Solubility Studies Utilizing a Phase Monitor

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Introduction

Supercritical Fluids (ScF) are attractive solvents due to their inherent properties:

Variability of Density,
Lower viscosity than liquids,
High Diffusivity, and
"Tunable" Solvation.

Interest in Supercritical Fluid technologies over the last few decades has been demonstrated by the extensive measurement of its properties. Information pertaining to solubility has been utilized to establish technical and economic feasibility, especially within the realm of SFE (Supercritical Fluid Extraction). However, solubility is also a formidable hurdle in the realm of preparative chromatography.

Most scaled-up techniques of chromatography are contingent upon analyte solubility. Liquid- phase chromatography readily affords the opportunity for bench top solubility studies.

Issue

Supercritical Fluid Chromatography (SFC) exploits the highly attractive properties of ScF. To achieve a pseudo-ScF phase, elevated pressures and temperatures are employed. Such a reality does not readily yield solubility

studies, of ScF systems, as a bench-top practice. Therefore, it is difficult for preparative scale SFC scientists to predict solubility of an analyte.

One “rule of thumb” stated by SFC manufacturers is:

“*any solute soluble in methanol or a less polar organic solvent will elute in SFC.”*

This rule certainly holds true for analytical SFC, however, some classes of compounds have limited solubility in carbon dioxide and modifier. This reality is, at times, not easily detectable on an analytical scale.

Over the past few decades, the solubility of commercially available solids and liquids in supercritical CO_2 have been measured extensively. However, preparative separation scientists in the pharmaceutical arena do not, usually, have the luxury of working with commercially available materials on a consistent basis.

Proposed Solution

Utilization of a bench-top phase monitor, a modified version of Supercritical Fluid Technologies’ design,(Figure 1) would serve a preparative-scale SFC chromatographer as a useful tool for determining optimum concentrations for a preparative scale-up.

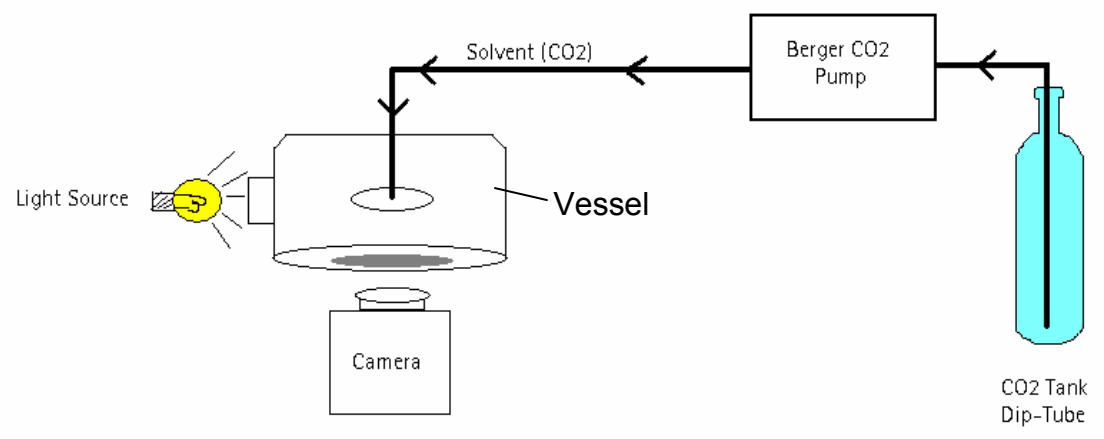
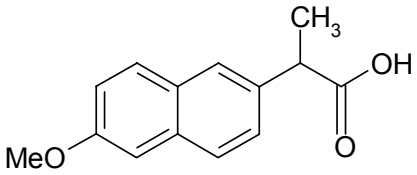


Figure 1: *General schematic of experimental apparatus, the phase-monitor. Sample would be introduced to the high pressure vessel at various concentrations in the organic modifier volume. Phase monitor modifications were provided through in-house fabrication and control of pressure was accomplished by means of a Berger CO_2 pump. Internal volume of modified vessel is 7 mL.*

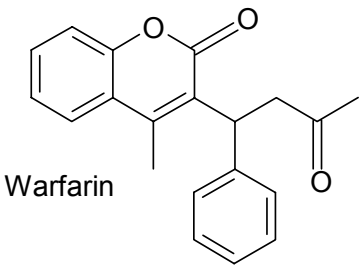
Proposed Experiments

Utilizing commercially available, "pharmaceutical-like" compounds (Figure 2), we wish to measure solubility under a variety of temperatures, pressures, modifier (I.e. IPA, MeOH, EtOH) and modifier concentrations. The data will assist an analyst to determine method parameters that are optimized for analyte solubility. The results optimized for solubility will be compared to resolution optimized chromatographic parameters to determine most efficient method to utilize for preparative chromatography.

The column utilized for this experiment is inconsequential due to the fact that the purpose of the study is to determine optimal mobile phase solubility for enhanced loadability on a preparative scale.



Naproxen



Warfarin

Figure 2: *Commercially available pharmaceutical molecules of various structure classes and solubility to be employed in our experiment.*

Results

The first stage of our experiment was to determine the analyte's solubility in three different organic phases (Table 1).

Sample	MeOH	IPA	EtOH
Naproxen	60.8 mg/mL	37.4 mg/mL	46.7 mg/mL
Warfarin	23.5 mg/mL	12.8 mg/mL	16.3 mg/mL
1:1 Mixture	33.3 mg/mL	14.3 mg/mL	20.0 mg/mL

Table 1: *Solubility of selected analytes in three organic phases at atmospheric temperature (30 degrees C) and pressure (Bench-top Technique).*

The solvent that yielded the best solubility for each analyte was utilized as the strong solvent or modifier in the analytical SFC method development on a Berger, 4.6x 250 mm, 60A, 6 μ Silica column (Figure 3).

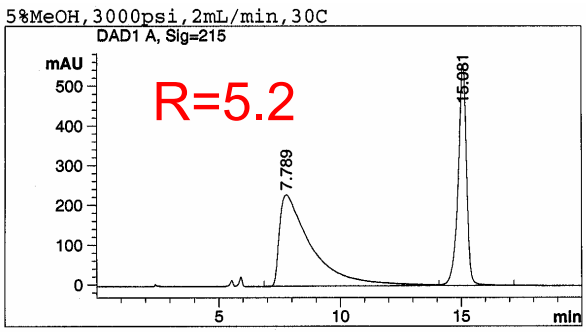


Figure 3: *Analytical SFC chromatogram with optimized resolution at 30 degrees Celsius. 16.9 μg injection.*

Based upon optimal solvent (determined in Table 1), the analyst determined solubility of the components at several solvent strengths and pressures via the Phase Monitor (Figure 4)

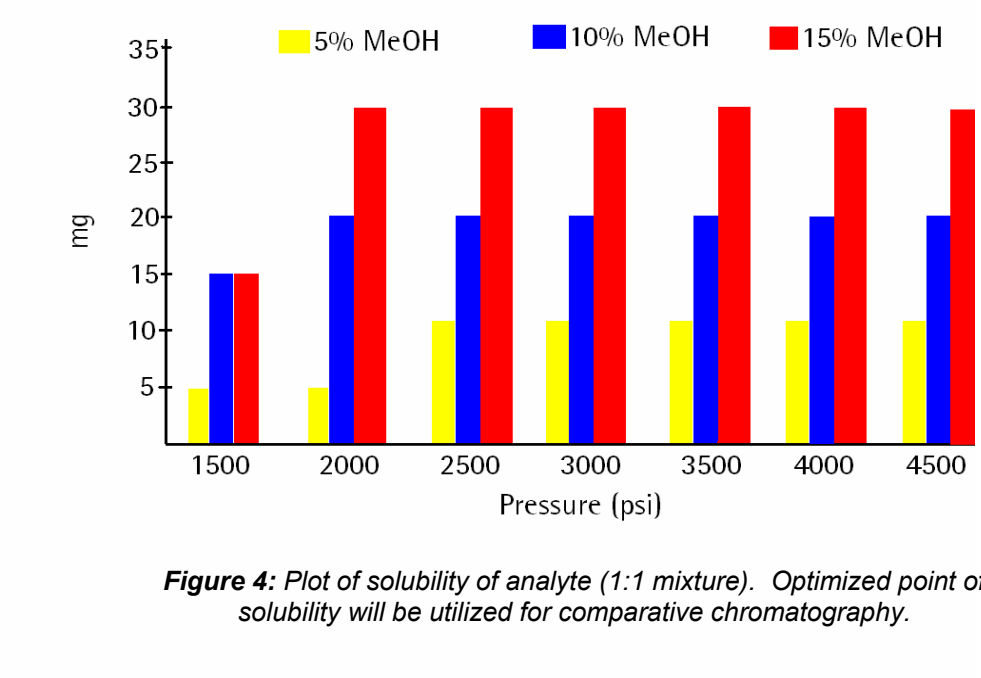


Figure 4: *Plot of solubility of analyte (1:1 mixture). Optimized point of solubility will be utilized for comparative chromatography.*

Solubility of this particular analyte, in the phase monitor, appeared to correlate to the solubility in the present volume of organic solvent.

Resultant solubility optimized conditions will be run on an analytical scale to measure the resolution of the components (Fig. 5).

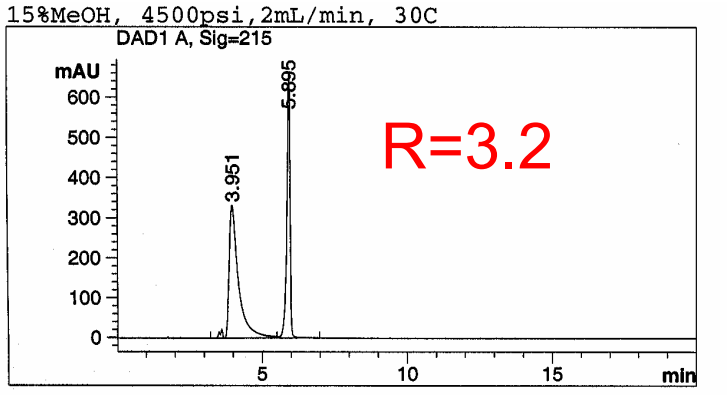


Figure 5: *Analytical SFC Chromatogram of conditions that are optimized, at 30 degrees Celsius, for solubility. 16.9 μg injection.*

The two methods were run on a Berger Mini-Gram system on a 10x 250 mm, 60A, 6μ Silica column, with an 200 μL loop Alcott autosampler, and Varian Pro-Star detector (λ=250 nm)(Table 2, Figure 6) for comparison.

Chromatogram Optimized for	Total Purified	Conc. Injection	# Inj. (clean -up)	Run Time (min)	Vol. of Organic solvent	Purity/ Recovery Peak 1	Purity/ Recovery Peak 2
Resolution	275 mg	55mg/200μL in DMSO*	5 (2)	119	59.5 mL	96.8%/ 86.3%	100%/ 69.2%
Solubility	275 mg	40mg/200μL in DMSO*	8 (0)	25	35.6 mL	98.9%/ 96.9%	99.7%/ 95.1%

Table 2: *Results of scaled-up purification efforts*
** sample injected in pure DMSO to maximize the loading*

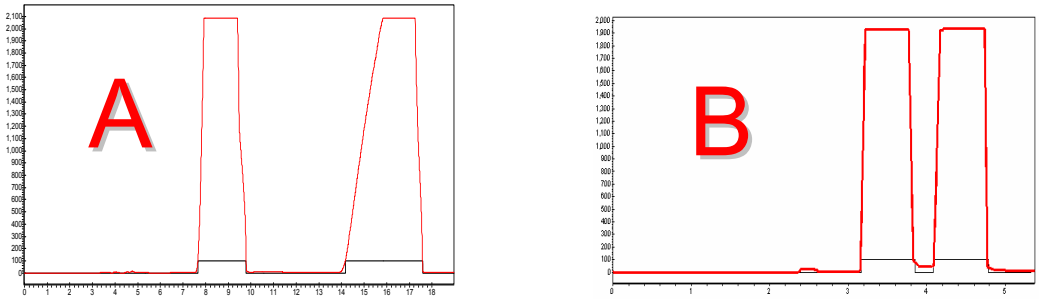


Figure 6: *Scaled-up SFC Chromatogram of conditions* ***A)*** Optimized for resolution (55mg/inj) and, ***B)*** Optimized for solubility (40mg/inj),

Note the poor peak shape in chromatogram Fig. 6A. Also the quantity injected with the resolution-optimized method was limited to solubility of analyte in DMSO and injection loop size.

One advantage demonstrated by the method optimized for solubility, chromatogram Fig. 6B, was the reproducibility of chromatography. Such reproducibility facilitates a sequence that stacked injections, thereby reducing purification time and solvent consumption.

Conclusions

It has, therefore, been demonstrated that when preparing to scale-up SFC chromatography, solubility studies via a phase monitor are critical in optimizing the efficiency of the purification. Further studies will need to include the effect of temperature in the optimization process.

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