CHAPTER II

TRAPPING CAPACITIES OF THREE SOLID-PHASES FOR SUPERCRITICAL FLUID EXTRACTION WITH PURE CARBON DIOXIDE

2.1 INTRODUCTION

A successful off-line supercritical fluid extraction experiment may be divided into two main processes. First, if we assume that the analyte is soluble in the supercritical fluid and the interaction between the analyte and matrix can be overcome, the analyte is removed from its matrix. Second, upon removal of the analyte from its matrix, the analyte is trapped or concentrated prior to analysis. Currently there are three main trapping systems commonly used today. The first and second involve the concentration of the analyte onto a solid surface. This solid surface is either a solid sorbent such as the chromatographic bonded phase octadecyl silica or an inert solid substrate such as stainless steel balls. The third trapping system commonly involves the collection of the analyte directly into a liquid solvent such as methanol. There have been numerous reports illustrating the importance of the solid-phase or liquid-phase composition, trapping temperature, flow rate, and the supercritical fluid composition (unmodified versus modified) on trapping. [1-15]

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Footnotes continued on next page

As SFE gains popularity and becomes a common industrial sample preparation technique, the optimization of the extraction and trapping parameters will continue to play an important role. If solid-phase trapping is to be used successfully on the preparative scale, it is important to determine the amount of solid-phase that is needed to retain all extracted analyte. This Chapter outlines the experimental determination of the trapping capacity of three solid-phases. A test mixture consisting of five different chemical classes which varied in polarity and volatility was spiked onto sand and extracted under identical conditions at three different spike masses. **Table 2.1** lists the analytes investigated as well as their corresponding molecular weights, boiling points, and melting points. [16] A tandem liquid trap was used to retain any analyte exiting from the solid-phase trap. Percent recoveries for the solid-phase rinses and tandem liquid traps at all spike masses are reported. Analyte breakthrough as a function of trap composition will be compared and discussed.

2.2 EXPERIMENTAL

Extraction

Extractions were performed on the Isco/Suprex Prepmaster (Lincoln, NE). Carbon dioxide (SFE/SFC grade) with helium headspace was donated by Air Products and Chemicals Co. (Allentown, PA).

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Table 2.1. Molecular weights, boiling points, and melting points of compounds investigated*

Compound	Molecular Weight, amu	Boiling Point, °C	Melting Point, °C
Acetophenone	120.16	202.6	20.5
N,*N*-Dimethylaniline	121.18	194	2.45
Naphthalene	128.19	218	80.5
2-Naphthol	144.19	288	96.0
n-Tetracosane	338.67	391.3	54

*Information taken from reference 16

A polarity test mixture consisting of acetophenone, *N,N*-dimethylaniline, naphthalene, 2-naphthol, and *n*-tetracosane (C_{24}) was prepared in methylene chloride. Approximately 5.4 grams of Ottawa Cement Testing sand (Fisher Scientific, Raleigh, NC) was placed in a 3.5 mL Keystone Scientific (Bellefonte, PA) extraction vessel. The test mixture was spiked onto the sand and extracted at three different total spike masses: 2.5 mg, 10.0 mg, and 20.0 mg or 0.5 mg, 2.0 mg, and 4.0 mg per component.

The three solid-phases investigated in this study were 80-100 mesh glass beads (Applied Science Laboratories, Inc., State College, PA), a 50/50 (w/w) mixture of 30 μm octadecyl silica (Keystone, Bellafonte, PA) and glass beads, and a 50/50 (w/w) mixture of 100-120 mesh Porapak Q® (Alltech, Deerfield, IL) and glass beads. Each solid-phase trap was prepared by filling the empty container provided by the vendor to approximately 90% with the appropriate phase(s). The glass beads trap contained approximately 1.5 g of glass beads, the 50/50 (w/w) octadecyl silica/glass beads trap contained approximately 0.5 g of octadecyl silica and 0.5 g of glass beads (total solid-phase mass of 1.0 g), and the 50/50 (w/w) Porapak Q®/glass beads trap contained approximately 0.4 g of Porapak Q® and 0.4 g of glass beads (total solid-phase mass of 0.8 g). A tandem liquid trap, **Figure 2.1,** consisting of 12 mL methylene chloride was employed to trap any unretained analytes exiting from the solid-phase trap. Upon completion of the extraction, approximately 4 mL of methylene chloride remained in the tandem liquid trap collection vial. All extractions were performed in two steps, at an oven temperature of 35 °C and at a liquid flow rate of 1.0 mL/min. The total mass of CO_2 delivered was 96.8 g (i.e. 39.8 g at a CO_2 pressure of 220 atm (0.88 g/mL CO_2 density) and 57.0 g at 340 atm (0.95 g/mL CO_2 density)). The solid-phase trap was cooled to +5 °C while the restrictor temperature was maintained at 100 °C. The tandem liquid trap was maintained at room temperature. Upon completion of the extraction, the trap temperature was raised to 25 °C while the restrictor temperature was lowered from 100 °C to 30 °C. The solid-phase was then rinsed twice with 5 mL aliquots of methylene chloride.

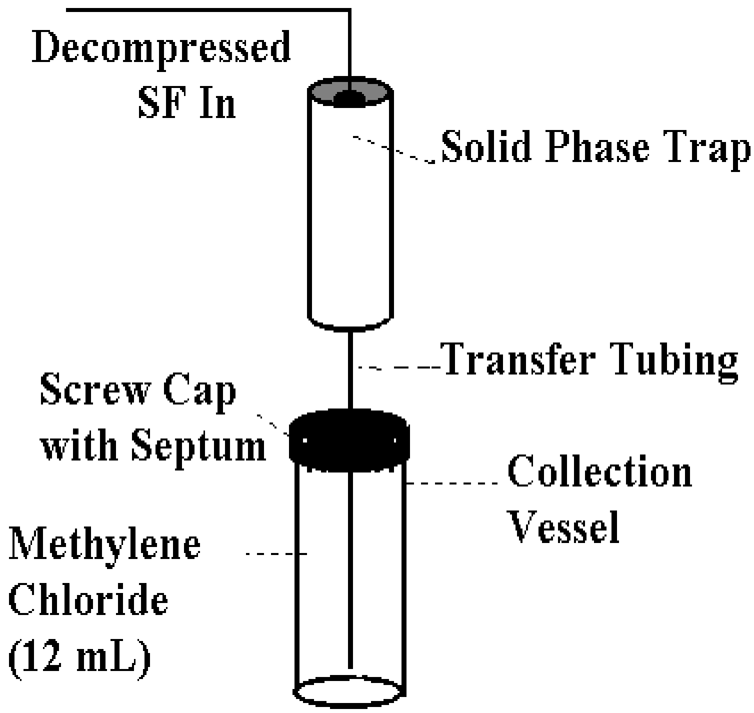

Figure 2.1. Tandem solid-phase/liquid trap

Extract Analysis

After completion of the extraction the solid-phase rinses and the tandem liquid trap were sonicated for 5 minutes to aid in the removal of any dissolved carbon dioxide. A 20,000 ppm pyrene internal standard (100 μL) was then added to the solid-phase rinses and the tandem liquid trap, and a portion of each solution was transferred to GC vials for analysis. The purpose of adding internal standard to the trap rinse and tandem liquid trap was to ensure good quantitation in the case of variation in solid-phase trap rinse volumes or evaporation loss in the tandem liquid trap during the extraction. Values corresponding to 100% recovery were obtained by adding 100 μL of the appropriate test mixture solution and 100 μL of internal standard to an empty collection vial and diluting to 5 mL with methylene chloride. Each solution corresponding to 100% recovery was run three times and the peak area ratios (peak area of analyte/peak area of internal standard) were averaged. Experimental percent recoveries were then calculated and reported.

A Hewlett Packard (Wilmington, DE) 5890 Series II GC equipped with a 7673 autosampler, a flame ionization detector, and a Vectra QS/20 Chemstation were used for all extract analyses. A DB-5 MS (5 % phenylmethylsiloxane) column (30 m long X 0.25 mm i.d.) with a film thickness of 0.25 μm was used. A 1 μL splittless injection was performed. The initial oven temperature was held at 50 °C for one minute and ramped to 140 °C at 15.0 °C/min., then ramped to 300 °C at 30.0 °C/min. and held for 3.0 min. The injector was maintained at 275 °C, and the flame ionization detector was maintained at 325 °C.

2.3 RESULTS AND DISCUSSION

There was several experimental difficulties encountered while performing this study. A one step extraction at high density was initially attempted; however, variable restrictor (100 °C) plugging resulted due to the high amounts of initially extracted analyte, therefore, a two step extraction was warranted which resulted in a long extraction time. The extraction was first performed at low densities to remove an initial amount of analyte followed by the second step at higher densities to remove the remaining analytes. The time needed to completely remove the analytes at the higher spike masses was 1.75 hours. Initially the trapping capacity at 1.0 mL/min and 4.0 mL/min. were to be determined; however, at a liquid flow rate of 4.0 mL/min (measured at the pump) complete loss of the tandem liquid trap solvent as well as restrictor plugging were observed. The flow rate was thus limited to 1.0 mL/min., resulting in an additional experimental difficulty.

With these restrictions, the trapping capacity or the total amount of analyte that is able to be retained on a solid-phase before significant breakthrough occurs has been examined for three different solid-phases. **Table 2.2** lists the recoveries obtained for the solid-phase rinses and tandem liquid traps for the 50/50 (w/w) octadecyl silica/glass beads trap. Each entry represents the average of at least two replicates. The solid-phase trapping capacity was shown to be a function of the chosen analyte. All test analytes showed quantitative recoveries of greater than 90% at the 2.5 mg total spike mass. At a total spike mass of 10.0 mg significant breakthrough from the solid-phase trap into the tandem liquid trap of a more volatile analyte, naphthalene, occurred; however, significant breakthrough of all other test analytes was not seen. Breakthrough or the appearance of analyte in the tandem liquid trap was seen for all analytes at the 20.0 mg total spike mass. Assuming that the trapping capacity of the glass beads is minimal at +5 °C, it would appear that the trapping capacity of octadecyl silica for all analytes except naphthalene was less than 4 mg/g of octadecylsilica. Since these five analytes were co-extracted, the trapping capacity might be higher if each analyte had been individually extracted. A lower solid-phase trap temperature may also lead to a greater net trapping capacity.

A glass beads trap was then investigated to better determine the role of the octadecyl silica phase on the trapping of the analytes on the octadecylsilica/glass beads

Table 2.2. Analyte percent recovery for the 50/50 (w/w) octadecylsilica/glass beads trap (1.0 g)

Solid-Phase Rinse**

Total Spike Mass, mg	Acetophenone	Dimethylaniline	Naphthalene	2-Naphthol	*n*-Tetracosane
2.5	96.2	90.0	96.1	100.4	94.7
10.0	87.8	86.2	34.0	82.1	88.7
20.0	75.8	47.9	17.0	89.0	86.6

Tandem Liquid Trap***

Total Spike Mass, mg	Acetophenone	Dimethylaniline	Naphthalene	2-Naphthol	*n*-Tetracosane
2.5	ND*	ND	ND	ND	ND
10.0	ND	ND	56.3	ND	ND
20.0	22.0	40.1	75.6	0.3	9.3

Mass Balance****

Total Spike Mass, mg	Acetophenone	Dimethylaniline	Naphthalene	2-Naphthol	*n*-Tetracosane
2.5	96.2	90.0	96.1	100.4	94.7
10.0	87.8	86.2	90.3	82.1	88.7
20.0	97.8	88.0	92.6	89.3	95.9

* ND indicates none detected
** Percent of extracted analyte retained on the solid-phase material
*** Percent of extracted analyte collected in tandem liquid trap
**** Percent retained on solid-phase + tandem liquid trap

SFE Conditions: CO_2 Pressure: 220 atm (step 1), 340 atm (step 2); Mass of CO_2 Delivered: 39.8 g (step 1), 57.0 g (step 2); Oven Temperature: 35 °C; CO_2 Liquid Flow Rate: 1.0 mL/min.; Restrictor Temperature: 100 °C; Solid-phase Collection Temperature: 5 °C; Tandem Liquid Trap: 12 mL Methylene Chloride (room temperature); Solid-Phase Desorption Temperature: 30 °C; Solid-Phase Rinse: 5 mL Methylene Chloride (2 times)

Assay - see Experimental

glass beads trap. **Table 2.3** lists the recoveries for the solid-phase rinses and tandem liquid traps for the glass beads trap. Acetophenone, *N*,*N*-dimethylaniline, and naphthalene could not be successfully retained on the glass beads trap at any spike mass, indicating their retention on the octadecylsilica/glass beads phase was aided by their interaction with the octadecylsilica material. The less volatile analytes 2-naphthol and *n*-tetracosane were retained successfully on the glass beads trap showing quantitative recoveries of greater than 92% at all spike masses. The total trapping capacity of the glass beads trap for the three test analytes was therefore less than 0.5 mg/1.5 g under the extraction and trapping conditions chosen. For 2-naphthol and *n*-tetracosane, the glass beads function as well or better than the octadecylsilica/glass beads trap (e.g. > 2 mg/1.5 g of glass beads).

The Porapak Q®/glass beads trap was shown to be in general the most successful solid-phase exhibiting the best trapping capacity of all three solid sorbents investigated. **Table 2.4** lists the recoveries for the solid-phase rinse and tandem liquid trap at all spike masses. Recoveries of greater than 88% on the solid-phase alone were seen for all test analytes at 0.5, 2.0, and 4.0 mg per analyte. Considering that the mass of Porapak Q® was 0.4 g in the trap, the trapping capacity for each analyte was at least 10 mg/g of Porapak Q®. Identical extraction and trapping conditions were used for all three solid-phases investigated. The appearance of test analytes in the tandem liquid trap after the Porapak Q®/glass beads at all spike masses was believed to be due to non-optimized trapping conditions rather than exceeding the solid-phase's trapping capacity. Higher spike masses could not be performed due to extraction difficulties such as restrictor plugging.

The trapping of the five test analytes when employing 100% CO_2 was shown to be easily achieved at the lower spike masses; however, the addition of modifier may be required for many real world analytes/matrices. It is believed that modified fluids will have an effect on the trapping capacity of both solid-phase and liquid phase traps. Numerous studies have shown that high trap temperatures and low flow rates favor successful trapping when employing modified fluids. On the other hand, liquid phase trapping must

Table 2.3. Analyte percent recovery for the 100% glass beads trap (1.5 g)

Solid-Phase Rinse**

Total Spike Mass, mg	Acetophenone	Dimethylaniline	Naphthalene	2-Naphthol	*n*-Tetracosane
2.5	2.5	1.4	3.1	92.1	93.5
10.0	1.7	1.0	4.6	94.2	98.6
20.0	4.3	3.1	19.9	92.5	93.7

Tandem Liquid Trap***

Total Spike Mass, mg	Acetophenone	Dimethylaniline	Naphthalene	2-Naphthol	*n*-Tetracosane
2.5	106.7	74.1	106.8	1.6	1.4
10.0	101.1	96.6	97.8	1.7	0.7
20.0	99.7	98.2	83.3	5.9	7.2

Mass Balance****

Total Spike Mass, mg	Acetophenone	Dimethylaniline	Naphthalene	2-Naphthol	*n*-Tetracosane
2.5	109.2	75.5	109.9	93.7	94.9
10.0	102.8	97.6	102.4	95.9	99.3
20.0	104.0	101.3	103.2	98.4	100.9

** Percent of extracted analyte retained on the solid-phase material
*** Percent of extracted analyte collected in tandem liquid trap
**** Percent retained on solid-phase + tandem liquid trap

SFE Conditions: CO_2 Pressure: 220 atm (step 1), 340 atm (step 2); Mass of CO_2 Delivered: 39.8 g (step 1), 57.0 g (step 2); Oven Temperature: 35 °C; CO_2 Liquid Flow Rate: 1.0 mL/min.; Restrictor Temperature: 100 °C; Solid-phase Collection Temperature: 5 °C; Tandem Liquid Trap: 12 mL Methylene Chloride (room temperature); Solid-Phase Desorption Temperature: 30 °C; Solid-Phase Rinse: 5 mL Methylene Chloride (2 times)

Assay - see Experimental

Table 2.4. Analyte percent recovery for the 50/50 (w/w) Porapak Q®/glass beads trap (0.8 g)

Solid Sorbent Rinse**

Total Spike Mass, mg	Acetophenone	Dimethylaniline	Naphthalene	2-Naphthol	*n*-Tetracosane
2.5	96.5	95.8	97.8	102.3	88.6
10.0	93.8	93.7	97.2	89.3	90.7
20.0	89.8	90.2	93.7	95.4	90.5

Tandem Liquid Trap***

Total Spike Mass, mg	Acetophenone	Dimethylaniline	Naphthalene	2-Naphthol	*n*-Tetracosane
2.5	6.5	4.9	3.4	2.4	1.7
10.0	11.1	7.8	4.3	6.4	7.1
20.0	7.3	6.9	3.5	1.9	6.9

Mass Balance****

Total Spike Mass, mg	Acetophenone	Dimethylaniline	Naphthalene	2-Naphthol	*n*-Tetracosane
2.5	103.0	100.7	101.2	104.7	90.3
10.0	104.9	101.5	101.5	95.7	97.8
20.0	97.1	97.1	97.2	97.3	97.4

** Percent of extracted analyte retained on the solid-phase material
*** Percent of extracted analyte unretained on the solid-phase and collected into tandem liquid trap
**** Percent retained on solid-phase + tandem liquid trap

SFE Conditions: CO_2 Pressure: 220 atm (step 1), 340 atm (step 2); Mass of CO_2 Delivered: 39.8 g (step 1), 57.0 g (step 2); Oven Temperature: 35 °C; CO_2 Liquid Flow Rate: 1.0 mL/min.; Restrictor Temperature: 100 °C; Solid-phase Collection Temperature: 5 °C; Tandem Liquid Trap: 12 mL Methylene Chloride (room temperature); Solid-Phase Desorption Temperature: 30 °C; Solid-Phase Rinse: 5 mL Methylene Chloride (2 times)

Assay - see Experimental

also be reoptimized with modified fluids although the protocols have not been set forth. Trapping capacity was determined to be a function of trap composition and is analyte dependent. Although high trap temperatures favor successful trapping when employing modified fluids, it is possible that the more volatile analytes will be lost from the solid-phase at elevated trapping temperatures, therefore decreasing the trapping capacity. If one lowers the trapping temperature to favor trapping of the more volatile analytes, loss of analyte may still result due to the mechanical rinsing of analyte off the solid-phase with the condensed modifier. It may be possible to increase the trapping capacity of the solid-phases by altering several parameters such as the trapping temperature and flow rate.

2.4 SUMMARY

The trapping capacities of three solid-phase traps consisting of either a 50/50 (w/w) mixture of octadecyl silica and glass beads, glass beads, or a 50/50 (w/w) mixture of Porapak Q® and glass beads for a mixture of components representing five different chemical classes were determined. It was seen that analyte breakthrough varied with trap and analyte composition. The trapping capacity for the octadecylsilica/glass beads trap was determined to be less than 8 mg/g of octadecylsilica for four analytes and less than 4 mg/g of octadecylsilica for naphthalene. The glass beads trap was less successful for the trapping of the three more volatile analytes resulting in a capacity of less than 0.5 mg/1.5 g. Consequently, the retention of acetophenone, dimethylaniline, and naphthalene on the octadecylsilica trap was due to adsorption on the octadecylsilica material. However, the glass beads are an effective trapping material for the two less volatile, higher molecular weight components, 2-naphthol and *n*-tetracosane. A trapping capacity for these two analytes was comparable to the octadecylsilica/glass beads trap of > 2 mg/1.5 g of glass beads. In this case, their retention was attributed to cryogenic freezing on the glass beads surface. Finally, the most successful trapping material was shown to be

the mixture of Porapak Q® and glass beads resulting in a trapping capacity of at least 10 mg/g of Porapak Q® per analyte.