Gel Permeation Chromatography

In The GC/MS Analysis Of Organics In Sludges

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One method for measurement of priority organics in sludges consists of sequential base/neutral and acid extractions with methylene chloride using a homogenization-centrifugation technique; gel permeation chromatograph (GPC) for removal of high-molecular-weight interferences from both extracts; and GC/MS identification and quantitation of the organics in the GPC fractions. Removal of interferences from the sludge extracts by semi-automated GPC produced an analyzable, but relatively contaminated, large-molecule (phthalate) fraction and a "clean" small-molecule fraction from base/neutral extracts and one "clean" phenol fraction from the acid extracts. The GPC clean-up removed between 48-65 percent by weight of the interferences from base/ neutral extracts of primary sludges and about 35 percent from base/ neutral extracts of activated sludges. GC/MS analyses confirmed low amounts of interferences in the small molecule GPC fraction of the base/-

neutral extract and in the phenol GPC fraction of the acid extract. The study also revealed that interferences in the extracts did not significantly alter the GPC elution position of representative organics as compared to their GPC elution position in pure methylene chloride.

Recovery studies on spiked sludge generally revealed satisfactory recoveries for most of the 21 representative organics. Representative pesticides, however, partitioned into both base/ neutral GPC fractions necessitating GC/MS analyses of both fractions for maximum pesticide recovery. Representative phenols exhibited erratic and sometimes low recoveries during the recovery studies. Significant amounts of the weak acid phenols were extracted inappropriately into the base/neutral extract. The study suggests that the main problem in analysis of phenols is in the extraction process.

Gel Permeation Chromatography in the GC/MS

Analysis of Organics in Sludges

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Introduction

.   The U.S. EPA in a 1976 Consent Decree (1) established, for

regulation, a list of 129 priority pollutants. The list includes

83 semivolatile (extractable) organics.   Measurement of these

semivolatile organics usually employs GC/MS methods (2) using

liquid/liquid extraction of the organics from the environmental

sample before the GC/MS analysis. In complex environmental samples

the extractable background organics can interfere with conventional

extraction techniques and with the subsequent GC/MS analyses of the

organics of interest.

Municipal wastewater sludges which contain a very complex

background of extractable organics (3) are important environmental

samples. The high organic content of municipal sludges prevents

efficient conventional extraction of organics. While continuous liquid-liquid extraction, microextraction, and extractive steam distillation techniques may have potential for extraction of sludges, homogenization-centrifugation and modified Soxhlet techniques have demonstrated efficient extraction capabilities (3). The homogenization-centrifugation technique was adopted as the extraction step in the EPA's interim procedures (4) for the analysis of sludges. The large amounts of organics extracted by the method necessitate separation and clean-up of the extract.

As further background, the principal classes of organic interferences (5) extracted from municipal sludge samples are:

- Lipids
- Fatty acids
- Saturated hydrocarbons

In the sludge samples the large amounts of extractable interferences overwhelm both the GC and the mass spectrometer. These interferences must, therefore, be decreased in the extract before injection into the GC/MS system in order to permit analysis. The interference of total organics with the GC/MS analysis of an extract can be reduced by removal of some of the interferences and by separation of the extract into multiple fractions such that the total amount of organics in any fraction permits satisfactory

GC/MS analysis.

Three conventional methods are available for this reduction and separation:

- Acid/base separation
- Polarity separation (silica gel chromatography, etc.)
- Molecular size separation (gel permeation chromatography)

Acid/base separation is the fundamental approach behind the Agency's basic GC/MS methodology for wastewaters (2). In this basic methodology, base/neutral extraction - followed by acid extraction - divides the amount of extracted interferences between acid and base extracts, separates the base/neutral organics from the acids (phenols), and thus tends to equalize the interferences in each extract. Polarity separation, with silica gel or florisil, and a sequence of eluting solvents of increasing polarity, is applied to organic extracts to separate the extract into multiple fractions (such that the total organics in any fraction does not prevent GC/MS analyses).

Molecular size separation removes the large lipids, large fatty acids, and large hydrocarbons as a discard from the extract. These

materials apparently thermally decompose in the GC system and create very complex GC chromatograms. Heavy loads of these materials also reduce the life of the GC columns and increase mass spectrometer down-time. The molecular size separation also separates extracts into multiple fractions.

The above techniques for separation of extractable organics in sludge have been incorporated into an "Interim Method for the Measurement of Organic Priority Pollutants in Sludges" (4) with two alternative approaches for the separation of extractable organics. One alternative consists of a base/neutral extraction followed by an acid extraction, where both extractions use methylene chloride as solvent and the homogenization-centrifugation technique. Gel permeation chromatography (GPC) is applied for removal of high molecular weight interferences in both base/neutral and acid fractions, and finally GC/MS identification and quantitation of the extractable organics in each GPC fraction is carried out. The other alternative approach employs silica gel or florisil chromatography of the base/neutral extract rather than gel permeation chromatography. The silica gel or florisil chromatography separates the extract into four fractions suitable for GC/MS analyses. Both alternatives analyze the pesticide and PCB subclass within the other base/neutrals. Thus each provides a consolidated analytical method for the extractables.

The objectives of this work were to evaluate the capability of gel permeation to remove and separate the interfering materials, to develop a semi-automated procedure of gel permeation chromatography and to evaluate the effectiveness of the Interim Method with the gel permeation separation in the analysis of priority organics in municipal sludges.

Organic Interferences

To gain a perspective on the organic interferences in municipal sludges, methylene chloride extracts were obtained by homogenization-centrifugation extraction of municipal sludges from the Cincinnati Metropolitan Sewer District's Mill Creek Sewage Treatment Plant. The extracts were extracted as either base/neutrals (B/N) at pH 11 (followed by acids (A) at pH 2) or as acid/neutrals (A/N) at pH 2 (followed as bases (B) at pH 11). Aliquots of the extracts were air dried in a solvent hood at ambient temperatures (22°C) for 24 hours to remove the methylene chloride and then weighed to estimate the amount of semivolatile organics extracted from the sludge by the homogenization-centrifugation technique.

The weighings (Table 1) revealed that more than 25% of the organics based upon dry weight of solids were extracted from raw primary sludge, about 18% from combined primary/secondary sludge

and 10% or less from activated sludge. These weights of extracted organics clearly indicate the large amounts of material in municipal sludge extracts that act as interferences in the subsequent analysis (GC/MS) of parts per billion levels of individual organics. The amounts extracted also clearly reveal that acid neutral extraction followed by base extraction places most of the mass of extracted organics in the acid/neutral extract whereas performing the base/neutral extraction first followed by acid extraction more evenly distributes the extracted organics between the base/neutral and acid extracts. In addition, the "clean" base extract in the acid/neutral followed by base extraction sequence should contain only two extractable priority organics. Thus the sequence of base/neutral extraction followed by the acid extraction results in significantly fewer background interferences in the extract containing most of the priority organics.

Our permeation chromatography for reduction and separation of interferences uses methylene chloride as the eluting solvent. In this work, 60$\overset{\text{o}}{\text{A}}$ Styragel of 37/75-μ particle size was selected for column packing to theoretically provide the best reduction of interferences. The column and system (described later) was sized to process extracts from 50 ml samples of primary sludges.

The separation of the interferences by gel permeation chroma-
tography was initially evaluated using UV absorbance and refractive
index measurements as relative indicators of the amounts of organic
material in the eluent from the GPC column. The smoothed (ideal-
ized) curve of either refractive index or ultraviolet absorption as
a function of GPC eluent volume (Figure 1) revealed the typical
spreading of the organics in sludge extracts when chromatographed.

Using a trial-and-error approach with GC/MS analyses, the EPA's
Interim Method had recommended two fractions to aid in the
separation of much of the interferences found in the base/neutral
extracts.

Since our semi-automated gel permeation procedure was to be
used in a planned study of toxic substances which involved the
spiking of 21 selected organics (including six phthalates), two GPC
eluent fractions were established for the base/neutral extract to
provide a phthalate fraction F_1 and a fraction F_2 containing the
organic molecules smaller than the smallest phthalate, dimethyl
phthalate. The location of the fraction positions in the eluent
profile (Figure 1), established using di-n-octyl phthalate and
dimethyl phthalate standards, produced a highly contaminated F_1
fraction and a "clean" F_2 fraction from sludge extracts. The
organics larger than the largest phthalate, di-n-octyl phthalate

were discarded. A fraction F_3 was similarly established for the acid (phenol) extract using a mixture of the eleven priority phenols. The refractive index indicated that the F_3 fraction was relatively "clean."

Ambient-temperature drying and subsequent weighing of the F_1 and F_2 GPC fractions (Table 1 and Figure 2) confirmed the presence of significant amount of interferences in the phthalate (F_1) fraction and the very low amounts of interferences in the clean F_2 fraction. The relative amounts of organics in each fraction, as the bar graph (Figure 2) reveals, support the use of the ultraviolet or refractive index measurements as simple tools for evaluating the GPC separation process.

Experiments were also performed in order to evaluate differing column packing materials in the gel permeation chromatography. This evaluation was done by passing 5-ml aliquots of combined base/neutral-acid extracts of Cincinnati Mill Creek primary sludge through two different sizes of Styragel. Appropriate F_1 and F_2 fractions, based upon calibration with the pure phthalate standards for each size of Styragel, were air dried and then weighed to determine the amounts of material in each fraction.

The results (Table 2) confirmed that the 60A^o Styragel produced fractions with the least amount of interferences. An interesting sequential GPC treatment of the extract, in which the aliquot was separated first by 60A^o Styragel, followed by 200A^o Styragel, revealed even further reductions of the interferences. This sequential approach with the two Styragel columns in series however, was not evaluated for its impact on recoveries of priority organics. It has not been determined whether or not the observed reduction in background organics is simply due to the doubling of the column length.

Semi-Automated GPC Procedure

Based on the above, a semi-automated gel permeation procedure was developed for which fractions F_1 and F_2 (Figure 1) are collected separately for each base/neutral extract, while a fraction F_3 is collected for the corresponding acid (phenol) extract. Under no circumstance should _absolute_ retention volumes shown in Figure 1 be accepted purely on face value; instead, they should be regarded as _relative_ retention volumes. These warnings also apply to the retention values (i.e., "cut points") quoted in the two procedures given below since the correct _absolute_ values will vary from column to column.

In our work with municipal sludge, cleanup of concentrated methylene chloride extracts was accomplished by means of pumped gel permeation chromatography (GPC) under semi-automated microprocessor control. For all of this work, the equipment consisted of the following:

Column: Styragel, 20-mm ID x 122 cm long, 60 $\overset{o}{A}$ pore size, 37/75-μ particle size. These columns were furnished by Waters Associates under their catalog No. 40966.

Pump: Altex Scientific, Model No. 101A, semi-preparative, solvent metering system. Pump capacity = 28 ml/min.

Detector: Altex Scientific, Model No. 153, with 254-nm UV source and 8-μl semi-preparative flowcells (2-mm pathlengths)

Microprocessor/controller: Altex Scientific, Model No. 420, Microprocessor System Controller, with extended memory.

Injector: Altex Scientific, catalog No. 201-56, sample

injection valve, Tefzel, with 10-ml sample loop.

Recorder: Linear Instruments, Model No. 385, 10-inch recorder.

Effluent Switching Valve: Teflon slider valve, 3-way with 0.060" ports.

Supplemental Pressure Gauge with connecting Tee: U.S. Gauge, 0-200 psi, stainless steel. Installed as a "downstream" monitoring device between column and detector.

Flowrate was typically 16.8 ml/min. of pure methylene chloride (MCB's "OmniSolv"). Recorder chart speed was 0.50 cm/min.

The GPC system was calibrated under manual control with standard solutions of select, individual, Consent Decree toxic organics. In carrying out all exploratory calibrations, this study relied heavily on relative retention data furnished by outside contractors; this permitted significant savings of time and chemicals.

Two microprocessor programs were developed for the base/
neutral and acid extracts. Program number I was based on our
knowing that (a) the first class of toxics to elute includes the
phthalate esters and (b) the phenols should not be in this base/
neutral extract, but rather should be in the subsequent acid
extract. Program number I therefore is designed to yield a forecut
for discard, a "first" fraction (F_1) containing all the phthalates
(plus other higher-molecular-weight neutral toxics), and a "second"
fraction (F_2) containing the remaining (i.e., non-phthalate) neu-
tral and basic toxics. Program number II is based on a knowledge
that prior to elution of the phenols, everything can be discarded
since all the non-acidic toxics of interest should already have
shown up in the preceding B/N extract.

The overall GPC system was sized to handle the extract from a
50-ml sample of sludge. All fractions of interest from the above
programs were concentrated with Kuderna-Danish evaporators to 3 ml,
then subjected to GC/MS/DS analysis. The concentration to 3 ml
should be carefully determined for GC/MS quantitation but the
actual final volume may be varied to prevent precipitation of
organics in the final extract fraction.

The detailed GPC procedures used were as follows:

Program No. I - With a methylene chloride flowrate of 16.80 ml/min. and a stabilized recorder baseline, the injector loop is filled (by suction, not pressure) with a maximum of 7 ml of the base/neutral extract; if at all possible, each extract should be preconcentrated to a volume of 7 ml, or less so that the GPC clean-up can be accomplished without overloading the sample loop or having to carry out repetitive sample injections.

The base/neutral extract is injected at what is arbitrarily designated as time t_0; for the following 9.0 min. (151 ml), the column effluent is diverted to waste. At time $t_{9.0}$, collection of fraction no. 1 (F_1) is started by means of the effluent switching valve; this is continued until F_1 = 102.5 ml. At time 15.1 min. ($t_{15.1}$), the effluent switching valve is again actuated to begin collecting fraction no. 2 (F_2); this is continued until time $t_{24.0}$ at which point F_2 = 149.5 ml.

The effluent is again diverted to waste, and pumping is continued until the recorder baseline has once more stabilized. During this column-flushing period, the injector loop is also flushed with clean CH_2Cl_2; then it can be loaded with the next sample.

Program No. II - Flowrate of CH_2Cl_2 is again 16.80 ml/min. Also

a maximum of 7 ml of (acid) extract is loaded into the injector loop while the recorder baseline is stabilizing.

The acid extract is injected at time t_0, and the column effluent is diverted to waste for a total elapsed time of 12.5 min. (210 ml). At time $t_{12.5}$, collection of fraction no 3 (F_3) is started; this is continued until $t_{24.0}$ at which point F_3 = 193.2 ml

Once again, the column effluent is diverted to waste until the recorder baseline has stabilized. At that point, the column is ready to receive the next sample, or it may be shut down if desired.

At least as frequently as every other day, accuracy of column flowrate (i.e., pumping accuracy) should be checked. In general, significant errors in column flowrate will cause greater deviations in separation reliability than any other single factor.

As an additional cross-check, column calibrations should also be repeated at least once every two or three months. Our experience has shown that calibration drift is not noticeable unless one of two things occurs:

1) Column flowrate has changed significantly, or

2) The GPC column is nearing "exhaustion" due to irreversible column contamination.

GPC Separation of the Priority Organics

In order to evaluate the impact of the extracted interfering organics on the GPC separation of the priority organics independently of extraction variability, a primary sludge containing 6% solids was extracted using the homogenization-centrifugation technique, and aliquots of the base/neutral and acid extracts were then "spiked" with a selected priority semivolatile organics as shown in Table III and IV. Spiked and unspiked aliquots were separated by the GPC procedure. The proper fractions were collected, concentrated and then analyzed using capillary GC/MS methods. Spiked GPC solvent samples were also carried through the GPC and GC/MS procedures.

The samples in this phase of the work and for all subsequent work were analyzed by gas chromatography/mass spectrometry (GC/MS) using a Finnigan 4000 GC/MS with an INCOS 2300 data system. The chromatographic column-a J and W. SE-54, fused silica capillary (30 m x 0.32 mm)-handled all the B/N and phenolic priority organics satisfactorily. Earlier versions of the column were plagued by progressive fragility and breakage problems. However, newer columns are sturdy and deteriorate chromatographically before they

become fragile. A typical column has lasted at least 4-6 months.

The gas chromatograph, after a grob-type splitless injection, was programmed from 60-270°C at 5°/min. The mass spectrometer, tuned to a satisfactory bis (pentafluorophenyl) phenylphospine spectrum (6) was scanned from 40-450 amu, using 1-sec. scans. Quantitation by the data system was performed using an internal standard of perdeuteroanthracene ($D_{10}A$) added to each sample. Response factors were determined by the injections of standard mixtures of the compounds to be determined.

The GPC separation of the selected extractable organics, spiked into the base neutral ($F_1 + F_2$) and acid (F_3) extracts fractions, are shown in Tables III and IV. The GC/MS chromatograms also confirmed that the F_2 and F_3 GPC fractions were "clean" with little or modest amounts of background organics. The chromatograms revealed that the GPC fraction F_1 contained substantial amounts of background organics. All six phthalates, isophorone, the two dinitrotoluenes, and N-nitrosodipropylamine separated into GPC fraction F_1 (large molecules). All sixteen PAH's, the 4-chlorophenyl phenyl and 4-bromophenyl phenyl ethers, all six chlorinated aromatics, the three chlorinated aliphatics, nitrobenzene, and N-nitrosodiphenylamine separated chiefly into GPC Fraction F_2 (Table III). As expected, all the phenols separated into the F_3 fraction (Table IV). The pesticides, PCB's, the two benzidines, and all

other extractable priority organics not referenced above were not tested. The above results and subsequent field tests revealed that the background organics in the Cincinnati sludge extracts did not significantly alter the elution position of the selected organics in the GPC separation as compared to the elution position of these same selected organics in methylene chloride alone.

Methods Recoveries

The overall utility of the analytical method with GPC clean-up and separation, plus capillary GC/MS for the final analyses was tested using a mixture of twenty-one representative priority organics spiked into organic free water and also into primary and activated sludges from our Cincinnati pilot plant.

These Cincinnati sludges were used because the GPC/GC/MS method is employed for priority pollutant research samples obtained from the EPA pilot plant in Cincinnati. The overall method (Figure 3) is similar to the Agency Interim Method (4) but is adapted for semi-automation using our available laboratory equipment and the $60\overset{o}{A}$ Styragel resin (i.e. S-X3 Bio Beads are used in the Interim Method).

The sludges were extracted three times with three 80-ml aliquots of methylene chloride (first at pH $\geq$ 11 and then at pH $\leq$ 2) using homogenization-centrifugation; and were then subjected to the semi- automated GPC separation. Each of the three GPC fractions

(F_1, F_2 and F_3) and the discarded forecuts were all analyzed for the twenty one spiked organics (Table V and VI) by the previously described capillary GC/MS procedure. The GC/MS analyses were applied to all fractions as well as discards to evaluate the distribution of each organic during extraction and GPC separation. Measurable amounts of the selected organics were not found in any discard.

In Table V the normalized distribution of the selected organic revealed that better than 90% of the measured (GC/MS) amounts of phthalates, polynuclear aromatic hydrocarbons, and Arochlor 1254 are found in a single GPC fraction. The total overall recoveries (Table VI) of the phthalates, representative polynuclear aromatic hydrocarbons and the Arochlor 1254 were generally satisfactory and comparable to those recently published (7) for the Interim Method (4).

The smallest phthalates, dimethylphthalate and (to a lesser degree) diethylphthalate exhibited low or very poor overall recoveries in the organic-free water and activated sludge samples. The recoveries were satisfactory in the primary sludge samples. Since spiking of phthalates directly into sludge extracts after extraction did not reveal recovery problems (Table III) for the small phthalates, the poor recoveries observed for the complete

method in the organic free water and activated sludge samples are probably associated with significant saponification losses of the small phthalate esters during extraction at pH 11.

Similar losses have been observed (8) for extractions of wastewaters at pH 11 by the standard EPA method (2). The presence of substantial amounts of fatty acids in primary sludges is likely to be responsible for the observed better recoveries of the small phthalates from the primary sludge. Since repetitive monitoring of sample pH during extraction was not performed, it is only postulated, however, that the fatty acids in the primary sludge consume enough base to reduce the saponification. Further work is needed to clarify the observed results more fully.

The three representative phenols exhibited erratic, and sometimes low, overall recoveries (Table VI). They tend to distribute into more than one GPC fraction (Table V). The very weak acid phenols were found both in the acid F_3 fraction and inappropriately in the base/neutral F_2 fraction. Pentachlorophenol, however, as a relatively strong acid was usually found appropriately in the F_3 (acid) fraction. Finally the recoveries of the weak acid phenols spiked directly into the acid extracts (Table IV) were superior to the same phenols spiked into the sludge before extraction. Thus the extraction process appears to be the main problem for the weak acid phenols.

The selected pesticides while generally exhibiting satis-
factory overall recoveries (Table VI) would at times distribute
substantially into both the small molecule (F_2) and the phthalate
(F_1) fractions. Indeed, the multi component toxaphene distributed
into each GPC base/neutral fraction (F_1 and F_2) approximately
equally. Clearly maximum recoveries of the selected pesticides
required GC/MS analyses of both GPC base/neutral fractions.

Discussion

The EPA provides two alternatives in the "Interim Method for
the Measurement of Priority Organic Pollutants in Sludges." The
determination of whether the polarity (silica gel or flourisil)
separation of the GPC separation of the base/neutral extract is the
more appropriate for use has not been fully evaluated. Rigorous
evaluation of the two alternatives is best achieved by measuring,
for the same sludge samples, the weights of total organics in the
various GC/MS fractions produced by each alternative, while also
determining total recoveries (accuracy) and precision of the
measurements of priority organics by each alternative using GC/MS.

While such a definitive study has not been performed, the
current work does provide insight into the utility of the al-
ternatives of the Interim Method. Both alternatives have similar

limitations in their common extraction approach and in their common phenols analyses. The sequence of the base/neutral extraction followed by the acid extraction, required to distribute the total amounts of interferences nearly equally into the two extracts, probably causes saponification losses of the small phthalate esters. The difficulties in the extraction and recovery of priority phenols also occurs as a common limitation in the method.

The GPC alternative for the separation and reduction of interferences in the base/neutral extract provides only two base/-neutral fractions for GC/MS analyses compared to the four fractions obtained by polarity separation. The GPC approach obviously requires fewer GC/MS injections if fractions are not combined before GC/MS analysis. While definitive studies to determine the actual reduction in organic interferences and the accuracy and precision of the two alternatives on the same sludges have not been performed, intuitive assessment indicates that the GPC separation and clean-up should produce greater reduction in the background organics. The polarity separation should only irreversibly remove the most polar organics from each extract fractions, but such polar organics tend to be preferentially excluded from the solvent during the solvent extraction step.

The distribution and nature of the background organics and the distribution of the priority pollutants into the various fractions,

however, can impact recoveries of the individual priority organics. The GPC separation of the base/neutral extract from Cincinnati sludges produced a very clean F_2 fraction with more than 90 percent of the extracted background organics separated from the priority organics in the F_2 fraction. The F_2 fraction also contained the major number of the tested neutral priority organics. In contrast, the F_1 fraction, which includes the six phthalates and a few additional priority organics, contained substantial amounts of background organics but was still analyzable by capillary GC/MS procedures. The GPC F_1 fraction, especially for primary sludges, thus may contain more background organics than any separate fraction from the polarity separation.

The collection of fractions in any separation and clean-up procedure unfortunately may permit the distribution of organics into more than one fraction. The results of GPC separation reveal that most of the tested priority organics distributed desirably, usually with 90% or better efficiency, into a single fraction. The two single component pesticides, lindane and heptachor, distributed with approximately an 80 percent efficiency into the "clean" F_2 fraction. The multi-component toxaphene, which appeared approximately equally in both fractions, obviously requires GC/MS analysis of both base/neutral fractions for reasonable recoveries. The observed distribution of phenols into the F_2 and F_3 fraction

occurred because of extraction difficulties and is not related to the GPC separation.

In contrast the four fractions from the polarity separation alternative increase the chances for distribution of organics into more than one fraction. Unpublished results (9) reveal that the distribution of the specific organics into more than one fraction is more of a problem in the polarity separation alternative.

Finally, automation of GPC separation with long term reuse of the packed column is simple and effective. The single solvent, methylene chloride, used in the GPC separation is the same as the extraction solvent and has a low boiling point for efficient Kuderna-Danish concentration. In contrast, the polarily separation requires changing of solvents (4 'solvent mixtures)' for the separation with one solvent, hexane, having a relatively high boiling point. The polarity separation also requires fresh deactivated silica gel or florisil not more than 5 days old for each separation. Such a procedure is not easily automated.

Summary

The Agency's "Interim Method for Measurement of Organic Priority Pollutants in Sludges," includes two alternatives, gel permeation chromatography and polarity (with either silica gel or

fliorisil) chromatography for separation and clean-up of the base/neutral extract before GC/MS analysis for organics. This study evaluated the effectiveness of the GPC alternative for reducing the background organics in the extract fractions before GC/MS analysis, and also developed a semi-automated GPC procedure for efficient laboratory operation.

Removal of interferences from the sludge extracts by semi-automated GPC produced an analyzable, but relatively contaminated, large-molecule (phthalate) fraction and a "clean" small-molecule fraction from base/neutral extracts, as well as one "clean" phenol fraction from the acid extracts. The GPC clean-up permitted the discard of 48-65 percent by weight of the interferences from base/neutral extracts of primary sludges and about 35 percent from base/neutral extracts of activated sludges. GC/MS analyses confirmed low amounts of interferences in the small-molecule GPC fraction of the base/neutral extract and in the phenol GPC fraction of the acid extract. The study also revealed that interferences in the extracts did not significantly alter the GPC elution positions of representative organics as compared to their GPC elution positions for standard mixtures prepared in pure methylene chloride.

Recovery studies on spiked sludges generally revealed satis-

factory recoveries for most of the 21 representative organics. Representative pesticides, however, partitioned into both base/-neutral GPC fractions, thus necessitating GC/MS analyses of both fractions for maximum pesticide recovery. Recovery of representative phenols was erratic and sometimes low during the evaluation. In addition, significant amounts of the weak acid phenols were extracted undesirably into the base/neutral extract. The study suggests that the main problem in analysis of phenols is in the extraction process.

Acknowledgements

Literature Cited

(1) Natural Resources Defense Council (NRDC) et al. vs. Train, 8 ERC 2120 (DDC 1976).

(2) Federal Register, 44 (233) December 3, 1979, "Guidelines Establishing Test Procedures for Analysis of Pollutants; Proposed Regulations," pp 69526-69558.

(3) Warner, J.S.; Jungclaus, G.A.; Engel, T.M.; Riggin, R.M.; Chuang, C.C.; "Analytical Procedures for Determining Organic Priority Pollutants in Municipal Sludge," EPA-600/2-80-030, Municipal Environmental Research Laboratory, U.S. EPA; Cincinnati, OH, March 1980.

(4) "Interim Methods for the Measurement of Organic Priority Pollutants in Sludge," U.S. EPA, Environmental Monitoring and Support Laboratory: Cincinnati, OH, August 1981.

(5) Bishop, D.F.; "GC/MS Methodology for Priority Organics in Municipal Wastewater Treatment," EPA-600/2-80-196, Municipal Environmental Research Laboratory, U.S. EPA: Cincinnati, OH, November 1980.

(6) Budde, W.L.; Eichelberger, J.W.; "Manual for Organic Analysis Using Gas Chromatography-Mass Spectrometry;" EPA-600/8-79-006, Cincinnati, OH, March 1979.

(7) Lopez-Avila, V.; Haile, C.L.; Goodard, P.R.; Malone, L.S.; Northcutt, R.V.; Rose, D.R.; Robson, R.L.; In "Advances in the Identification and Analysis of Organic Pollutants in Water," Vol. II; Keith, L.H.; Ed. Ann Arbor Publishers: Ann Arbor, MI. 1981; pp 793-828.

(8) Longbottom, J.E.; Environmental Monitoring and Support Laboratory: U.S. EPA; Cincinnati, OH, personal communication, 1982.

(9) Haile, C.L.; and Lopez-Avila, V.; "Development of Analytical Test Procedures for the Measurement of Organic Priority Pollutants in Sludge;" Final Report in Preparation, EPA Contract 68-03-2695; Environmental monitoring and Support Laboratory: Cincinnati, OH.
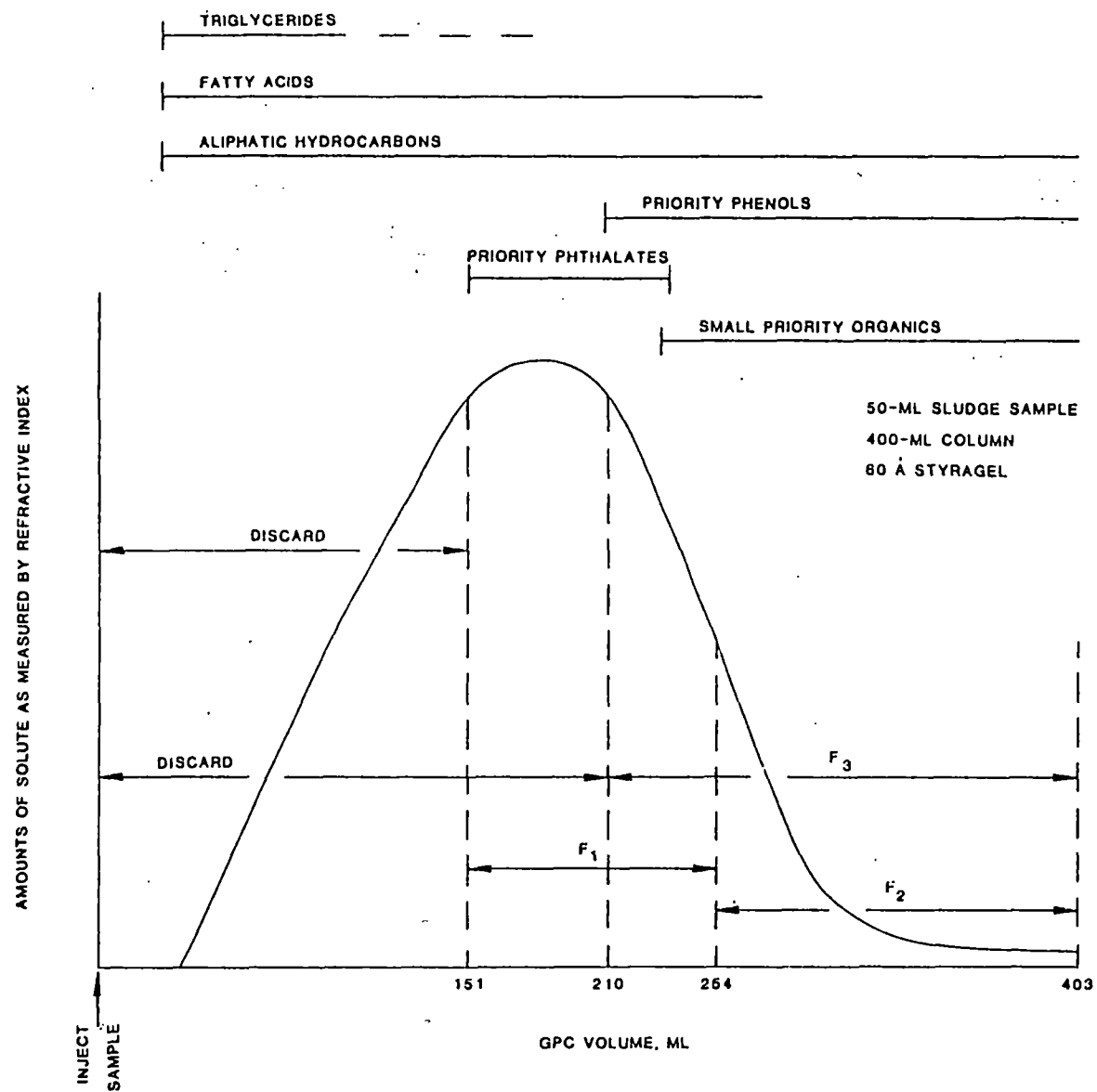
Figure 1. GPC separation of organics.
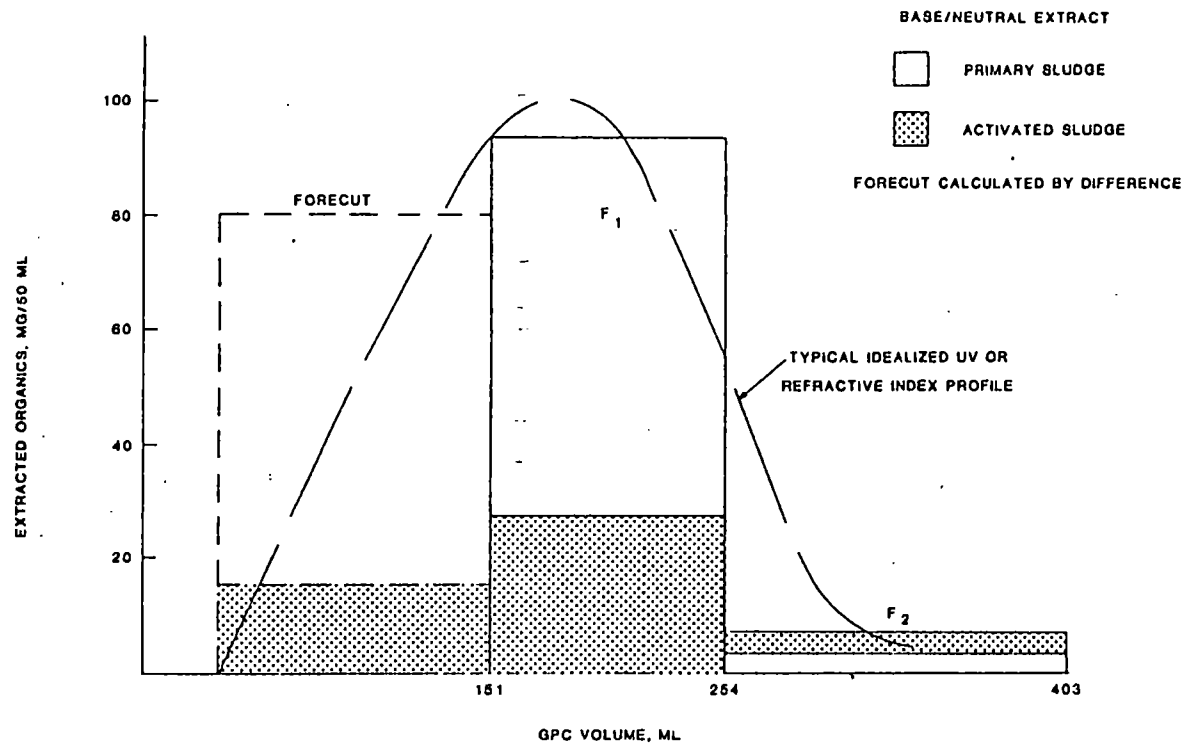
Figure 2.' Amounts of extracted organics in the GPC base/neutral fractions.

Sludge
(50 ml)

Adjust to pH ≥ 11
with 6N NaOH

Extract 3 times with 80 ml
CH_2Cl_2 by homogenization/
centrifugation

Dry with Na_2SO_4

Kuderna-Danish concentration
to 7 ml. Separate by semi-
automated GPC into fractions
F_1 and F_2

Forecut
discard

Adjust to pH ≤ 2
with 6N HCl

Extract 3 times with 80 ml
CH_2Cl_2 by homogenization-
centrifugation

Concentrate F_1 and F_2
to 3 ml. Capillary
column GC/MS analysis
with SE-54 WCOT column

Dry with Na_2SO_4

Kuderna-Danish concentration
to 7 ml. Cleaning by semi-
automated GPC into fraction
F_3

Forecut
discard

Kuderna-Danish concentration
to 3 ml. Analyze for phenols
by GC/MS with SE-54 WCOT column

Figure 3. Scheme for analysis of extractable organics in sludges.

Table I. Extractable Organics From Municipal Sludges.

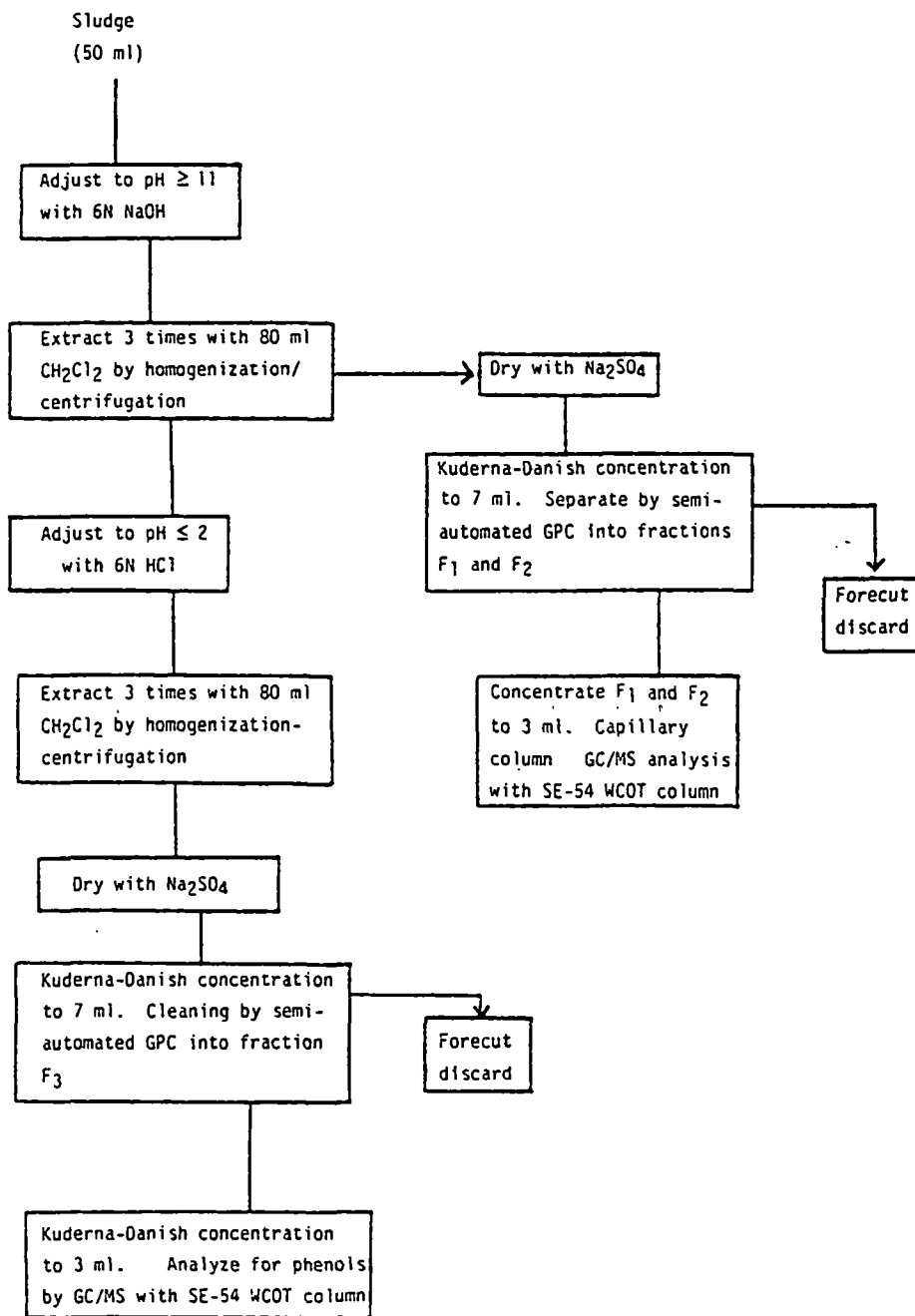|			Weight of Extractable Organics, g/l				
Sludge Type	Sludge, Percent Solids	Extraction Sequence	CH_2Cl_2 Extract	GPC Separation		% Solids Extracted	% of Extracted B/N Solids Removed by GPC
				F_1	F_2		
activated,	2.0	B/N	0.62	0.34	.055		36
1/6/80		A	0.53				
		Combined	1.15			6	
activated,	2.0	A/N	1.02				
1/6/80		B	0.08				
		Combined	1.10			6	
activated,	1.5	B/N	0.78	0.46	.055		34
1/6/80		A	0.73				
		Combined	1.51			10	
activated,	1.5	A/N	1.22				
1/6/80		B	.10				
		Combined	1.32			9	
primary,	1.5	B/N	3.47	1.79	.029		48
1/6/80		A	2:11				
		Combined	5.58			37	
primary,	3.5	A/N	9.22			26[b]	
8/13/79							
primary,	4.5	B/N	10.50	3.21	0.44		65
3/20/79							
primary,	4.5	A/N	15.98			36[b]	
3/20/79							
primary/ activated,	5.0	A/N	8.59	$F_1+F_2 = 5.56$			35
11/15/78		B	0.23				
		Combined	8.82			18	

[a] B/N is base/neutral extraction at pH 11; A is acid extraction at pH 2 after B/N extraction. A/N is acid/neutral extraction at pH 2; B is base extraction at pH 11 after A/N extraction. [b] Since the base extraction after acid/neutral extraction removes very little additional extracted material, the percent solids extracted can be estimated using only the weight of the acid/neutral extract.

Table II. Selection of GPC Packing[a]

Styragel Packing	Total Solute in	
	Phthalate Fraction [b] F_1, mg	Small Molecule Fraction [b] F_2, mg
60$\overset{o}{A}$	138	11.4
200$\overset{o}{A}$	197	12.3
60$\overset{o}{A}$ + 200$\overset{o}{A}$ [c]	67.8	5.8

[a] 5-ml aliquots of combined base/neutral - acid extract from a Cincinnati Millcreek Primary Sludge were used in study. [b] Phthalate fraction is based upon elution volumes for largest (di-n-octylphthalate) and smallest (dimethyphthalate) phthalates. Small-molecule fraction contains all elutable molecules smaller than dimethylphthalate. [c] These special F_1 and F_2 fractions generated by the two columns in series.

Table V. Fraction of Organics in Each GPC Fraction[a]

	Distilled Water Mean[b]			Primary Sludge Mean[b]			Activated Sludge Mean[b]		
	F_1	F_2	F_3	F_1	F_2	F_3	F_1	F_2	F_3
dimethyl phthalate	-	-	-	.935	.005	.059	.999	.001	-
diethyl phthalate	.948	.052	-	.905	.028	.052	.915	.033	.052
di-n-butyl phthalate	.946	.052	.004	.904	.043	.052	.889	.035	.076
butylbenzyl phthalate	.968	.032	-	.919	.040	.040	.962	.022	.016
di-n-octyl phthalate	.975	.025	.001	.952	.044	.004	.974	.025	.004
bis(2-ethylhexyl)phthalate	.968	.032	.001	.946	.042	.012	.903	.028	.070
naphthalene	-	1.000	-	.007	.909	.083	-	.974	.026
acenaphthene	-	.986	.015	.005	.906	.089	-	.947	.054
fluorene	-	.980	.021	-	.931	.069	.001	.950	.049
phenanthrene	-	.980	.020	.004	.916	.080	.003	.928	.069
anthracene	-	.950	.050	.002	.926	.073	.005	.945	.050
fluoranthene	-	.970	.031	-	.907	.093	.002	.936	.062
chrysene	-	-	-	-	-	-	-	-	-
pyrene	-	.970	.031	-	.907	.093	.002	.940	.059
phenol	-	.667	.334	.001	.879	.119	.005	.467	.528
2,4-dimethylphenol	-	1.000	-	-	.869	.131	-	-	-
pentachlorophenol	-	-	1.000	-	.274	.726	-	-	1.000
lindane	.186	.805	.010	.033	.956	.011	.157	.808	.036
heptachlor	.257	.731	.013	.188	.812	-	.229	.753	.019
toxaphene	.543	.458	-	.528	.315	.157	.526	.475	-
arochlor 1254	-	1.000	-	-	1.000	-	-	1.000	-

[a] F_1 is the phthalate fraction of the base/neutral extract; F_2 is the small molecule fraction of the base neutral extract; F_3 is the phenol fraction of the acid extract. [b] The mean is the average weight fraction of the specific organic found in each GPC fraction by GC/MS analysis; the mean is normalized and is usually based upon 4 determinations in distilled water, 6 determinations in primary sludge, and 8 determinations in activated sludge.

Table VI. Percent Recoveries

Priority Organic[c]	Spiked Distilled H_2O mean[a]	S^b	Primary Sludge mean[a]	S^b	Activated Sludge Mean[a]	S^b
dimethyl phthalate	-	-	64	11	3	4
diethyl phthalate	43	31	60	10	30	14
di-n-butyl phthalate	100	42	96	52	100	48
butylbenzyl phthalate	79	47	74	12	75	34
di-n-octyl phthalate	112	57	72	35	85	37
bis(2-ethylhexyl)phthalate	122	69	45	29	137	67
naphthalene	82	15	47	26	60	18
acenaphthene	74	26	73	19	72	14
fluorene	73	22	76	19	74	16
phenanthrene	77	23	76	17	73	21
anthracene	86	20	78	18	78	23
fluoranthene	77	15	105	29	78	23
chrysene	94	35	111	64	88	42
pyrene	82	14	108	34	80	21
phenol	56	-	56	6	39	20
2,4-dimethylphenol	25	18	25	17	-	-
pentachlorophenol	38	-	152	113	78	43
lindane	80	25	62	20	91	57
heptachlor	71	28	35	21	89	57
toxaphene[d]	120	38	130	44	91	25
arochlor[d] 1254	71	11	95	44	69	28

[a] The mean is usually an average percent recovery of four determinations in distilled water, 6 determinations in the primary sludge and 8 determinations in the activated sludge.
[b] S is an estimate of the standard deviation of the $\frac{S}{\sqrt{n}}$ where n is the number of determinations. [c] Except for Toxaphene and Arochlor 1254, the sludges and distilled water were spiked with 1500 µg/l of each organic. [d] The sludges and distilled water were spiked with 4500 µg/l of Toxaphene and Arochlor 1254.