

METALLOORGANIC, ORGANIC AND MUTAGENIC
PROPERTIES OF OIL SHALE RETORT WATERS

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INTRODUCTION

Considerable technological research is currently underway to develop a shale oil industry in the United States. The feasibility of shale oil production is being tested using various technologies, most of which are presently at the bench-level to pilot-plant stages of development. Concern about the potential health and environmental impacts of such an industry has, in turn, prompted intensive biomedical and environmental research, much of it directed at chemically and biologically evaluating the genotoxicity/carcinogenicity potential of shale oils. It has already been demonstrated that certain crude and chemically fractionated shale oil samples are mutagenic using carcinogen-screening bioassay systems such as the Ames Salmonella assay.(1-6)

Retort waters are co-produced with shale oil during the retorting of oil shale. The quantity and chemical properties of the retort water vary depending upon the retorting technology used;(7) aboveground retorting processes generate much less retort water than shale oil, whereas in situ processes generate approximately equal amounts of retort water and shale oil. All of the retorting processes designed to date provide for complete recycling of all process waters, with no discharge into the environment. Nevertheless, considering the enormous amounts of retort waters that may be produced in a large-scale shale oil industry, there is considerable concern about the health and environmental impacts associated with the recycling of these waters and any accidental release into the environment.

Much is known about the inorganic properties of retort waters, notably the metal and trace metal content.(8) Less is known about their organic content. Information about specific classes of organic compounds (9,10) and organometallic species (11) is available; even a few comprehensive studies of numerous classes of organics have been reported.(12) Much remains to be elucidated, however, about the relationship of the retort waters' organic content to mutagenicity and chelation of the metals present, i.e. metallorganics.

The primary goal of this study was to evaluate the mutagenic, organic, and metallorganic properties of oil shale retort waters. Four retort water samples were analyzed in the mutagenesis/organics study: a storage water and a condensate water from the Paraho aboveground retort; a retort water from the Occidental vertical, modified in situ retort; and a retort water from the horizontal, true in situ retort at Vernal, Utah.

A second goal of this study was to develop and evaluate improved methods of chemically fractionating the complex organic content of retort waters to facilitate their chemical and mutagenic characterization. To begin the mutagenesis study, we tested several methods for extracting hydrophobic organics from the retort waters: 1) solvent extraction with pH adjustment; 2) XAD-4 partition chromatography; and 3) C_{18} -partition chromatography. We then tested the usefulness of high-performance liquid chromatography (HPLC) for fractionating the hydrophobic organic fraction. Each method was evaluated both chemically and biologically. For the metallorganics/organics study we decided to test steric-exclusion chromatography as a means of fractionating metal-organic chelates.

EXPERIMENTAL

Mutagenesis/Organics Studies

C₁₈-Partition Chromatography. Each retort water used in the mutagenesis studies was initially fractionated by partition chromatography on a C₁₈-cartridge based on a method described by Riggin and Howard.(13) In our procedure, the C₁₈-cartridge was pre-conditioned by eluting 2 ml of methanol through it, followed by 4 ml of milli-Q-purified water. Each retort water (24-150 ml) was then loaded onto the C₁₈-cartridge. The organic-loaded C₁₈-cartridge was then washed with 4 ml of milli-Q water. The hydrophobic organic fraction of the retort water was then eluted from the C₁₈-cartridge with 2 ml of methanol.

HPLC Analysis. The hydrophobic organic fraction of each retort water was further fractionated on the basis of polarity by normal phase HPLC using an NH₂-column and a three-solvent mobile phase system. Hexane flowed through the column isocratically at 5 ml/min. for the first 5 min., followed by a 10 min., linear gradient to 100% methylene chloride; 100% methylene chloride flowed isocratically for 5 min., followed by a 10 min., linear gradient to 100% isopropanol; finally, 100% isopropanol flowed isocratically for 5 min. The NH₂-column was reconditioned by cycling back to hexane using a 10 min., linear gradient, followed by 100% hexane isocratically for 10 min.

Fifty μ l of sample (50 to 100 mg/ml) were injected onto the NH₂-column per HPLC run; 10 to 20 runs of each sample were repetitively collected into 250 ml roundbottom flasks. The HPLC effluent was monitored by UV absorbance at 250 nm. Each HPLC fraction was concentrated by rotary evaporation, transferred to a pre-weighed vial, evaporated to dryness under N₂, weighed, and redissolved in 1 ml of methylene chloride. Half of the sample was set aside for combined gas chromatography-mass spectrometry (GC-MS) analysis; the other half was redried and dissolved in 0.5 ml DMSO for the Ames bioassay.

GC-MS Analysis. The organics in the HPLC fractions were analyzed on a Hewlett Packard 5982 GC-MS instrument in the electron impact (70 eV) mode. A 15 m x 0.30 mm I.D. glass capillary column coated with 0.25 μ m film thickness of SE-52 was programmed from 40°C to 100°C at 32°C/min., then programmed at 8°C/min. to 290°C, where it was maintained isothermally for 30 min. A Grob splitless injection system was used. The GC column was interfaced to a mass spectrometer via platinum-iridium tubing. The mass range of 50 to 300 a.m.u. was scanned by computer (HP5934A) every 1.8 sec.

Mutagenesis Assay. Agar-plate mutagenicity assays were carried out essentially as described by Ames et al.(14) The TA98 strain of Salmonella typhimurium was used with the S9 fraction from rat liver homogenate (induced with Aroclor 1254) as a metabolic activator. Dimethylsulfoxide (DMSO) was used as the solvent for all of the standards and HPLC fractions that were bioassayed. After 24 to 36 hr. incubation of the inoculated plate, revertant colonies of TA98 were counted on a New Brunswick Scientific Company Biotran II automated colony counter.

Metallorganics/Organics Studies

Sephadex G-15 Chromatography. Each retort water (2-10 ml) was loaded on a Sephadex G-15 column (2.5 cm x 31 cm) and chromatographed at a specific flow rate ranging from 23-31 ml/hr. with milli-Q-purified water. Replicate runs were made with each sample, with and without Blue Dextran as a void volume marker. The column effluent was monitored by UV absorbance at 254 nm and collected in 4 ml fractions on an Isco fraction collector. Each column fraction was divided in half: one aliquot was set aside for metals analysis by plasma emission spectroscopy (PES); the other half was methylated, prior to GC and GC-MS analysis.

Methylation. Each column subfraction was extracted with an equal volume of chloroform to remove hydrophobic organics. The chloroform extract was set aside for GC analysis. The aqueous fraction, believed to contain polar organic compounds which

might chelate metals, was evaporated to dryness under N_2 at $\sim 100^\circ C$. The dried residue of each fraction was then methylated by adding 1 ml of BF_3 /methanol (14% w/v) and incubating at $100^\circ C$ for 40 min. in a sealed vial. After cooling, chloroform (1 ml) was added and the mixture was transferred to a test tube containing buffer solution (3 ml of 1M KH_2PO_4 , pH 7). After vortexing for one min. and centrifugation, a specific amount of the chloroform layer (0.4-0.7 ml) was transferred to a glass vial and the chloroform was evaporated under N_2 at R.T. The residue was redissolved in chloroform (100-200 μl) and an aliquot (1-2 μl) was analyzed by GC and GC-MS.

GC Analysis. The organics in the methylated fractions were initially analyzed on a Hewlett Packard 5880 gas chromatograph equipped with a flame ionization detector. A fused silica capillary column (30 m x 0.25 mm I.D.) coated with 0.25 μm of SE-52 was programmed from $40^\circ C$ to $300^\circ C$ at $4^\circ C/min.$, where it was maintained isothermally for 10 min.

GC-MS Analysis. Following the GC analysis, the organics in the methylated fractions were analyzed on a Hewlett Packard 5985 GC-MS instrument in the electron impact (70 eV) mode. A 60 m x 0.25 mm I.D. fused silica capillary column coated with 0.25 μm of SE-54 was programmed from $40^\circ C$ to $300^\circ C$ at $5^\circ C/min.$, where it was maintained isothermally for 8 min. A splitless injection system was used to introduce the sample onto the GC-MS instrument. The GC column was interfaced directly to the mass spectrometer. The mass range of 50 to 400 a.m.u. was scanned every 1 sec. by computer (HP 2100MX equipped with the HP 7920 Large Disc Drive).

PES Analysis. Each Sephadex column fraction was analyzed for metals (Fe, Mo, Ni, Mn and Zn) on a Spectrometric Spectra Span III, three-jet, direct-current plasma emission spectrometer equipped with a multi-element cassette. The instrument was operated according to the manufacturer's recommendations. A standard solution consisting of 1 ppm each Fe, Mo, Ni, Mn and Zn (dissolved in a buffer of 2000 ppm Li, 1% HNO_3) was used to calibrate the instrument. A solution of Li-buffered (with 1% HNO_3), deionized water was used to blank the instrument. The instrument's detection limits were 2 ppb for Mn and 5-10 ppb for Fe, Ni, Mo and Zn.

Materials

Samples. The Paraho storage water was sampled on August 26, 1977, and the condensate water was sampled on October 13, 1980, at the Paraho aboveground retort (Anvil Points, Colorado). The Occidental retort water was sampled on March 7, 1979, at the Occidental vertical, modified in situ retort (Room 6) at Logan Wash, Colorado. The Vernal retort water was sampled on July 11, 1978, at Vernal, Utah, horizontal, true in situ retort.

Standards. Most of the standards used in the HPLC and GC studies were purchased from Aldrich Chemical Company (Milwaukee, Wisconsin) and RFR Corporation (Hope, Rhode Island).

Chromatographic Columns. The C_{18} -Sep Pak cartridges used for the C_{18} -partition chromatography and the μ Bondapak NH_2 -columns and precolumns used in the HPLC analyses were purchased from Waters Associates, Inc. (Milford, Massachusetts). The NH_2 -column was a semi-preparative column (7 mm x 30 cm). The glass columns (2.5 cm x 45 cm) and Sephadex G-15 used in the metallorganics/organics study were purchased from Pharmacia Fine Chemicals (Piscataway, New Jersey).

Solvents and Glassware. All of the solvents used in the chromatographic analyses were redistilled-in-glass solvents purchased from Burdick and Jackson Laboratories, Inc. Deionized water was passed through a milli-Q system (Millipore) containing two ion exchange resins and two charcoal filters. All glassware was acid cleaned.

RESULTS

Mutagenesis/Organics Study

All of the various methods tested for extracting organics from retort waters

TABLE 1. Organics in Oil Shale Retort Waters.

RETORT WATER	ORGANIC ¹ FRACTION (mg/ml)	TOTAL ORGANIC CARBON (mg/ml)	pH
PARAHO	29.85 ± 0.08	42.75	8.4
PARAHO (CONDENSATE)	1.28 ± 0.13	4.08	8.7
OCCIDENTAL	1.47 ± 0.01	4.23	8.9
VERNAL	1.02 ± 0.15	2.93	8.5

1 EXTRACTABLE BY C₁₈ PARTITION CHROMATOGRAPHY WITH MeOH ELUTION.

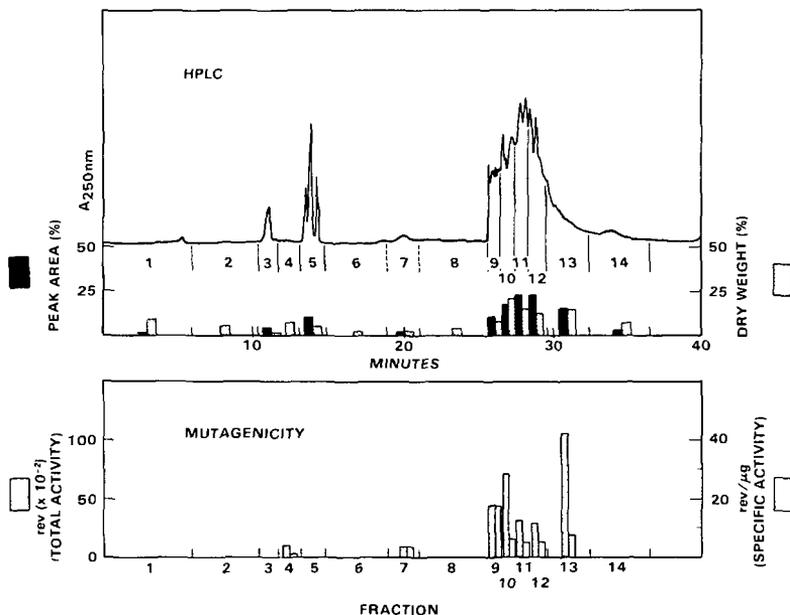


FIGURE 1. HPLC of Hydrophobic Organic Fraction from Paraho Storage Retort Water.

extracted some mutagenic activity. C_{18} -partition chromatography yielded the highest recovery of mutagenic activity (2-4 rev/ μ g); the amounts of organics extracted were also quite reproducible (Table 1). Solvent extraction with pH adjustment also yielded appreciable mutagenic activity (2.6 rev/ μ g) but the efficiency of extraction varied considerably. HPLC fractionation of the hydrophobic organic fraction or C_{18} -extractable organics, resulted in considerable fractionation of the organics and mutagenic activity for all of the retort waters studied (Figure 1).

On the basis of the HPLC fractionation, most of the species in the hydrophobic organic fraction of each retort water chromatographed in the polar HPLC region but some species chromatographed in the moderately polar HPLC region (Figure 1). The organic content of the various samples appeared to be quite heterogeneous. GC-MS analyses of the HPLC fractions of the oil shale retort waters revealed that the moderately polar HPLC region contained mainly nitrogenous compounds: pyridine, alkyl-pyridines, aniline, alkyl-anilines, quinolines and alkyl-quinolines. The polar HPLC region mainly contained oxygenated and mixed-function compounds: carboxylic acids, dicarboxylic acids, phenols, alkyl-phenols, and amides.

The mutagenic activity of each retort water was confined to its hydrophobic organic fraction. The Paraho storage water was the most mutagenic (1.29 rev/ μ g), followed by Occidental (0.06 rev/ μ g), Vernal (0.04 rev/ μ g) and Paraho condensate water (0.02 rev/ μ g). No mutagenic activity was detected in the complementary hydrophilic, or aqueous, fraction. After HPLC fractionation of the hydrophobic organic fraction, virtually all of the mutagenic activity was concentrated in the polar HPLC region (Figure 1). The mutagens of the Paraho storage water chromatographed throughout the polar HPLC region, indicating a high degree of heterogeneity. The mutagens of the Paraho condensate water appeared to be less heterogeneous. The mutagens of the Occidental and Vernal retort waters were even less heterogeneous, confined exclusively to one region of the polar HPLC region.

Metallorganics/Organics Study

Sephadex G-15 chromatography fractionated the organics in retort waters into several discrete peaks (Figure 2). Each of the waters studied yielded a distinct chromatographic pattern. Detailed GC and GC-MS analyses revealed considerable fractionation of the organics (Figure 3). On the basis of the GC analysis, the first peak fraction (Number 27) contained quite a variety of polar organics. The second peak fraction (Number 36) was easily the most complex, containing a broad spectrum of organics ranging from N-heterocycles to carboxylic acids. Interestingly, Fraction 39, a non-UV-absorbing region, contained a homologous series of organics. Finally, the third peak fraction (Number 41) contained a moderate variety of polar organics. The metals analysis performed by PES indicated that metals were fractionated as well (Figure 3). Fe and Zn chromatographed over a wide region, most of which was also associated with organic species. In contrast, most of the Mn chromatographed in very narrow regions associated with organics (Fractions 27 and 29).

DISCUSSION

The chromatographic procedures developed for the mutagenesis and metallorganics studies yielded considerable fractionation of the retort waters' organics. On the basis of the mutagenesis study, C_{18} -partition chromatography appears to be well-suited for extracting mutagens from retort waters and probably other aqueous samples as well. It is noteworthy that all of the retort waters' mutagenicity was confined to the C_{18} -extractable, or hydrophobic organic, fraction. As Table 1 indicates, this fraction generally constituted a small percentage of the retort waters' total organics. Normal phase HPLC with the NH_2 -column proved useful for further fractionation of the hydrophobic organic fraction. As a result of the HPLC analyses, it is clear that the retort waters' mutagens are polar and, in some cases, quite heterogeneous. The exact identity of the mutagens remains a mystery, however. None of the numerous organic species identified to date by GC-MS are known mutagens. On the basis of the HPLC studies, polar, mixed-function compounds, perhaps N- and O-containing species, are likely candidates.

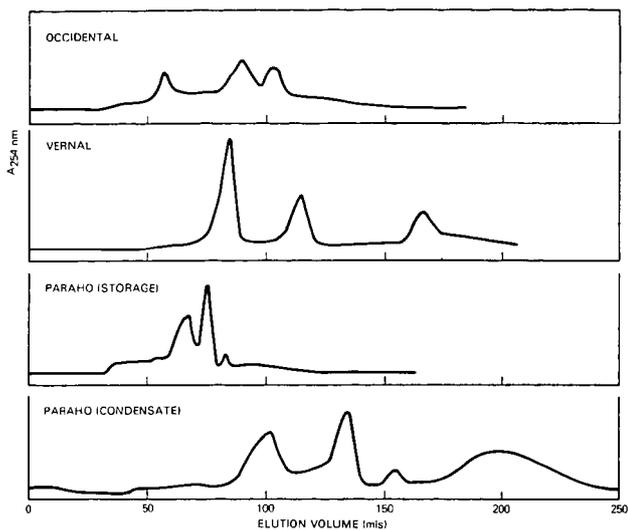


FIGURE 2. Steric Exclusion Chromatography of Oil Shale Retort Waters with Sephadex G-15.

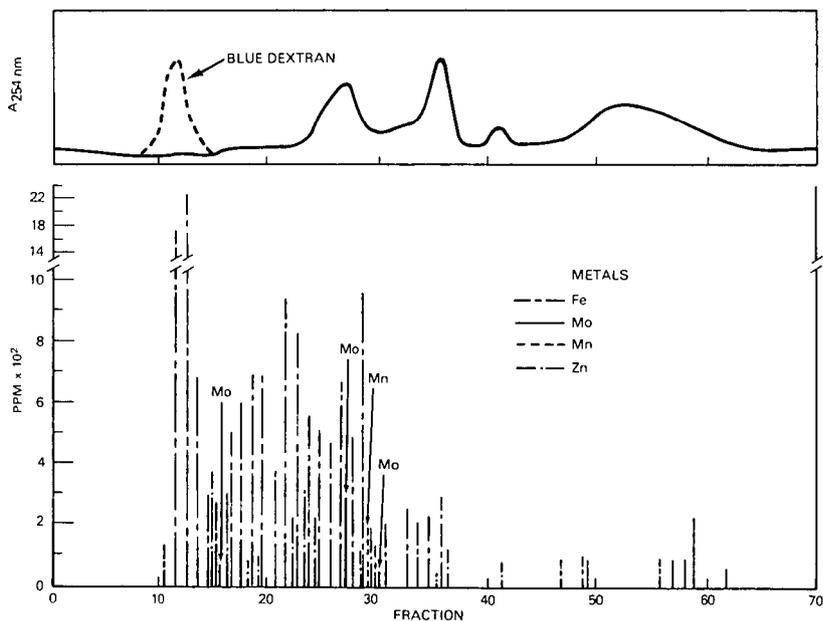


FIGURE 3. Fractionation of Organics and Metals in Paraho Condensate Retort Water with Sephadex G-15.

Steric-exclusion chromatography with Sephadex G-15 also provided considerable fractionation of the retort waters' organics. This type of chromatography appears to be amenable to the studies such as the metallorganics study (15) in that it constitutes a rather mild form of chromatography, unlike ion-exchange chromatography, for example. One might reasonably expect organic-metal chelates to survive the chromatography. Indeed, in our studies to date with the retort waters, certain metals, and organic species appear to co-elute, presumably as distinct complexes or aggregates. Interestingly, each retort water appears to contain several distinct aggregates with nominal MW's ≤ 1500 , based on Sephadex G-15's nominal fractionation range as calibrated against Dextran standards.

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