

Solute Recovery from Equilibrium Solubility Determinations Using a Modified Supercritical Fluid Extractor

Robert J. Maxwell, James W. Hampson, and Miriam Cygnarowicz-Provost

U.S. Department of Agriculture, ARS, Eastern Regional Research Center, 600 East Mermaid Lane, Philadelphia, Pennsylvania 19118, USA
Address correspondence to Robert J. Maxwell.

INTRODUCTION

Equilibrium solubilities of compounds in supercritical fluids are determined using flow-through (dynamic) (1), static (2), or recirculating systems (3,4). In a flow-through system, the supercritical fluid is passed through the sample at various flow rates, and instantaneous equilibrium between the fluid and the sample is assumed. Static determinations are performed in a vessel containing a fixed amount of solute, and the pressure or temperature is adjusted until all of the sample dissolves in the supercritical phase. Recirculating devices operate with a fixed fluid volume that recirculates continuously throughout the system until equilibrium is achieved.

Until recently, researchers measured most solubilities in supercritical fluids using laboratory-built devices. In 1988, LDC Analytical (Riviera Beach, Florida) introduced a commercial instrument (Sample Preparation Accessory [SPA]) that enabled such measurements to be made in either dynamic or recirculating modes (5). The unit's recirculation system comprises an extraction chamber for the sample, an in-line UV-vis monitor, a sample loop, a recirculation pump, and two automated injection valves. As the analyte is extracted from the sample, its presence in the supercritical fluid is detected by the UV-vis monitor. The UV-vis readings are used to determine when equilibrium solubility has been achieved, at which point 10–20 μL of the solution is transferred on-line to the mobile-phase stream of a liquid chromatograph through the automated injection valves.

This system can be used in its original configuration for solubility studies if the compound to be extracted has moderate to high solubility in the supercritical medium and a strong UV-vis chromophore (4); compounds that have low solubility or no chromophoric groups, however, cannot be analyzed using

this system in the direct high performance liquid chromatography (HPLC) takeoff mode. Instead, to recover the solute, the system must be depressurized through the orifice restrictor. To analyze compounds that have wide solubility ranges or that lack chromophores without complete system decompression, we modified the design of this extractor. With these modifications in place, we can obtain highly reproducible, rapid recovery of compounds of varying solubility, including those with and without chromophoric groups.

EXPERIMENTAL

Replacement parts and standards: We obtained the supercritical fluid extractor from LDC Analytical and modified the unit as outlined below. The replacement rotor seals used for these modifications came from two sources: a loop control-valve rotor seal (Figure 1) (part number K-1182) was obtained from Rheodyne (Cotati, California), and rotor seals for injection valves 1 and 2 (part numbers SSAC6UWLDC-E and SSAC8WE, respectively) were from Valco Instruments (Houston, Texas). (When ordering the injection-valve rotor seals, indicate that they should be as wide and deep as possible so that they can be used at pressures as great as 5000 psi [34.5 MPa].)

For injection valve 1, we used three sample loops from Valco Instruments (2 mL, $1/16$ – $1/8$ in. o.d. [part number SL2KC6U]; 5 mL, $1/16$ – $1/8$ in. o.d. [part number SL5KC6U]; and 10 mL, $1/16$ – $1/4$ in. o.d. [part number SL10KC6U]) and a 30-mL loop from LDC Analytical. We obtained HPLC-grade solvents from Burdick & Jackson (Muskegon, Michigan) and anthracene (>99.9%) and *o*-anisic acid (99%) from Aldrich Chemical Co. (Milwaukee, Wisconsin). Before use, the *o*-anisic acid was further purified by recrystallization from a water-ethanol solution. Carbon dioxide and 10% methanol in carbon dioxide (both SFC grade) were obtained from Scott Specialty Gases (Plumsteadville, Pennsylvania).

Refitting the extractor for off-line collection: Before refitting the extractor, we made off-line measurements of the flows at various points in the system (6) and ensured that the recirculating pump was operating within the range of 90–100 mL/min before proceeding. Next, we installed the appropriate replacement rotor seals in the loop control valve, in-

jection valve 1, and injection valve 2 and then remeasured the flows in the loop (6).

To further improve flows in the recirculation system and to prepare the instrument for off-line takeoff, we made the following changes to the tubing and connections: We removed the manifold assembly (A–C, Figure 1) from the loop control valve, which was connected to the manifold tee by two lengths of $1/16$ -in. o.d. tubing (A, 0.03-in. i.d., and B, 0.01-in. i.d.). We removed the 0.01-in. i.d. section (B) and into its place silver-soldered a section of $1/16$ -in. o.d., 0.04-in. i.d. tubing of the same length. The manifold assembly was then reconnected to the loop control valve and to the fitting on the recirculation pump. Next, we removed the tubing connection between injection valves 1 and 2 (F, Figure 1) and the two connections to injection valve 2 (G and H) and replaced them with appropriate lengths of $1/16$ -in. o.d., 0.04-in. i.d. tubing. The original 10- μL sample loop was replaced with a 2-, 5-, or 10-mL loop, depending on the loop volume to be recovered. All of the other original fittings and connections in the system were used without further alteration. A model 114M solvent delivery module (Beckman Instruments, Columbia, Maryland) with a solvent reservoir was connected to the external outlet of tube J (Figure 1), and a 250-mL shielded volumetric flask was placed at the end of tube H.

Measuring solubility: Figure 1 shows the instrument setup for measuring solubility. With the sample in the extractor, the unit is charged with carbon dioxide, and the pressure and temperature settings are specified. To begin the recirculation process, the loop control valve is moved from the "charge loop" position to the "closed loop" position, and injection valve 1 is set in sequence position 1 on the central panel. (Valves 1 and 2 are switched in tandem by pneumatic activators to provide four flow configurations. In position 1, fluid is sent through the sample loop on valve 1, and in position 2, the contents of the sample loop are directed into the solvent stream through valve 2. Positions 3 and 4 are used to clean valve lines between injections.)

When the recirculation pump is turned on, the experiment starts. Extraction continues until equilibrium solubility is reached as de-

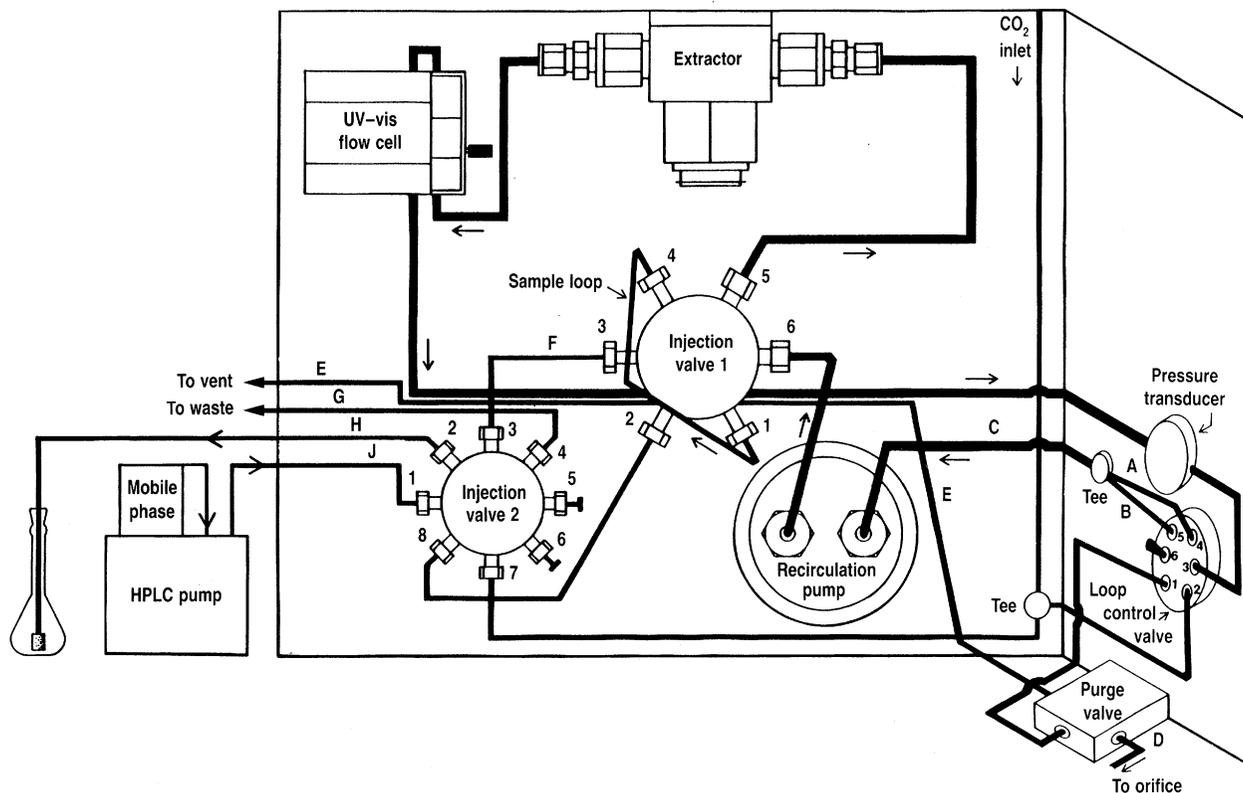


FIGURE 1: Schematic of the modified supercritical fluid extractor showing the direction of flow through the recirculating loop.

scribed below, and then the HPLC solvent pump is activated. Solvent is pumped through injection valve 2 until the first drops appear at the external tip of tube H, which has an HPLC low-pressure inlet filter that acts as a gas diffuser. Injection valve 1 is then switched to position 2 on the control panel, and the contents of the sample loop are expelled through injection valve 2 into the receiver. (With the diffuser in place on tube H, gaseous carbon dioxide slowly expands into the receiver with minimal analyte loss.) Liquid solvent is pumped through the system until all of the analyte has been recovered (2–5 min). The injection valves are switched to positions 3 and 4 to purge the lines of any residual solvent and then are returned to position 1. In this position, the set pressure is quickly reestablished, and the system is ready for the removal of another fraction as soon as equilibrium solubility is established at the new set point.

Equilibrium solubility for compounds with chromophoric groups is determined by a constant response from the in-line UV-vis detector (Figure 1). For samples with no chromophoric groups, however, the recirculation time required to reach equilibrium must be determined off-line. This can be accomplished by repeatedly collecting a sample off-line at

a given temperature and pressure for increasing recirculation times and then measuring solute concentration. At the maximum recirculation time, solute recovery no longer increases.

The recirculation system must be thoroughly cleaned between experiments to avoid cross-contamination. To accomplish this, we installed quick-connect fittings (part number QF-4-316, Swagelok Co., Solon, Ohio) on the carbon dioxide cylinder and on a cylinder containing 10% methanol in carbon dioxide. After completing an experiment, we disconnect the carbon dioxide cylinder from the line and switch to the cylinder that contains 10% methanol in carbon dioxide. The extractor is then pressurized, the oven is heated, and this mixture is recirculated. We perform this procedure several times, purging the spent mixture after each charge. Finally, a slight vacuum is applied at vent E (Figure 1) to remove traces of methanol.

The extractor can be reconfigured quickly for conventional analyte takeoff through the standard 10- μ L analytical loop to an HPLC system without reversing all of the design changes outlined above. The enlarged rotor seals for the three valves contribute to improved system operation and can be used for virtually all applications. Because the modified manifold assembly (A–C, Figure 1) improves the flow characteristics of the cir-

culating loop, it should be left in place (6). Restoring the unit's original capabilities requires reinstallation of only the following factory-supplied 1/16-in. o.d. tubing parts: the 10- μ L loop on injection valve 1, tubing section F (Figure 1) between injection valves 1 and 2, and tubing section H on port 2 of injection valve 2.

RESULTS AND DISCUSSION

Extractor modifications: The modifications described above for the supercritical fluid extractor require few changes to the original instrument design. Three wider-bore rotor seals are substituted for the standard narrow-bore seals, and 0.01-in i.d. tubing sections are replaced by tubing of larger internal diameters. These modifications enable the extractor to perform general equilibrium solubility measurements more easily and to be used for several applications that cannot be performed using the original design.

The major difference between the two designs is the way in which analyte is recovered from the recirculation loop. In the original design, solute can be recovered by one of two methods: injecting an aliquot of solute directly into the mobile-phase stream from an HPLC pump or venting the contents of the recirculation loop through an orifice restrictor.

TABLE I: Solute Recovery for Equilibrium Solubility Measurements Using Modified and Unmodified Supercritical Fluid Extractors

Performance Variable	Unmodified		
	HPLC Takeoff	Orifice Restrictor	Modified
Sample constraints	Requires chromophore	Does not require chromophore	None
Percent of total loop volume recovered per aliquot	0.06–0.12%	100%	11–65%
System decompression required for solute takeoff at increasing pressure intervals*	No	Yes	No
Solute recovery time*	2–4 min	30–60 min	2–4 min
Repeatability*	Good†	Variable‡	Good§

*If extracted compound exhibits low solubility or has no chromophore.
 †SD = 3–10% (4).
 ‡Results were limited by instrument design difficulties.
 §See statistical results for *o*-anisic acid under "case studies" in text.

In contrast, the extractor modifications described in this report exploit the HPLC capability of the original design and allow for off-line solute recovery into a receiver without the need to discharge the solute through the orifice (D, Figure 1).

Table I compares the two analyte recovery methods. For compounds that exhibit moderate solubility in supercritical carbon dioxide and have chromophoric functional groups, the instrument can be used in its original configuration; for all other compound types, the modified design should be used to ensure reproducible results. The table compares the repeatability and solute takeoff and recovery that can be obtained using the orifice-restrictor recovery system and using the modified extractor.

In the original design, if solute cannot be recovered through injection into the HPLC mobile phase, it must instead be captured by decompressing the system through the restrictor orifice. This time-consuming method produces variable results because a pressure drop occurs throughout the entire system during decompression, not solely at the restrictor, resulting in some analyte precipitation throughout the system. In contrast, the modified extractor uses pneumatic valves to inject the solute into a receiver as part of the mobile-phase stream in a process that requires a few minutes to complete and that causes minimal loss of system pressure.

The volume of the unmodified extractor recirculation system with the original 10- μ L sample (*t*_{recovery}) loop on valve 1 (Figure 1) is

~16 mL. In the modified system, we have used analyte recovery loops with volumes ranging from 2 to 30 mL, thereby increasing the overall system volume to 18–46 mL. Significant portions of the system volume can be removed in each aliquot — an important advantage when an analyte has limited solubility. Using the 2–30 mL recovery loops, for example, 11–65% of the total system volume can be sampled as one aliquot. By contrast, when the original 10- μ L loop is used with HPLC takeoff, only a small portion of the system volume (0.06%) can be recovered in each aliquot (Table I).

Case studies: We used the modified extractor for solubility measurements on several compound classes. We chose two compounds, anthracene and *o*-anisic acid, to study the repeatability of the proposed method and to compare the results obtained with those reported using different measuring techniques.

In the first study, we measured the equilibrium solubility of anthracene using a 2-mL loop (11% of the total recirculation loop) on injection valve 1 (Figure 1). Aliquots of anthracene in carbon dioxide were removed at pressures of 16.5–32.0 MPa. Methylene chloride was the HPLC mobile phase used for solute recovery. We determined the concentrations of recovered solute by diluting the solutions to a known volume and reading the absorbance on a model DU-70 UV spectrophotometer (Beckman) at 256 nm. The resulting data were plotted with those published by Zerda et al. (7), who measured the solubility of anthracene in supercritical carbon dioxide using a Fourier transform infrared cell in a static configuration. Although limited, the results of these studies show good agreement between the two sets of data (see Figure 2), even though the instruments used to measure the solubilities differed significantly.

To determine whether varying the size of the sample loop on injection valve 1 (Figure 1) would result in wide discrepancies between recoveries, we measured the equilibrium solubility of *o*-anisic acid using both 2-mL and 5-mL sample loops. The experiments were performed at 40 °C over a pressure range of 12.0–33.8 MPa. Methanol was pumped through injection valve 2 (Figure 1) to sweep the solute into the receiver. Methanol solutions that contained the solute were diluted to a known volume, and concentrations were determined using a UV spectrophotometer at 230.5 nm.

The results of these determinations are shown in Figure 3. In most cases, we made no attempt to obtain 2-mL and 5-mL aliquots of *o*-anisic acid at the same pressure. Instead, we observed trends over the entire pressure range. The effects of increasing pressure follow the same general pattern for both loops. In one instance, four 2-mL aliquots were removed at 18.6 MPa (arrow, Figure 3). The concentration of *o*-anisic acid at this pressure was 0.0094 ± 0.0007 mol/L; thus, good re-

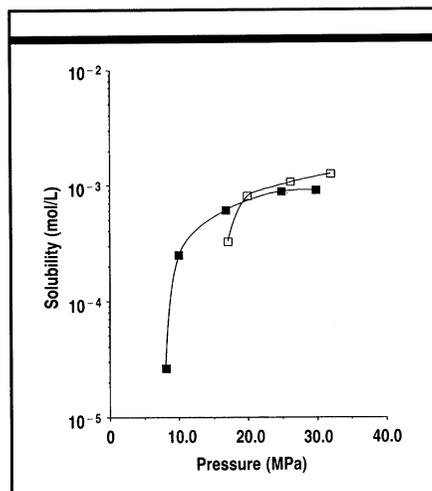


FIGURE 2: Solubility of anthracene in supercritical carbon dioxide measured using the modified supercritical fluid extractor (open squares) and a static system (8) (closed squares). Other conditions are described in the text.

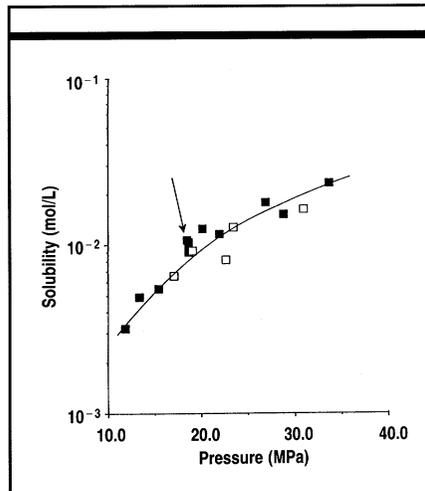


FIGURE 3: Solubility of *o*-anisic acid in supercritical carbon dioxide as measured using the modified supercritical fluid extractor with 2-mL (closed squares) and 5-mL (open squares) sample loops. Other conditions are described in the text.

producibility can be achieved when the aliquots are recovered at identical pressures. These data demonstrate that reproducible results are obtainable for analyte recovery when the modifications we have discussed are incorporated into the instrument.

In other studies performed in this laboratory, the solubilities of several lipid classes were measured using sample loops as large as 30 mL to determine concentrations gravimetrically (8). Solubilities were also determined for polycyclic ether antibiotics, an important class of veterinary drugs. Concentrations of these nonchromophoric compounds were measured off-line using HPLC with a refractive index detector (9).

ACKNOWLEDGMENTS

We wish to thank the following individuals for their willing support and assistance in completing this project: Karen Barabutes and Robert Keltchen of LDC Analytical, Barbara Spruce of Rheodyne, and Mack Harvey of Valco Instruments. We also thank Drs. Jerry King and Favio Favati of NC AUR-U.S. Department of Agriculture (Peoria, Illinois) for many helpful discussions.

REFERENCES

- (1) J.M. Dobbs, J.M. Wong, R.J. Lahiere, and K.P. Johnston, *Ind. Eng. Chem. Res.* **26**, 56-65 (1987).
- (2) J. Chrastil, *J. Phys. Chem.* **86**, 3016-3021 (1982).
- (3) T. Klein and S. Schulz, *Ind. Eng. Chem. Res.* **28**, 1073-1081 (1989).

- (4) K. Schäfer and W. Baumen, *Fresenius Z. Anal. Chem.* **332**, 122-124 (1988).
- (5) J.B. Nair and J.W. Huber III, *LC•GC* **6**, 1071-1073 (1988).
- (6) R.J. Maxwell, J.W. Hampson, R.W. Garber, and B. Hunter, unpublished results.
- (7) T.W. Zerda, B. Wiegand, and J. Jonas, *J. Chem. Eng. Data* **31**, 274-277 (1986).
- (8) J.W. Hampson, R.J. Maxwell, and M. Cygnarowicz-Provost, unpublished results.
- (9) R.J. Maxwell, J.W. Hampson, and M. Cygnarowicz-Provost, unpublished results. ■

Reprinted from

LC•GC

VOLUME 9, NUMBER 11 (1991)
788-794

THE MAGAZINE OF SEPARATION SCIENCE