT-IR) spectrometry. Capillary SFC is used for high

separation power and is more suited for fluids with low density. However, capillary columns have some limitations these include sample loading capacity, detection limits and quantitation [6, 7].

As mentioned FID is mostly used for capillary SFC, although in certain cases FID can be used with packed column SFC when a non-flammable mobile phase is used. However, the mobile phase that is used is usually carbon dioxide which requires an organic modifier to deactivate any unbounded silanol groups in the stationary phase [10] thus causing the mobile phase to become flammable this in turn causes a high background signal and a loss of sensitivity. Alternatively, modifiers like esters or lower alcohols can be used in packed column SFC in order to improve the elution of polar compounds [9]. However, to avoid the use of modifiers, open-tubular capillary columns can be used, since silanol groups are not present in the stationary phase [10].

Compared to capillary columns, packed columns display higher efficiency per unit time; also separations can be transported directly from analytical or preparative liquid chromatography (LC) to SFC. Moreover, a standard liquid chromatograph can easily be converted into a supercritical fluid chromatograph [11].

It has was found that certain separations that were developed on a 50 Î¼m i. d. capillary column can be repeated with the same or better performance on a 1 mm i. d. (“ microbore”) packed column. The packed column system has the additional advantage of yielding excellent peak area precision. It is also

shown that the combination of water and formic acid is an effective modifier for CO2 which can be used with FID [6].

A study using the water and formic acid modifier was conducted by H. E. Schwartz et al. formic acid is used as it has low background noise and therefore is more favourable, however another problem arises when using this modifier and that large gradient ‘ humps’ appeared during the run, these were most probably because of organic impurities in formic acid. A way round this problem is that water is added to the carbon dioxide via the use of an ‘ aquafier’ system, the ‘ aquafier’ system used by H. E. Schwartz et al. was a “ 15 cm x 4. 6 mm i. d. silica column (100-200 mesh) that was saturated with ca. 40% w/w water”. The column was placed between the pump outlet and injection valve. A test mixture of the formic acid and water modifier was performed by H. E. Schwartz et al. and prodcued the chromatogram as seen in Figure 1[6].

Figure 1 – Chromatogram of a test mixture of formic acid/water/CO2 mobile phase.

Peak identification (from left to right): n-eicosane, anthraquinone, n-triacontane, tocopherol acetate, cholesterol [6].

In Figure 1 the baseline rises this was due to the pressure program used, however due to the addition of water to the mobile phase which prevented the accumulation of formic acid on the head of the column no ‘ hump’ is visible. In Figure 1 it can also be seen that all the peaks have good shape and resolution even for the more polar compounds like anthraquinone, tocopherol acetate and cholesterol [6].

Mobile phases and stationary phases used in SFC

In SFC the density of the mobile phase is about 200-500 times greater to that in gas chromatography. Compounds with high molecular weights are not usually detectable in gas chromatography, however with the density of the mobile phase being greater they can therefore be chromatographed [12]. A wide range of compounds have been tested for use as SFC mobile phases, however, a variety of these required special conditions, and would therefore not be suitable. This resulted in carbon dioxide CO2 being used as it was easily obtainable, low cost and safe [13], along with the critical temperature being 31oC and critical pressure being 73. 8 atm [14]. A problem with CO2 as a mobile phase in a packed column is that if CO2 mobilizes a species then there is a possibility that the compound will be irreversibly adsorbed onto the column, this is because of the high activity of most sorbents, this does not happen in capillary SFC as inert fused silica open-tubular column are used. To avoid adsorption onto the column, surface activity needs to be decreased; this has been achieved by using modifiers [14].

There are two main reasons why modifiers are added to the mobile phase, first is that only a small amount of modifier is added in order to deactivate the sorbent active sites, second is when the modifier is added in higher concentrations (level of modifier needed is â‰¥1%)it improves the solubility of the analyte in the mobile phase [14].

One problem with using modifiers is they have a high response when a FID is used; this high response causes an increase in the baseline. The alternative to using FID which helps relieve this problem is the use of a ultra-violet absorption detector, although it is not as applicable to organic compounds

compared to FID [14]. This is only true for packed SFC, as when capillary SFC is used most separations are done using only CO2, which is compatible with FID. Having only CO2 as the solid phase can cause slight defects on the chromatograms such as very broad peaks and not well resolved, as well as longer retention times, this is solved by adding a small amount of water to the mobile phase, hence improving the peaks and decreasing the retention time [12].

Modifiers which can be used with the mobile phase include methanol, acetonitrile, chloroform and formic acid. Methanol is the most popular modifier being used in both packed and capillary SFC, even though the addition of water speeds up elution of polar compounds in capillary SFC [12]; methanol has a greater effect when used with silica-packed columns [14]. The solubility of methanol, acetonitrile and chloroform in CO2 was studied by K. L. Maguire and R. B. Denyszyn, they found out that when the pressure is below 90 for methanol/CO2 there was little effect on solubility, but when raised above 90 there was a substantial increase. Acetonitrile/CO2 had very little pressure dependence but small temperature dependence. Finally, chloroform/CO2 both pressure and temperature had a small effect on solubility, when either was raised the solubility of chloroform increased [14].

Research by G. L. Pariente and P. R. Griffiths showed when carboxylic acid groups were present in the analyte the retention time was greatly increased while still using CO2 mobile phase. The cause of this could be due to that the solubility of these polar molecules is low and the solvation is not great enough to overcome the strong hydrogen bonds. The alternative mobile phase used was chlorofluorocarbon (CCl2F2), in comparison to CO2 which

had a capacity factor greater than 20 for isophthalic acid; CCl2F2 had a capacity factor of 3. 9. These results suggest that CCl2F2 has sufficient free energy of solvation to overcome the hydrogen bonds [14].

Even though CO2 is the most extensively used mobile phase it is no more polar than hexane [15], so alternatives including CCl2F2 have been investigated, however the critical temperatures must not be too high as one of the main advantages of SFC is that elution can take place at mild temperatures. Another example is ammonia (NH3), as it possesses a high dipole moment and relatively low critical temperature, however supercritical NH3 reacts with siloxane linkages and when left for an extended time the siloxane stationary phase for capillary SFC breaks down too [14]. Therefore, a more useful way of eluting polar compounds is CO2 and the use of a modifier [15].

For packed SFC more or less all of the stationary phases used in HPLC are used in SFC, most of these are ‘ silica-based, chemically bonded or encapsulated, or polymeric’ [8]. Evaluation of stationary phases of SFC was originally carried out by Schoenmakers et al. this was however, only done using pure CO2 as the mobile phase, and certain phases did not perform well, if a modifier was used these phases would have performed better.

When CO2 and a modifier is used as a mobile phase the stationary phase also becomes modified in that both CO2 and the modifier adsorb onto the stationary phase. Depending on the stationary phase depends on the level of adsorption, for CO2 all phases adsorb the same but more polar phases adsorb more modifier than less polar phases. This causes the stationary

phase to become more polar than the mobile phase, which in turn will cause polar solutes to interact more with stationary phase increasing retention time.

Other stationary phases that have been studied include ‘ octadecylsiloxane-bonded silica (ODS), cyanopropylsiloxane- bonded silica, divinylbenzene-ODS, polydimethylsiloxane and porous graphitic carbon (PGC) stationary phases in supercritical’ [8].

In capillary SFC a problem arises in that normal GC stationary phases dissolve in the supercritical fluid mobile phase as they have a high solvating power. In order to correct the problem a non-extractable stationary phase is needed, examples of this are bonded phase where the stationary phase is attached to the column to surface groups via covalent bonds and cross linked phase where polymer chains within the stationary phase are attached to each other.

In order to create non-extractable stationary phase, the process of ‘ coating’ must be undertaken, there are two types of coating, dynamic and static. The most favoured for SFC is static, as dynamic can lead to poor column efficiency and a thick stationary phase is not possible. In static coating the stationary phase is first dissolved in supercritical fluid and forced into the column, to avoid the removal of the phase cross link phase is used as it occurs between the polymers and not between polymer and substrate, and therefore can be applied to glass and fused silica columns [16].

Conclusion