

**SUPERCRITICAL CARBON DIOXIDE  
EXTRACTION OF RESIN AND FATTY ACIDS  
FROM SEDIMENTS AT PULP MILL SITES**

H.B. Lee and T.E. Peart

NWRI Contribution No. 91-123

TD  
226  
N87  
No. 91-  
123  
c. 1

**SUPERCRITICAL CARBON DIOXIDE EXTRACTION OF RESIN AND  
FATTY ACIDS FROM SEDIMENTS AT PULP MILL SITES**

**H.B. Lee and T.E. Peart**

**Research and Applications Branch  
National Water Research Institute  
Burlington, Ontario L7R 4A6**

**NWRI Contribution No. 91-123**

## **MANAGEMENT PERSPECTIVE**

Until recently, the use of organic solvents in the extraction of samples for chemical analysis has been an inevitable step even though they are a potential health hazard and can create pollution and disposal problems. With the advent of the supercritical fluid extraction (SFE) techniques, the usage of organic solvents can be drastically reduced. This report summarizes the successful application of modified supercritical carbon dioxide for the extraction of toxic resin and fatty acids from sediments collected at pulp mill locations. While the environmentally friendly SFE technique produces equal or better recovery of the above acids, it is also more cost efficient in the long run as it requires only ca. 5% of the solvent and 10% of the time in comparison to the classical soxhlet extraction.

## **SOMMAIRE À L'INTENTION DE LA DIRECTION**

Jusque tout récemment, l'emploi de solvants organiques pour l'extraction d'échantillons aux fins d'une analyse chimique a été une étape inévitable même si ces produits peuvent constituer un risque pour la santé et créer des problèmes de pollution et d'élimination. Grâce aux techniques d'extraction par liquide supercritique, il est possible de réduire énormément la quantité de solvants organiques utilisée. Le présent rapport résume l'application couronnée de succès de dioxyde de carbone supercritique modifié pour l'extraction d'acides résiniques et d'acides gras toxiques contenus dans des sédiments recueillis aux usines de pâte à papier. Alors que la technique écologique d'extraction par fluide supercritique permet une récupération égale ou supérieure des acides mentionnés ci-dessus, il s'agit d'une technique également plus rentable à la longue car elle n'exige que 5 % environ de solvant et 10 % du temps comparativement à l'extraction classique par la méthode Soxhlet.

## **ABSTRACT**

A rapid and efficient method for the extraction of resin and fatty acids commonly found in sediments collected from pulp mill locations was developed by using modified supercritical carbon dioxide. In the presence of a 1:1 mixture of methanol and formic acid, quantitative recovery of all acids except for palustric and neoabietic acids was achieved with a 5 min static and 10 min dynamic extraction with carbon dioxide at 365 bar and 80°C. Although the above two resin acids were only 40% recovered from spiked samples, these values were at least 250% better than those obtained by the classical soxhlet technique. The cleaner SFE extract permitted a less stringent cleanup after the off-line derivatization of the acids, thus it further reduced analytical time and the use of solvent. An in-situ extraction and on-line derivatization of the resin and fatty acids also proved feasible for the semi-quantitative screening of the toxic acids in sediments near pulp mill locations.

## RÉSUMÉ

Une méthode rapide et efficace d'extraction d'acides gras et résiniques généralement présents dans les sédiments recueillis aux usines de pâte à papier a été élaborée grâce à l'emploi de dioxyde de carbone supercritique modifié. En présence d'un mélange 1:1 de méthanol et d'acide formique, une récupération quantitative de tous les acides, à l'exception des acides palustrique et néoabiétique, a été réalisée par une extraction statique de 5 minutes et une extraction dynamique de 10 minutes par le dioxyde de carbone à 365 bars et à 80 °C. Même si la récupération des deux acides résiniques mentionnés ci-dessus n'a été que de 40 % dans des échantillons dopés, ces valeurs étaient au moins 250 % supérieures à celles obtenues par la méthode classique Soxhlet. L'extrait plus pur obtenu par la technique du fluide supercritique a permis une purification moins poussée après la récupération des acides du système d'extraction et la préparation de leurs dérivés, ce qui diminue le temps d'analyse et la quantité de solvant utilisée. L'extraction des acides et la préparation des dérivés effectuées dans le même système d'extraction s'avèrent un moyen pratique pour le dépistage semi-quantitatif des acides gras et résiniques dans les sédiments à proximité des usines de pâte à papier.

## INTRODUCTION

A large number of environmental pollutants have been identified in the discharges from the pulp and paper industry. Chlorinated phenols, guaiacols, catechols, aliphatic neutrals and acids, as well as furans and dioxins have been identified from chlorobleaching mills [1,2]. Resin acids, natural products derived from wood and pulp, occur in effluent samples from every paper mill [3,4]. Many of the above chemicals are toxic to fish and have a life time long enough for bioaccumulation in aquatic organisms. Among them, resin acids and a few unsaturated fatty acids have been identified as the major components of effluents which contribute to the toxicity to fish [5-7]. The pulp and paper industry in Canada and elsewhere has implemented various techniques to detoxicate the effluents before they are discharged into the receiving waters. However, effluent levels of resin and fatty acids (RFA) from those mills without an effective secondary (microbiological) waste treatment are so high that they can be acutely toxic to fish. Owing to their low solubilities, resin acids are readily adsorbed by sediments and are easily detected in samples downstream of the paper mills.

RFA in sediments are extracted by using the classical soxhlet technique with polar organic solvents [8,9]. In a recent study, we have found that, by addition of a trace amount of concentrated hydrochloric acid to the polar solvents, the recoveries of RFA in sediments were improved by 200 to 300% [9]. However, the presence of a strong acid caused degradation of palustric and neoabietic acids into abietic acid. Therefore this technique would produce biased low results for the above two unstable resin acids and biased high results for abietic acids in sediment samples.

Supercritical fluid extraction (SFE) has been applied to many organic pollutants in various environmental matrices [10-13]. In general, supercritical carbon dioxide produces good recoveries for non-polar compounds such as PCBs [10]. However, for the extraction of more polar compounds, carbon dioxide modified by methanol or other polar

solvent or supercritical nitrous oxide is required to improve the recoveries to a level comparable to soxhlet extraction. Until recently, there were few reports on the supercritical fluid extraction of organic acids from sediments. This work describes the optimization of the extraction of resin and fatty acids from sediments using modified supercritical carbon dioxide.

## EXPERIMENTAL SECTION

All resin acids were obtained from Helix-Biotech Scientific Ltd. (Vancouver, B.C., Canada) and used without further purification. Fatty acids and  $\alpha$ -bromo-2,3,4,5,6-pentafluorotoluene (pentafluorobenzyl bromide, PFBBBr) were purchased from Aldrich Chemicals Co. (Milwaukee, WI). Stock solutions of individual resin and fatty acids were prepared in acetone at 1000  $\mu\text{g/mL}$  and kept at  $-20^{\circ}\text{C}$  in the dark. Spiking solutions of mixed RFA also in acetone were stored at  $4^{\circ}\text{C}$  in the dark. A PFBBBr solution was prepared by dissolving 1 g of the reagent in 20 mL of acetone and kept at  $-20^{\circ}\text{C}$  until use.

All solvents used were distilled-in-glass grade supplied by Burdick and Jackson. SFC grade carbon dioxide with a helium head pressure of 10500 kPa was purchased from Scott Specialty Gases (Troy, MI).

Several river sediment samples were collected from different locations near an Ontario paper mill in September 1990. Among them, a sample obtained from a site approximately 2 km downstream of the mill was, as shown by previous analysis using soxhlet extraction, contaminated with RFA at levels typically found in paper mill sediments. This sediment was air-dried, crushed, homogenized and used for the development of the extraction method.



Supercritical carbon dioxide extraction of the sediment was performed with the Hewlett-Packard 7680A extraction module and the available 7.0 mL stainless steel thimbles and caps. To minimize contamination and plugging of the bottom cap by the sample, two circles of Whatman GF/C filter paper of the same diameter as the thimble were cut by pressing the edge of the thimble against the paper and placed above the bottom cap after it was screwed in. A sediment sample typically of 500 mg was weighed and 25  $\mu$ L of water and 300  $\mu$ L of modifier added directly to the sample. In some cases, the sample was placed in between two layers of Celite of 200 mg each (see later discussion). A 5 min static and a 10 min dynamic extraction was carried out at a chamber temperature of 80°C using supercritical CO<sub>2</sub> of 0.80 g/mL density (approximate pressure 365 bar) at a flow rate of 2.0 mL/min. The extract was deposited onto an octadecylsilane (ODS) trap which was maintained at 15°C with cryogenic CO<sub>2</sub> during the extraction stages. At the end of the extraction, the RFA were collected in glass vials by eluting the trap with two 1 mL aliquots of acetone. The total time for the entire extraction cycle was ca. 35 min.

The acetone extracts containing the RFA were combined and reduced to 1 mL before the acids were converted into their pentafluorobenzyl (PFB) esters as described before [9]. After solvent exchange into petroleum ether (b.p. 30-60°C), the derivatized products were cleaned up on a 5 cm 5% deactivated silica gel column prepared in a 20 cm x 0.7 cm I.D. disposable Pasteur pipet. Following the application of the derivatized extract to the column prewashed with 2 mL of petroleum ether, the column was eluted with 5 mL of 5+95 (v/v) dichloromethane and petroleum ether and then with 7 mL of 1+1 dichloromethane and petroleum ether mixture. The last fraction was saved and the solvent exchanged into iso-octane for GC-ECD analysis using a 30 m x 0.25 mm ID DB-17 column as described before [14].

## RESULTS AND DISCUSSION

### *The HP 7680A SFE module*

The Hewlett-Packard SFE module (Figure 1) is a standalone extractor for solid and semi-solid samples using modified or non-modified supercritical carbon dioxide. It is controlled by an Intel 80386 class personal computer with dedicated software running under the Microsoft Windows/286 environment for multi-tasking purposes. This system provides a user-friendly interface through extensive use of graphics and pull-down menus for sample log-in, method documentation, routine operation and instrument maintenance. Although all extractions in this work were done with the 7.0 mL extraction thimbles, thimbles of 1.5 mL volume are also available for smaller samples. Instead of using a restrictor to depressurize the supercritical fluid and deposit the extract into a test tube containing an organic solvent, the HP 7680A extractor employs a unique nozzle/trap assembly. The nozzle allows instant depressurization of the carbon dioxide, and at the same time permits the decoupling of flow and pressure; thus, the density can be set independent of the flow of the fluid. The SFE extract is deposited onto a packed trap made either of stainless steel or ODS material. The analytes are then rinsed off the trap with a predetermined amount of solvent into a glass vial and the extract is ready for analysis, cleanup, or further workup. The operation is fully automated from the point where the thimble is placed into the extraction chamber and on. Other salient feature of the Hewlett-Packard extractor has been described in an earlier article [15]. An animated diagram displaying the instrument status of the HP 7680A is shown in Figure 2.

### *Supercritical carbon dioxide extraction of RFA from sediments*

RFA in sediment were poorly extracted by supercritical carbon dioxide. Even at the maximum extraction chamber temperature of 80°C and a fluid density of 0.80 g/mL, only a small amount of palmitic acid yet no resin acids were recovered from the

reference sample after a 5 min static and another 10 min dynamic extraction at a flow rate of 2 mL/min. Addition of 300  $\mu$ L of methanol improved the recoveries of the total fatty and resin acids to 65 and 34%, respectively, of the soxhlet values (Table 1). Analogous to the fact that the presence of an acid substantially improved the recovery of RFA in the soxhlet extractions, the SFE recovery of RFA was also greatly improved by the presence of 300  $\mu$ L of acetic acid in the sample. Using a stronger acid such as formic acid further enhanced the recovery of total RFA to ca. 80%, however, it was noted that the recoveries of palustic and neoabietic acids were lower when the stronger acid was used. The use of dichloroacetic acid, 10% hydrochloric acid in methanol and a 1:1 mixture of acetic acid and methanol as modifiers also proved to be less effective than formic acid for the extraction of all RFA, although a 1:1 mixture of methanol and formic acid provided the best recovery of RFA in sediment. The effect of each modifier on the recovery of the major RFA in sediments is shown in Table 1.

The effect of the amount of modifier used on the recovery was also studied. Based on a 500 mg sample size, 300  $\mu$ L of a 1:1 mixture of methanol and formic acid mixture was found to produce the optimal recovery of RFA. Smaller amounts such as 100 or 200  $\mu$ L of the modifier were insufficient and a larger volume such as 500  $\mu$ L did not further improve the recovery.

#### *Other factors affecting the recovery of RFA in sediments*

At the early stage of our work, extraction of sediments was carried out at either 50 or 60°C. Within the working temperature range of 40 to 80°C for the HP 7680A, recovery of RFA from the reference sediment was found to increase with increasing chamber temperature. Therefore all subsequent extractions were done at a temperature of 80°C. Note that supercritical carbon dioxide of the maximum density attainable by the HP 7680A at each temperature was used in each case so that highest extraction efficiency could be achieved.

The moisture content of a sample also plays an important role in the extraction of RFA from sediments. The results from our experiments indicated that the best recovery of RFA was obtained from samples containing 5 to 10% moisture content. If freeze-dried sediments were used, a reduction of 25 to 40% in the recovery of the RFA was observed. However, an addition of 5% by weight of water to the dry sediment prior to the extraction would bring the recovery back to quantitative.

It was also observed that, an improvement of ca. 10% in the recovery of RFA was achieved by sandwiching the sediment with 200 mg layers of Celite and spiking each layer with half the amount of modifier. Presumably the slightly better recovery was attributed to the longer retainment of the modifier with solids during the dynamic extraction stage. While longer extraction times (both static and dynamic) did not further improve the recovery, shorter extraction times resulted in incomplete recovery of RFA.

Under the optimized conditions, a second extraction of the sediment with fresh modifier recovered less than 5% of additional RFA, indicating that the first extraction was essentially complete.

#### *Cleanup of derivatized extracts*

In comparison to the exhaustive but often non-selective soxhlet extraction, supercritical carbon dioxide extraction of RFA from sediment produced a much smaller amount of coextractives in the extract. The cleaner extract enabled us to employ a smaller (0.8 g vs. 5.0 g) column for sample cleanup after the derivatization [9], and thus it further improves the saving in time and the amount of solvent used. This cleanup step effectively removed interferences deriving from sediment coextractives as well as the blank of the SFE grade CO<sub>2</sub> which was found to be present in all samples from three different suppliers.

### ***Method performance***

With the exception of palustric and neoabietic acids, the recoveries of other major fatty and resin acids found in pulp mill sediments from spiked samples were better than 85% at fortification levels of 10 and 1  $\mu\text{g/g}$  (Table 2). Recoveries for palustric and neoabietic acids were between 35 and 40% at the same levels, presumably due to degradation of these two acids under acidic extraction conditions. It should be noted that, with soxhlet extraction, the recoveries were even poorer for palustric (5 to 15%) and neoabietic (<5%) acids [9]. This extraction procedure has been successfully applied to sample sizes from 25 mg to 1 g. Larger sample sizes were not tried since with a 1 g sample, a detection limit of 0.05  $\mu\text{g/g}$  can easily be achieved [9].

### ***In-situ extraction and derivatization of RFA in sediments***

Although derivatization analysis of polar organics enjoys many advantages such as improved chromatographic properties and enhanced detector response of the derivatives, this approach is more cumbersome because of the extra step. Therefore, an ideal method would be one which combines the extraction and derivatization steps into one. In our case, experimental conditions had to be modified since the SFE conditions and the esterification reaction with the PFBBBr reagent were incompatible with each other. The esterifying agent reacts with acids and methanol and the reaction requires a base to catalyze the formation of esters. This problem was solved by replacing the methanol/formic acid modifier with a 250  $\mu\text{L}$  5% solution of the PFBBBr reagent in acetone and 50  $\mu\text{L}$  of triethylamine. An initial extraction was attempted by using a 10 min static and 5 min dynamic extraction time. Although the recoveries of RFA by this in-situ method (Figure 3) were only 35 - 45% of the off-line derivatization technique (Figure 4), the results nevertheless indicated that the in-situ derivatization was feasible for the determination of RFA in sediments. The lower recoveries were not unexpected since acetone was a less effective modifier than the 1:1 methanol/formic acid mixture for

the extraction of RFA and also the complete conversion of the acids into their PFB esters required 1 to 2 hr at 60°C. Indeed, by extending the static extraction time from 10 to 60 min, the recoveries were improved to ca. 60% for the RFA by comparison to the best off-line SFE extraction and derivatization results. However, the amounts of palustic and neoabietic acids extracted by the in-situ method were proportionally higher, since these two acids are less stable under acidic conditions. Doubling the amounts of PFBBBr and triethylamine only improved the recovery of RFA by another 5 to 10%. Further extension of the static extraction time is impractical since the sample throughput would be severely reduced.

## CONCLUSION

Most RFA commonly found in sediments downstream of pulp and paper mill locations are quantitatively extracted by supercritical carbon dioxide in the presence of methanol and formic acid as modifiers. Although the recoveries of the unstable palustic and neoabietic acids are ca. 40% as indicated by the recovery experiments for the spiked sediments, the SFE results of the above two acids are at least 250% better than the soxhlet values on both spiked and naturally contaminated samples. Because of the feasibility of a rapid, one-step in-situ extraction and derivatization of RFA, this technique is most suitable for the semi-quantitative screening of the toxic RFA in sediments for quick sample turn around time.

## ACKNOWLEDGEMENT

The authors are grateful to Hewlett-Packard (Canada) Ltd. for the generous loan of the HP 7680A Supercritical Fluid Extractor module. We also thank W. Pipkin of Hewlett-Packard, Avondale, PA, for helpful discussions.

## REFERENCES

1. R.H. Voss, J.T. Wearing, R.D. Mortimer, T. Kovacs and A. Wong, *Pap. Puu.*, 62:12 (1980) 809.
2. K. Lindström and F. Österberg, *Environ. Sci. Technol.*, 20:2 (1986) 133.
3. A. B. Mckague, J.M. Leach, R.N. Soniassy and A.N. Thakore, *Pulp Pap. Can. Trans. Tech. Sect.*, 3:3 (1977) TR75.
4. I. Rogers, H. Mahood, J. Servizi and R. Gordon, *Pulp Pap. Can.*, 80:9 (1979) T286.
5. C.C. Walden and T.E. Howard, *Tappi*, 60:1, (1977) 122.
6. C.C. Walden and T.E. Howard, *Pulp Pap. Can.*, 82:4 (1981) T143.
7. M.H. Priha and E.T. Talka, *Pulp Pap. Can.*, 87:12, (1986) T447.
8. B. Brownlee, M.E. Fox, W.M.J. Strachan and S.R. Joshi, *J. Fish. Res. Board Can.*, 34 (1977) 838.
9. H.B. Lee and T.E. Peart, *J. Chromatogr.*, 547 (1991) 315.
10. M.M. Schantz and S.N. Chesler, *J. Chromatogr.*, 363 (1986) 397.
11. S.B. Hawthorne and D.J. Miller, *Anal. Chem.*, 59 (1987) 1705.
12. N. Alexandrou and J. Pawliszyn, *Anal. Chem.*, 61 (1989) 2770.
13. S.B. Hawthorne, *Anal. Chem.*, 62 (1990) 633A.
14. H.B. Lee, T.E. Peart and J.M. Carron, *J. Chromatogr.*, 498 (1990) 367.
15. W. Pipkin, *Amer. Lab.*, Nov. (1990) 40D.

TABLE I

Effect of modifier on the recovery of RFA in sediment

	None	MeOH	HOAc	HCOOH	MeOH/HCOOH H	Soxhlet $\mu\text{g/g}$
		% Recovery <sup>1</sup>				
Palmitic	3	63	71	74	94	18.0
Stearic	<1	64	69	73	94	6.4
Oleic	<1	65	78	82	94	6.8
Linoleic	<1	73	68	88	90	13.9
Pimaric	<1	44	90	87	102	12.7
Isopimaric	<1	33	67	84	88	40.2
Palustric	<1	117	204	141	267	4.6
Abietic	<1	37	64	73	89	52.7
Dehydroabietic	<1	44	76	84	102	65.8
Neoabietic <sup>2</sup>	<1	(1.9 $\mu\text{g/g}$ )	(3.8 $\mu\text{g/g}$ )	(0.7 $\mu\text{g/g}$ )	(4.4 $\mu\text{g/g}$ )	<0.1
Chlorodehydroabietic <sup>3</sup>	<1	23	71	81	89	48.9
Total fatty acids <sup>4</sup>	3	65	70	80	92	55.3
Total resin acids <sup>5</sup>	<1	34	66	79	94	232

<sup>1</sup>All SFE recoveries were relative to soxhlet results.<sup>2</sup>The recoveries of neoabietic acid under various SFE conditions were given in  $\mu\text{g/g}$  since this acid was not recovered by the soxhlet method.<sup>3</sup>Sum of 12- and 14-chlorodehydroabietic acids.<sup>4</sup>Sum of the fatty acids listed together with lauric, myristic, linolenic and eicisanoic acids.<sup>5</sup>Sum of the resin acids listed together with sandaracopimaric and dichlorodehydroabietic acids.



TABLE II

% Recovery of resin and fatty acids from fortified sediment samples  
by supercritical fluid extraction

RFA	10 $\mu\text{g/g}$	1 $\mu\text{g/g}$
Palmitic	99 $\pm$ 7	94 $\pm$ 10
Stearic	97 $\pm$ 7	85 $\pm$ 7
Oleic	94 $\pm$ 7	105 $\pm$ 5
Linoleic	88 $\pm$ 8	107 $\pm$ 7
Pimaric	91 $\pm$ 6	98 $\pm$ 6
Isopimaric	90 $\pm$ 7	95 $\pm$ 3
Palustric	38 $\pm$ 8	35 $\pm$ 4
Abietic	90 $\pm$ 10	98 $\pm$ 10
Dehydroabietic	108 $\pm$ 5	104 $\pm$ 8
Neoabietic	36 $\pm$ 7	40 $\pm$ 5
Chlorodehydroabietic	89 $\pm$ 6	96 $\pm$ 9

Recoveries and standard deviations were calculated from replicate determinations of six identical samples.

### **LIST OF FIGURES**

**Figure 1. The Hewlett-Packard 7680A Supercritical Fluid Extraction Module.**

**Figure 2. Animated instrument status display of the HP 7680A.**

**Figure 3. GC-ECD chromatogram of the RFA PFB esters in the reference sediment sample as recovered by the in-situ extraction/derivatization route.**

**Figure 4. GC-ECD chromatogram of the RFA PFB esters in the reference sediment sample as recovered by the off-line derivatization route.**

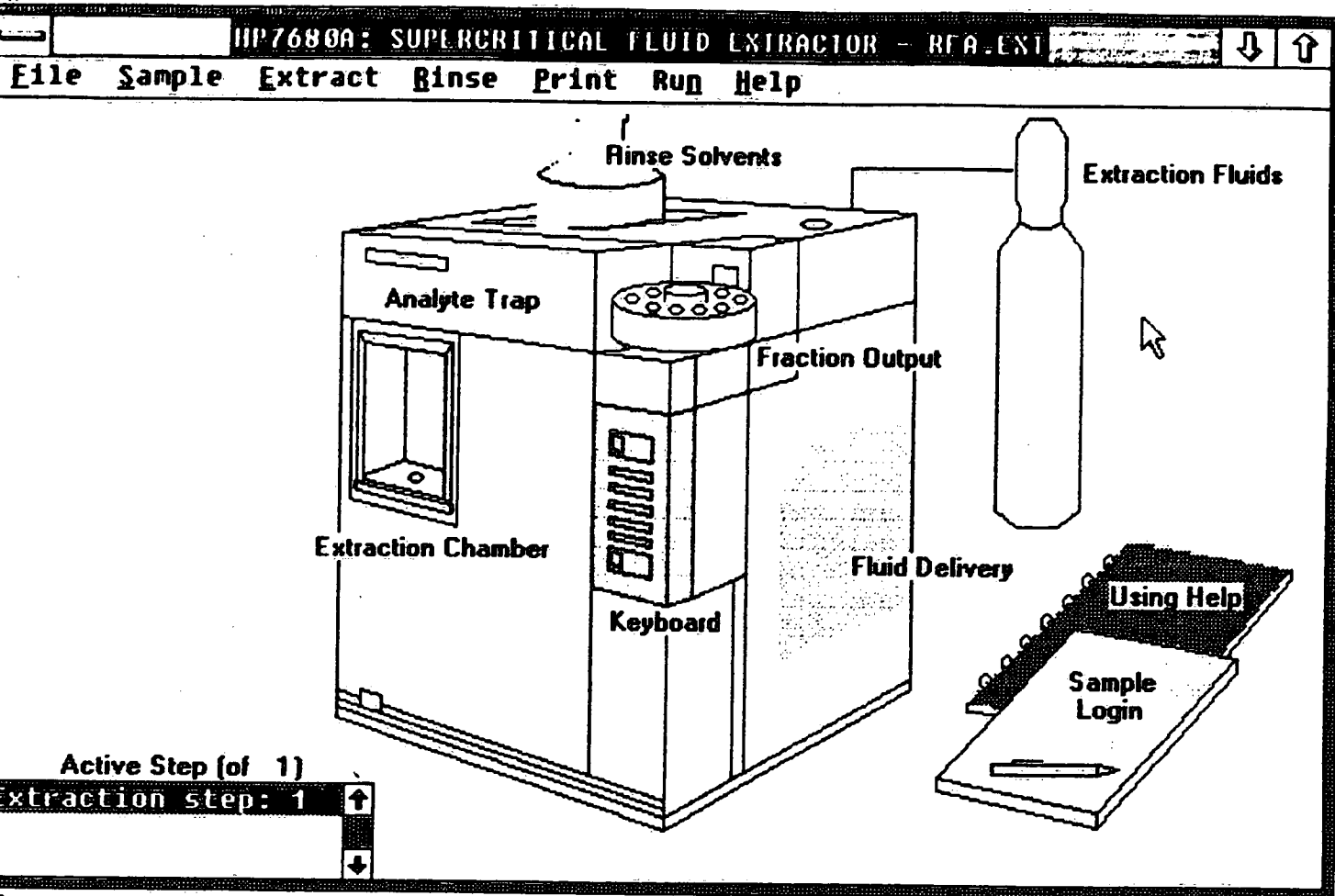


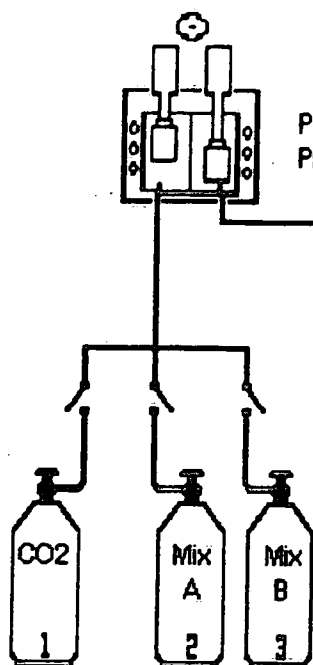
FIGURE 1

Instrument Status

CO2 Density:  
Flow rate:

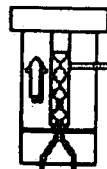
HP7680A Flow Diagram

Status:  
Extraction step:  
Time remaining:  
Rinse substep:



Pump head temp:  
Pressure:

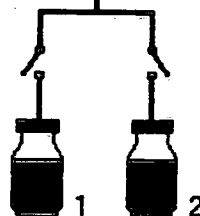
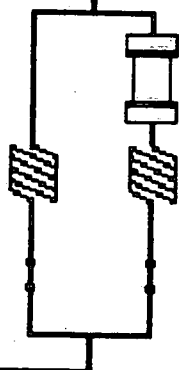
Nozzle temp:  
Trap temp:



Rinse rate:  
% dispensed:  
Vial number:

Vent/Waste Port

Chamber:  
Chamber temp:  
Thimble vol:



Rinse solvent: Acetone

Bypass preheat: 75  
Chamber preheat: 25

Extraction fluid: CO2

OK

Help

FIGURE 2

FIGURE 3

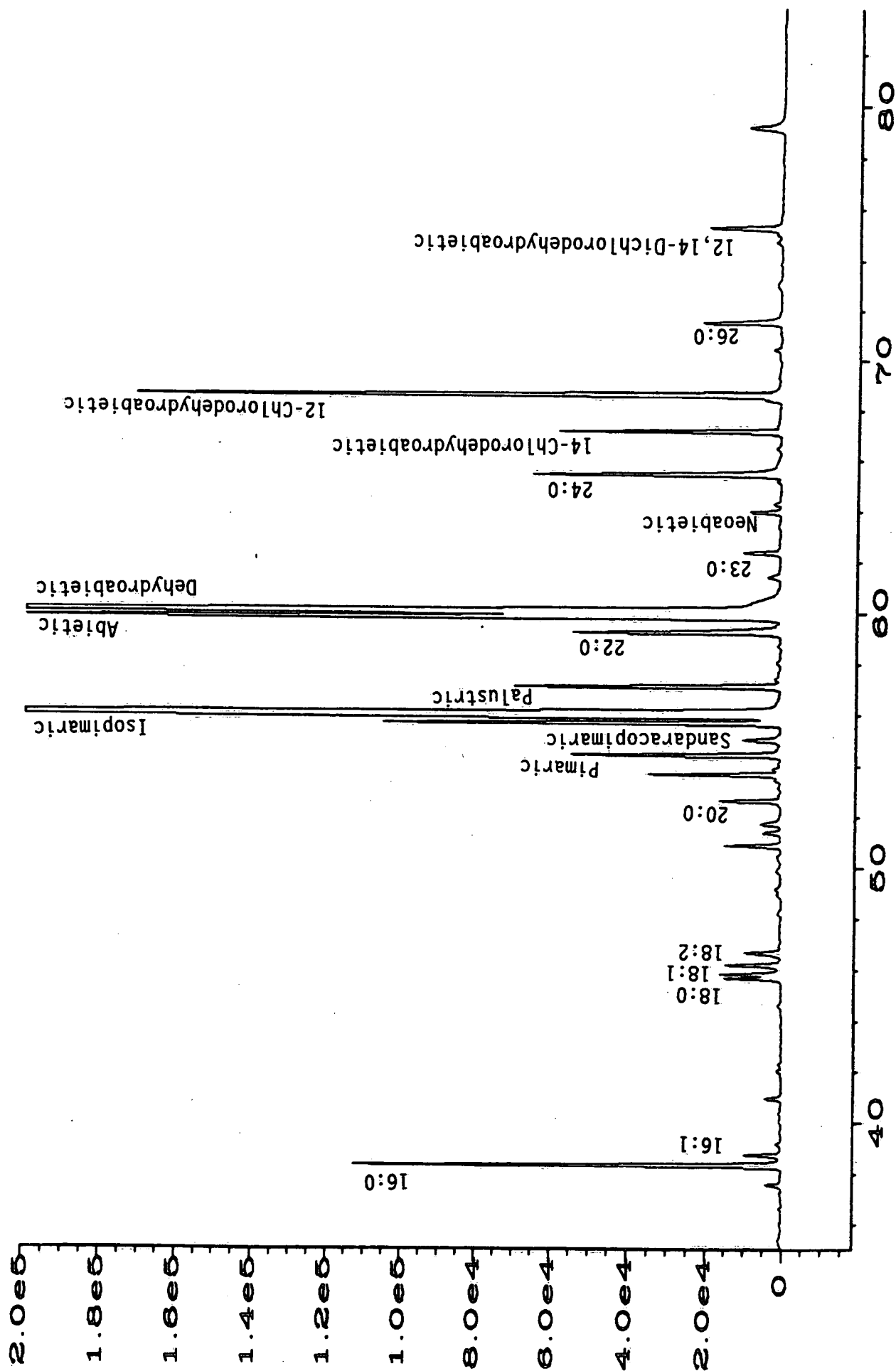
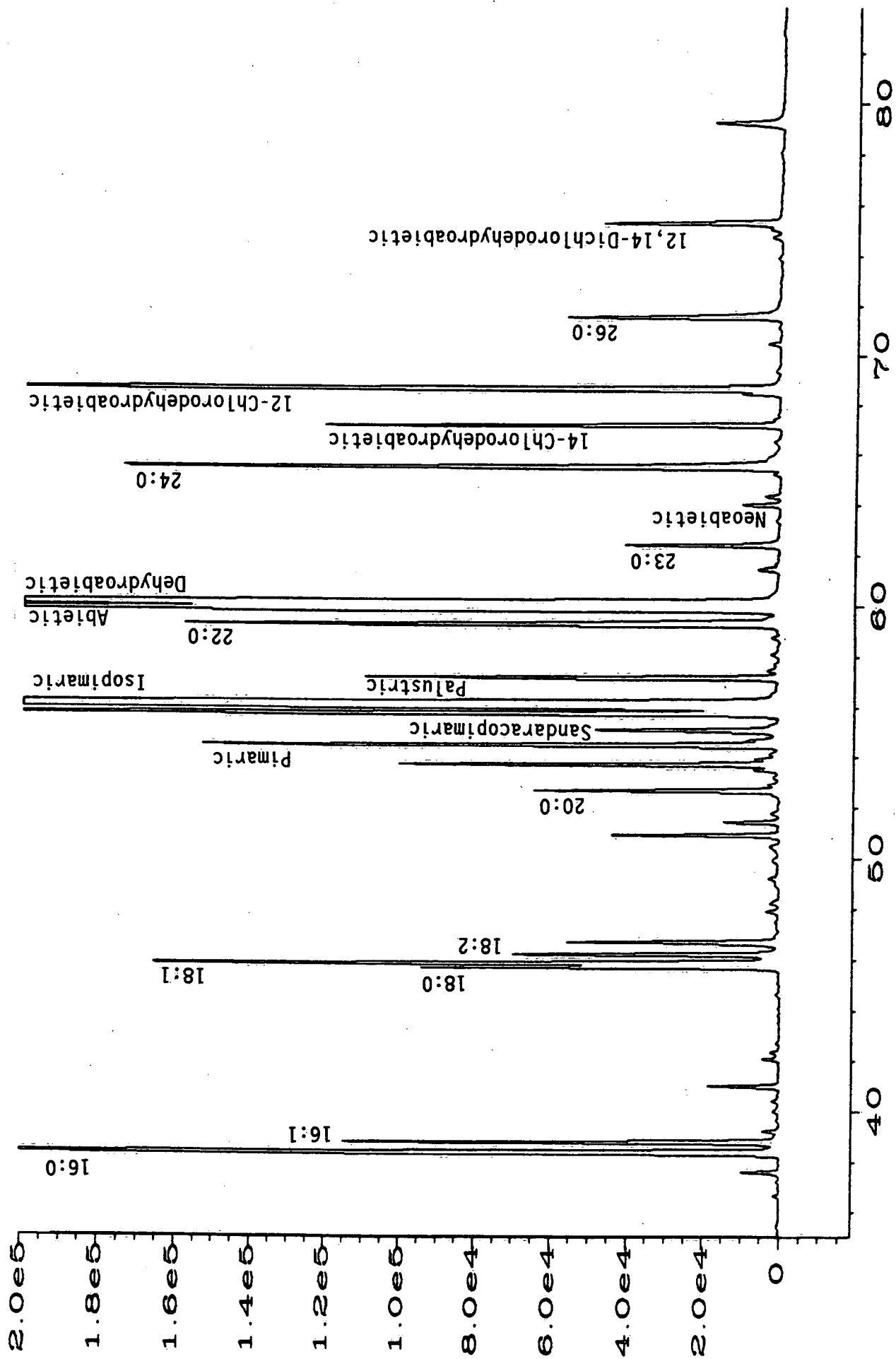
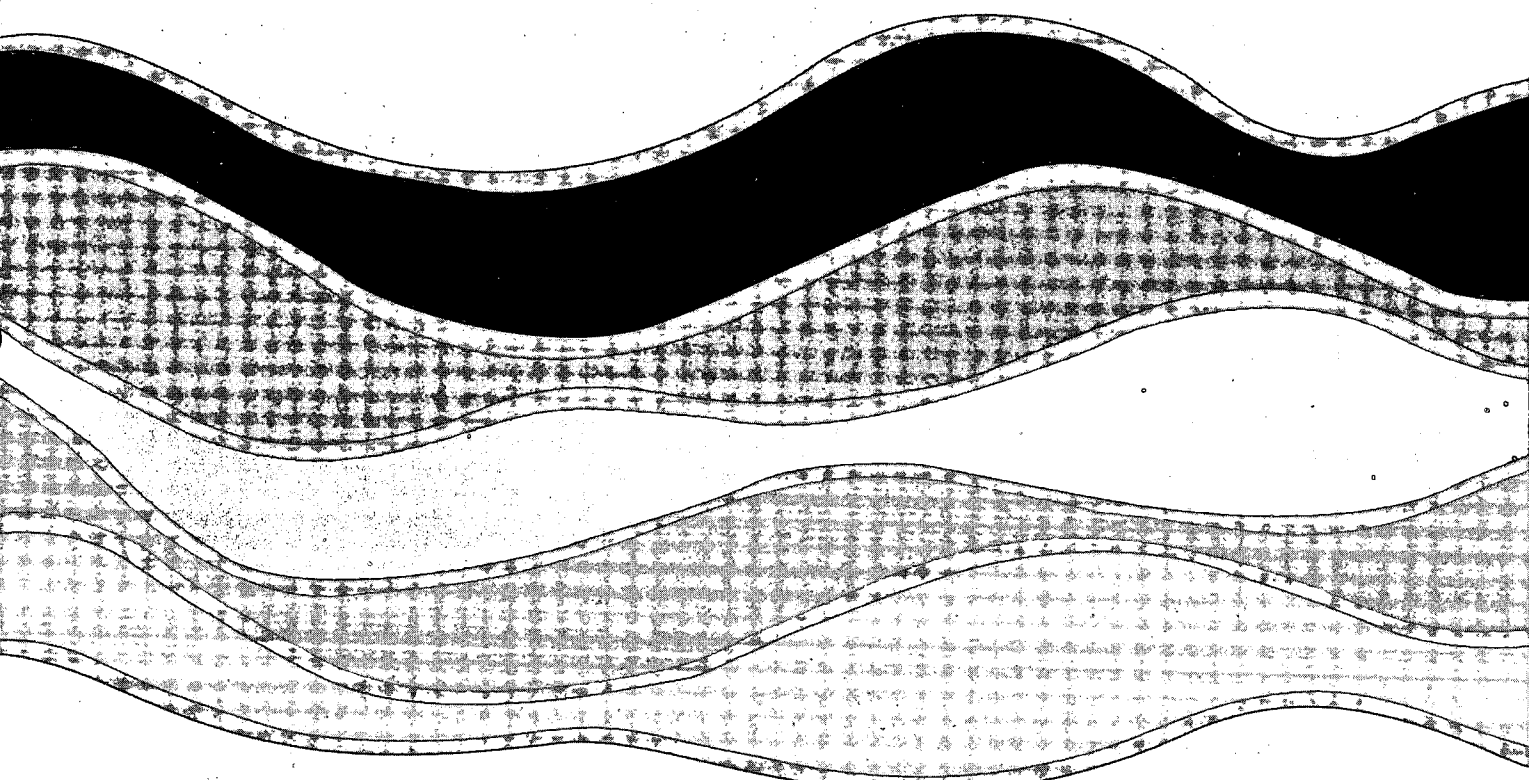


FIGURE 4





3 9055 1017 0259 4



NATIONAL WATER RESEARCH INSTITUTE  
P.O. BOX 5050, BURLINGTON, ONTARIO L7R 4A6



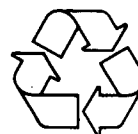
Environment  
Canada

Environnement  
Canada

Canada

INSTITUT NATIONAL DE RECHERCHE SUR LES EAUX  
C.P. 5050, BURLINGTON (ONTARIO) L7R 4A6

*Think Recycling!*



*Pensez à recycler!*