Supercritical Fluid Chromatography

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In supercritical fluid chromatography, a supercritical fluid is used as the mobile phase and the stationary phase is usually a thin liquid film which is bonded to the surface of support materials or the capillary column wall. Super critical fluid chromatography inherits the best features of the gas and liquid chromatography and results in efficient separations.

A super critical fluid is formed by heating a substance above its critical temperature. At or above the critical temperature of a substance, its vapor cannot be liquefied by applying pressure. Therefore, above the critical temperature, a distinct liquid phase does not exist. Critical pressure is the vapour pressure of a substance at its critical temperature. The densities and viscosities of super critical fluids are intermediate to those of gaseous and liquid states.

Carbon dioxide, nitrous oxide, ammonia and n-butane have critical temperatures of 31.3 ^{o}C, 36.5 ^{o}C, 132.5 ^{o}C and 152.0 ^{o}C, respectively and critical pressures of 72.9 atm, 71.7 atm, 112.5 atm, and 37.5 atm respectively. Out of the above compounds, carbon dioxide is preferred as a mobile phase in super critical fluid chromatography as reaching the critical temperature and pressure is easier and economical in practical operations. Moreover, as carbon dioxide is readily available in the atmospheric air, it is a cheaper source. Carbon dioxide is safe in handling and can be recycled. Therefore, in industrial and academic usage, carbon dioxide is more often used as the super critical fluid mobile phase.

A super critical fluid acts as a solvent when an analyte is dissolved. However, the intermolecular forces of a supercritical fluid are weak. Therefore, the analyte or the solute that dissolves in the supercritical fluid diffuse rapidly through the fluid. Due to this property, the super critical fluid mobile phases dissolve large non-volatile molecules such as n-alkanes (having 5 to 30 or more carbon atoms), di-n-alkyl phthalates (having 4-16 carbon atoms), and polycyclic aromatic hydrocarbons. When using a supercritical fluid as a mobile phase, analytes are easily recovered compared to the liquid chromatographic techniques. For example, when carbon dioxide is used as the mobile phase, after the analysis, pressure is reduced and fluid is evaporated under ambient temperature to recover the analyte with much ease.

Super critical fluid chromatographic mobile phases have low viscosity. Therefore, the columns are longer than those used in liquid chromatography. The liquid film stationary phases which are used in liquid chromatography are most often used in super critical chromatographic stationary phases as well. Both open tubular and packed columns are used in super critical fluid chromatography although open tubular columns are more common. Internal coating consists of polysiloxanes which are bonded or cross linked to surface of silica particles or to the inner silica wall of capillary tubing. The polar stiationary phases consists of cyano, diol and amino groups and low polarity stationary phases consistes of C_{18}, C_8 and C_4 groups.

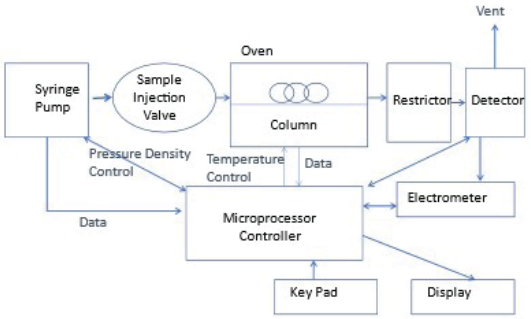

Figure 1. Schematic diagram illustrating the instrumentation of super critical fluid chromatography.

A thermostated column oven which is similar to gas chromatographic column oven is used so that the temperature of the mobile phase can be controlled. Restrictor or back pressure regulator is used to control the pressure in the column. The back pressure regulator also convert the eluent from a super critical fluid to a gas before transfering to the detector.

The density of a super critical fluid can be varied by varying pressure. When the pressure is increased, the density increases and it in turn increases the solvent

power. This results in reducing the retention factor of the analyte. (Figure 2)

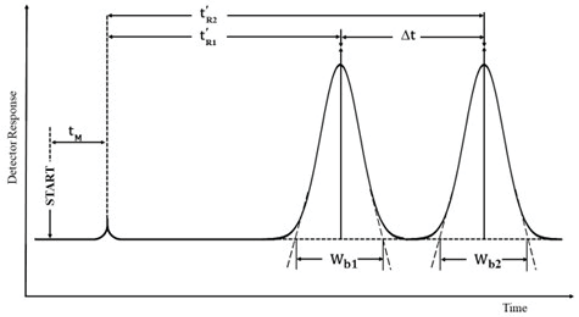

Figure 2. Peak separation in a chromatogram.
t_M:Column hold up time.
t'_{R1}, t'_{R2}: Adjusted retention times.
Retention factors for two compounds are $t'_{R1}/_{tm}$ and $t'_{R2}/_{tm}$.

Therefore, in order to obtain good resolution, (Figure 3) pressure programming is used in super critical fluid chromatography.When the separation of analytes are carried out with isobaric pressure, it is possible to have overlaps of peaks, higher retention times amd braodning of peaks. Therefore, a linear pressure gradient can be applied in pressure programming which provides high resolution, low retention times and efficient peaks.

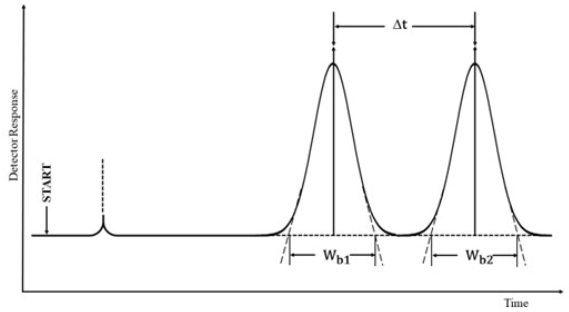

Figure 3. Resolution between two peaks in the chromatogram equals to $2\Delta t/(W_{b1}+W_{b2})$ where W_{b1} and W_{b2} are peak widths at the base.

Due to above reasons, the mobile phase in the super critical fluid chromatography plays a role in both the transport of molecules and the retention factor. Analyte gets soluble in the mobile phase due to solvent-solute interactions. The solvent power is a function of both the chemical composition and the density of the fluid. Therefore, the selectivity factor, α, (Equation 1) can be changed by changing the mobile phase and the column pressure.

$$\alpha = \frac{t'_{R1}}{t'_{R2}} \qquad (1)$$

As carbon dioxide is a non polar molecule, polar organic modifiers are introduced in small concentrations to modify the selectivity factor values. A modifier is needed to obtain a good resolution when the mobile phase is non polar and the analyte is polar. Gradient elution is used with low to high modifier concentrations. Under these conditions, the low polarity compounds may elute first followed by high polarity compounds. Methanol, isopropanol, acetonitrile, tetrahydrofuran and dichloromethane are used as modifiers. Methanol is the most commonly used modifier due to the reasons like availability, low cost, complete miscibility with carbon dioxide, low toxicity and the low UV cut off range (205 nm) where solvent absorption will not interefere with the detection process. Addition of about 5.5% methanol reduces the diffusion coefficient of the mobile phase by around half. However, when the modifier concentration increases, the viscocity of the mobile phase and the column pressure drop increases. Therefore, the modifier concentration has to be kept around an optimum value suitable for the particular separation.

In some occasions, with the usage of the column, the surface silanol groups can be exposed to the mobile phase. When a highly polar molecule is analyzed, they can retain in the silanol active sites, without proper elution. As a solution to this problem, a highly polar molecule which is often an acid or a base is added to the mobile phase as a co-solvent. The additive can cover the surface site. Moreover, the additive can form an ion pair with the solute and suppress the solute ionization. Therefore, the solute elutes without retaining in the surface active sites.

Many detectors are compatible with super critical fluid chromatography including flame ionization detector, mass spectrometric detector, flurescence emision, thermionic, flame photometric, UV and IR detectors.

Super critical fluids have intermediate properties to those of gases and liquids. Therefore, supercritical fluid chromatography comprise of the best feature of gas chromatography and liquid chromatography. The low viscocity of the super critical fluid mobile phase

results in high flow rates and faster separations. The diffusion rates of the analytes in super critical fluids are intermediate to those of gases and liquids. Therefore, the rate of band broadening in supercritical fluid chromatography is less compared to gas chromatography but high compared to liquid chromatography. In super critical fluid chromatography, minimum plate height (maximum efficiency) is achieved in less time, so the separation requires only small retention times.

When an analyte dissolves in the super critical fluid after injection to the instrument, it dissolves in the super critical fluid at much lower temperatures than a liquid, which resembles volatilization. The vapour pressure may get 1010 greater than its original solid or liquid state. Therefore, the technique is useful to separate high molecular weight compounds, thermally unstable compounds, polymers and biological molecules at low temperatures.

In super critical fluid chromatography, the solvation properties are similar to liquid and viscosities are similar to gases.Therefore it has many advantageous over gas chromatography and liquid chromatography. Super critical fluid chromatography using carbon dioxide is performed between 40-60 ^{o}C. Therefore, it is relatively convenient to analyze non-volatile and thermally labile compounds. There are inherent features arising from the low viscosity of the super critical fluids such as fast equilibriation than high performance liquid chromatography, faster separations, less pressure drops across the column, easy analysis of otherwise adsorptive compounds, ability to use open tuular columns and excellent reproducibility. The super critical fluid chromatography has a wide range of detector compatibility. The resolving power is also greater than five times that of high performance liquid chromatography. As less hazardous mobile phases and modifiers are used, the generation of toxic waste is less. Super critical fluid chromatography has wide applications in separations including drugs, foods, pesticides, herbicides, polymers, fossil fuels, explosives, propellants, polycyclic aromatic hydrocarbons, natural products and also the ability to perform chiral separations.

Problems:

1. Draw and label the phase diagram for carbon dioxide.
2. Draw the graph of viscocity versus mole fraction of the modifier concentartion when methanol is used as the modifier in super critical fluid chromatographic separations where carbon dioxide is used as the mobile phase.
3. Write down the factors which affect the retention in super critical fluid chromatography.
4. Briefly explain why the super critical fluid chromatography is preferred over high performance liquid chromatography for the analysis of natural product extracts consisting long chain alkanes in food manufacturing industry.
5. Briefly explain why the supercritical fluid chromatography is preferred over gas chromatography for the analysis of high molecular weight polycyclic aromatic hydrocarbons.

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