United States
Environmental Protection
Agency

Research and Development

Health Effects
Research Laboratory
Research Triangle Park NC 27711

EPA-600/S1-84-028 Jan. 1985

EPA

Project Summary

Isolation and Concentration of Organic Substances from Water — An Evaluation of Supercritical Fluid Extraction

Daniel J. Ehntholt, Christopher P. Eppig, and Kathleen E. Thrun

This study describes the use of supercritical fluid carbon dioxide (SCF CO_2) as an extraction solvent for the isolation and concentration of 23 specified organic solutes in water at trace levels. Direct extraction using a non-toxic, non-hazardous solvent such as carbon dioxide has not previously been applied to the isolation and concentration of trace levels of organic compounds from water. Most of the recovery studies performed on the model compounds in this research were conducted on 400 mL aqueous samples in a stainless steel extractor operated at 2,500 psi and 45°C.

The ability of SCF CO_2 system to extract and subsequently trap model solutes with widely varying chemical and physical properties was generally found to be lacking. Recovery values of greater than 40 percent were demonstrated for only four of the model solutes, 2,4-dichlorophenol, isophorone, phenanthrene and stearic acid. The low recoveries were attributed to the inability of SCF CO_2 to extract highly water soluble or alkaline solutes such as glucose, glycine, trimesic acid, quinaldic acid, humic acid, caffeine, 5-chlorouracil and quinoline. Mass balance studies also indicated losses resulting from an ineffective trap system for volatile solutes (chloroform, furfural and methylisobutyl ketone) and adsorption of hydrophobic compounds (biphenyl, 1-chlorododecane, 2,4'-dichlorobiphenyl and 2,2'5,5'-tetrachlorobiphenyl) to the extraction system.

This Project Summary was developed by EPA's Health Effects Research Laboratory, Research Triangle Park, NC, to announce key findings of the research project that is fully documented in a separate report of the same title (see Project Report ordering information at back).

Introduction

One means of understanding and evaluating the possible toxicological effects of organic substances in drinking water is through biological tests Many of these tests however, require significantly higher concentrations of organic compounds than those normally found in drinking water because exisiting test systems are not sufficiently sensitive to contaminants at trace levels. In addition, although hundreds of organic compounds have been identified and quantified in samples of natural waters, much of the organic matter present cannot readily be characterized using currently available analytical protocols Without such characterization the substances cannot be purchased or synthesized for use in preparing of the concentrated solutions required for health effects testing Therefore, direct concentration/isolation of organic contaminants in aqueous samples for biological testing offers a potential solution.

The Health Effects Research Laboratory (HERL) U S EPA has funded several independent studies in an effort to determine the effectiveness of different isolation/concentration techniques

Systems or techniques investigated include reverse osmosis, vacuum distillation, solid adsorbents, and supercritical fluid carbon dioxide (SCF CO_2) extraction For purposes of comparison, a mixture of 23 model compounds was chosen by the HERL—U S EPA to evaluate each system.

While solubility data in supercritical fluids were reported as early as the late 1800s, commercial applications of supercritical fluids (e.g , hops extraction and the decaffeination of coffee) did not come on stream until the 1970s The renewed interest in superficial fluid extraction was largely spurred by the increased scrutiny of industrial solvents because of health and safety considerations and increasing costs associated with energy-intensive separation processes such as evaporation and distillation. Use of a nontoxic, non-hazardous, volatile solvent such as carbon dioxide offers several distinct advantages in the extraction of organic substances from water for biological testing.

Experimental Procedures

Preparation of Model Compound Test Solutions

Test solutions of model compounds used in the small scale extractor studies (400 mL) and the 10 liter extractor studies were prepared by simply diluting the required volume(s) of stock solution with organic free water containing an inorganic salt matrix. The salt matrix consisted of 70 ppm $NaHCO_3$, 120 ppm $CaSO_4$ and 47 ppm $CaCl_2 \cdot 2H_2O$ Table 1 lists the final concentration at which each model compound was tested

Small Scale Extraction

Recovery studies were conducted on 400 mL aqueous samples in a stainless steel extractor (extractor capacity was approximately 600 mL) operated at 2500 psi and 45°C. Supercritical conditions are achieved for CO_2 at pressures >1,070 psi and temperatures >31.1°C. Approximately 300 standard liters of CO_2 were passed through the aqueous solutions into the trapping system via a pressure reduction valve. While various systems were evaluated, the trapping system used for the recovery studies consisted of a set of three sequential glass U-tubes in series, maintained at -76°C by a dry ice-acetone bath. Operation at this temperature prevented clogging of solid CO_2.

To enhance the CO_2/aqueous phase interfacial area and facilitate contact by dispersion of the CO_2 as fine bubbles, a plug of silanized glass wool was placed in the bottom of the extraction vessel. After the vessel was charged with 400 mL of aqueous feedstock solution, it was slowly pressurized to the extraction pressure and simultaneously heated to the desired temperature. Carbon dioxide was then passed through the aqueous phase at a velocity of slightly more than 10 cm/min (about 10 standard liters/min at 1 atm., 70°F). After the pre-determined amount of carbon dioxide (300 standard liters) flowed through the sample, the system was depressurized and the extracted aqueous raffinate was drained into a collection vessel. Analyses of the extracted aqueous raffinate and the residue in the trapping system were used for mass balance determinations.

Ten Liter Extractor

An original objective of this effort was the extraction of a single five-hundred liter sample at the conclusion of the program. During the course of the project, however, it was decided that a smaller-scale run combined with additional trapping experiments would yield more useful results. A final series of ten liter extractions was therefore carried out on solutions containing all of the organic compounds of interest and the three inorganic compounds specified. The extraction apparatus used in these studies was similar to that used for the small-scale work, but it had an internal

Table 1. Summary of Small Scale Extraction Study

Compound	Concentration (µg/L)	Number of Determinations	% Recovery		
			Trap Mean Recovery	Raffinate Mean Recovery	Mass Balance
Anthraquinone	50	2	21 4	84 6	106.0
Biphenyl	50	1	3 8	23.4	27.2
Bis(2-ethylhexyl)-phthalate	50	3	15.4	11 3	26 7
Caffeine	50	2	*	81 4	81.4
Chloroform	50	*	—	—	—
1-Chlorododecane	5	1	20.7	*	20 7
5-Chlorouracil	50	1	*	96.0	96.0
Crotonaldehyde	50	1	7.8	31 0	38.8
2,6-Di-t-butyl-4-methylphenol	50	1	32 7	*	32 7
2,4'-Dichlorobiphenyl	50	3	20.3	8.5	28.8
2,4-Dichlorophenol	50	1	45 4	28.0	73 4
Furfural	50		22 3	10.8	33.1
Glucose	50	*	—	—	—
Glycine	50	*	—	—	—
Humic Acid	2000	2	*	42.0	42 0
Isophorone	50	1	40.4	24.5	64.9
Methyl Isobutyl Ketone	50	1	17 3	11.4	28.7
Phenanthrene	1	1	97.0	*	97.0
Quinaldic Acid	50	1	*	85.0	85.0
Quinoline	50	2	3 4	46 1	49.5
Stearic Acid	50	1	47.5	22.0	69.5
2,2',5,5'-Tetrachlorobiphenyl	5	3	18 7	12.0	30 7
Trimesic Acid	50	1	*	91.0	91.0

* = not detected.
\- = not analyzed.

volume of approximately fifteen liters The traps used were stainless steel impingers with a volume capacity of approximately one liter

Results and Discussion

General

A series of SCF CO_2 extractions of model solutes was conducted. In all instances, the organic free water used to prepare the model compound test solutions contained a salt matrix (70 ppm $NaHCO_3$, 120 ppm $CaSO_4$ and 47 ppm $CaCl_2 \cdot 2H_2O$) to simulate the salt content of drinking water Analysis of the trapping system after extraction indicated that these salts as well as lead nitrate were not extracted by SCF CO_2. Experiments that were conducted to determine whether artifacts were produced by the presence of a chlorine residual (2 ppm) also showed that no new compounds were formed

Small Scale Extraction

Table 1 details the experimental results obtained for the SCF CO_2 extraction of the model compounds The compounds selected for investigation, the nominal spiking levels, and the number of experiments performed are provided in the first three columns The mean trap recoveries representing the sum of the three U-tube trap in series are then presented along with the mean raffinate recovery (SCF CO_2 extracted feedstock) and the mass balance (mean trap recovery plus mean raffinate recovery). While values for mass balance determinations exceeded 40 percent for 11 of the model compounds, only 2,4-dichlorophenol, isophorone, phenanthrene and stearic acid could be extracted and recovered from the trapping system at levels >40 percent.

The low recoveries were largely attributed to the inefficiency of SCF CO_2 as an extraction solvent for highly water soluble or alkaline solutes such as glucose, glycine, trimesic acid, quinaldic acid, humic acid, caffeine, and quinoline. Poor extraction efficiency was also demonstrated for anthraquinone and 5-chlorouracil as indicated by the high recoveries for these substances in the raffinate. Mass balance determinations suggested losses resulting from an ineffective trap system for volatile compounds (chloroform, furfural and methylisobutyl ketone) and adsorption to he extraction system for hydrophobic solutes (biphenyl, 1-chlorododecane, 2,4'-dichlorobiphenyl and 2,2',5,5'-etrachlorobiphenyl

Ten Liter Extraction

Based on scale-up considerations from the 400 mL runs, each sample extraction with SCF CO_2 was conducted at 1950 ± 50 psi and 37-45°C, and involved passing approximately 11,200 standard liters of carbon dioxide through the aqueous solution in about 110 minutes Since pressure/flow rate fluctuations might occur in the large scale apparatus that could lead to the rupture of the glass traps, a series of three stainless steel impingers maintained at -76°C were used to collect the organics present in the effluent carbon dioxide stream.

Because the small scale extraction experiments had shown that quantitative removal of organics from the traps was a problem, a trap rinse sequence was designed to assure the dissolution of all of the organic compounds from the traps. The solvents used were compatible with any derivatization/sample preparation steps necessary before analysis. Thus, at the conclusion of each experiment, the three traps were rinsed sequentially with methylene chloride, methylene chloride/base (5N NH_4OH added dropwise to each trap), and Milli-Q water. The first methylene chloride trap rinse yielded some aqueous phase extract (approximately twenty milliliters) which was added to the Milli-Q rinse. Aliquots of the trap rinses, raffinate, and feedstock were analyzed according to methods previously developed specifically for this project. Table 2 summarizes the results obtained from these runs. In general, the types of compounds which were extracted and trapped were the same as those found in the small scale experiments. In particular, the hydrocarbons and phenols were collected in the traps, whereas the more water soluble compounds were not detected in the trapping system. The mass balances for some types of materials (e g., 5-chlorouracil and the humic acid) were poorer in the 10 liter extraction; however, these runs contained all 23 compounds at the same time and the extractions were also conducted for a longer period of time It is possible that the interactions between compounds under the acidic extraction conditions accounts for the low total recoveries in certain of these cases. For example, the absence of humic acid in the raffinate and the observation of a brown organic material upon cleaning the extractor suggested that this material was precipitated.

Conclusions

This study demonstrated the utility of supercritical fluid carbon dioxide for the isolation and concentration of selected compounds present in water at low concentrations Compounds exhibiting greater solubility in water (e.g., trimesic acid, glucose, and glycine) do not show evidence of extraction; in addition, those materials which tend to precipitate (humic acid) or form more soluble species (caffeine) under acidic conditions were not extracted.

An extraction conducted on an aqueous solution containing a two part-per-million chlorine residual did not indicate the generation of any new species in the extract. All of the tests in this program were conducted on aqueous solutions containing $NaHCO_3$, $CaSO_4$, and $CaCl_2$ added at concentration levels typical of drinking water Experiments were also conducted to determine whether or not these inorganic materials or $PbNO_3$ (added to several solutions as a surrogate for possible toxic metal concentration) were extracted. Results indicated that the inorganics were not isolated or concentrated.

The extraction conditions used in the study were determined based on approximately seventy percent extraction of phenolic compounds in early runs. While additional treatment with supercritical fluid carbon dioxide might increase the extraction efficiency of the process, additional trapping (recovery) problems may occur

Although the aqueous extraction sampling and analysis procedures were well developed for the study, the trap systems and trap rinse procedures for the small scale extractions (0.4 L) and the 10 L extractions were different. Therefore, the results obtained for trap recoveries are not directly comparable, but the raffinate analysis results are

The overall conclusion from this study was that the supercritical fluid carbon dioxide extraction of drinking water represents an alternative path for selected organic compounds which are not highly soluble in water It can be used in lieu of organic solvents or membrane techniques when those interfere with biological tests.

Recommendations

Since the concept should be adaptable to large scale extraction of certain types of organic compounds from water, further study of the supercritical fluid extraction concept is recommended. Specifically, the efficiencies of alternative trapping systems should be defined For example, complete trapping of all effluent carbon dioxide in a vessel from which fractional distillation of CO_2 can take place is likely to yield higher recoveries of

Table 2. *Summary of Ten Liter Extraction (Avg. 3 Runs)*

Compound	Concentration (μg/L)	% Recovery		
		Trap Mean Recovery	Raffinate Mean Recovery	Mass Balance
Anthraquinone	50	32	31	63
Biphenyl	50	15	*	15
Bis(2-ethylhexyl)-phthalate	50	30	*	30
Caffeine	50	6	71	77
Chloroform	50	NA	*	0
1-Chlorododecane	5	25	*	25
5-Clorouracil	50	9	13	22
Crotonaldehyde	50	3	2	5
2,6-Di-t-butyl-4-methylphenol	50	31	*	31
2,4'-Dichlorobiphenyl	50	45	*	45
2,4-Dichlorophenol	50	26	*	26
Furfural	50	3	*	3
Glucose	50	*	*	0
Glycine	50	*	*	0
Humic Acid	2000	1	+	1
Isophorone	50	28	*	28
Methyl Isobutyl Ketone	50	5	4	9
Phenanthrene	1	14	*	14
Quinaldic Acid	50	*	89	89
Quinoline	50	4	31	35
Stearic Acid	50	*	27	27
2,2,'5,5'-Tetrachloro-biphenyl	5	30	*	30
Trimesic Acid	50	*	84	84

* = *not detected.*
NA = not analyzed.
\+ = *none detected, brown precipitate was recovered from the extractor*

organics. In addition, the use of "closed systems" in which the effluent CO_2 stream is recycled through the aqueous stream after removal of some portion of the dissolved organic compounds may permit more efficient collection of those organics. If these studies are conducted on a small scale, particular attention should be paid to irreversible adsorption to the traps and inefficient removal of the organics from the effluent CO_2 stream as likely causes of low organic compound recovery

Daniel J. Ehntholt, Christopher P. Eppig, and Kathleen E. Thrun are with Arthur D. Little, Inc., Cambridge, MA 02140.
***H. Paul Ringhand** is the EPA Project Officer (see below).*
The complete report, entitled "Isolation and Concentration of Organic Substances from Water—An Evaluation of Supercritical Fluid Extraction," (Order No. PB 85-138 899; Cost: $10.00, subject to change) will be available only from:
National Technical Information Service
5285 Port Royal Road
Springfield, VA 22161
Telephone: 703-487-4650
The EPA Project Officer can be contacted at:
Health Effects Research Laboratory
U.S. Environmental Protection Agency
Research Triangle Park, NC 27711

United States
Environmental Protection
Agency

Center for Environmental Research
Information
Cincinnati OH 45268

BULK RATE
POSTAGE & FEES PA
EPA
PERMIT No G-35

Official Business
Penalty for Private Use $300

☆U S GOVERNMENT PRINTING OFFICE, 1985 — 559-016/789