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PARTITION CHROMATOGRAPHY–HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY FACILITATES THE ORGANIC ANALYSIS AND BIOTESTING OF SYNFUELS

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SUMMARY

Partition chromatography (Sephadex LH-20 or C_{18}-partition) followed by high-performance liquid chromatography facilitated the organic and mutagenic characterization of synfuel samples. The mutagens in oil shale retort waters were polar, whereas those in a shale oil range

heterogeneous than those of an SRC-I solid
aromatic amines and aza-polynuclear aromatic hydrocarbons) were ident
SRC

INTRODUCTION

try in the United States. The feasibility of shale oil production and coal liquefaction is being tested using various technologies, most of which are presently at the bench-level to pilot-plant stages of development. A wide variety of experimental synfuel samples are currently being produced, along with by-products associated with the various synfuel processes, *e.g.*, retort waters from oil shale retorting. Concern about the potential health and environmental impacts of a synfuels industry has, in turn, prompted intensive biomedical and environmental research, much of it directed at chemically and biologically evaluating the genotoxicity/carcinogenicity potential of synfuels. Research has already demonstrated that certain crude and chemically fractionated synfuel samples are mutagenic using carcinogen-screening bioassay systems such as the Ames *Salmonella* assay[1–7]. Known carcinogens such as polynuclear aromatic hydrocarbons and primary aromatic amines have been identified in various synfuel samples[6–9].

The primary goal of this study was to compare the organic and mutagenic

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properties of a shale oil, oil shale retort waters, and selected coal liquefaction products. The following synfuel samples, representing several technologies, were analyzed: (1) a shale oil from the Paraho aboveground retort; (2) four oil shale retort waters from various retorting technologies [Paraho, aboveground; Occidental, modified *in situ* (vertical); and Geokinetics, true *in situ* (horizontal)]; (3) a distillate blend from the solvent refined coal-II process (SRC-II); and (4) two samples from the SRC-I process, a process solvent and a solid product.

A secondary goal of this study was to evaluate the effectiveness of partition chromatography and high-performance liquid chromatography (HPLC) in fractionating synfuel samples. The various synfuel samples studied to date are extremely complex, containing a plethora of organic species[6–8,10]. In general, these samples have defied anything but rudimentary characterization without some type of fractionation. A number of different fractionation methods have already been evaluated, all aimed at facilitating the chemical and biological characterization of synfuel samples, *e.g.*, solvent extraction[10,11], thin-layer chromatography (TLC)[7], Sephadex LH-20 partition chromatography[5,12], HPLC[8,9], and alumina–silicic acid chromatography[13]. In this study, we decided to test the effectiveness of partition chromatography coupled with HPLC in facilitating the organic and mutagenic characterization of synfuel samples.

EXPERIMENTAL

Initial fractionation of shale oil and SRC samples

The Paraho shale oil and SRC-II distillate blend were initially fractionated by Sephadex LH-20 partition chromatography based on a method developed by Klimisch and Stadler[14] and modified by Jones *et al.*[12]. In our procedure, the Sephadex LH-20 was swelled in methanol–water (85:15 v/v) and packed in a 30 × 2.5 cm glass column. Each synfuel sample (1.5 g) was diluted with hexane and loaded on the column. Sequential step-wise elution with hexane, 10% (v/v) toluene in hexane and methanol (≈300 ml each) yielded a general separation based on polarity. The SRC-I samples were not fractionated prior to HPLC because they appeared to be much less complex chemically compared to the shale oil and SRC-II samples.

Initial fractionation of oil shale retort waters

Each retort water used in the mutagenesis studies was initially fractionated by partition chromatography on a C_{18}-cartridge based on a method described by Riggin and Howard[15]. In our procedure, the C_{18}-cartridge was preconditioned 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 C_{18}-cartridge; the organic-loaded cartridge was then washed with 4 ml of milli-Q-purified water. The C_{18}-extractable, or hydrophobic organic, fraction of the retort water was then eluted from the C_{18}-cartridge with 2 ml of methanol. The exact volume of each retort water loaded per C_{18}-cartridge was determined by constructing binding curves (dry weight of hydrophobic organics plotted *versus* volume of retort water). A volume corresponding to the early, linear part of the curve was selected to avoid overloading the cartridge.

HPLC analysis

Each Sephadex LH-20 fraction of the shale oil and SRC-II distillate blend, the hydrophobic organic fraction of each retort water and the SRC-I samples were fractionated on the basis of polarity by normal-phase HPLC using an NH_2-column and a three-solvent mobile-phase system (Fig. 1). 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 NH_2-column was reconditioned by cycling back to hexane using a linear gradient of 10 min, followed by 100% hexane isocratically for 10 min.

A sample of 50 μl (50–100 mg/ml) was injected onto the NH_2-column per HPLC run; four to twenty runs of each sample were repetitively collected into 250-ml round-bottom flasks. The HPLC effluent was monitored by UV absorbance at 250 nm. Each HPLC fraction was concentrated by rotary evaporation, transferred to a preweighed vial, evaporated to dryness under nitrogen, 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 dimethyl sulfoxide (DMSO) for the Ames bioassay.

GC–MS analysis

Samples were analyzed on Hewlett-Packard 5982 and 5985 GC–MS instruments in the electron-impact (70 eV) mode. The 5982 instrument was equipped with a 15 m × 0.30 mm I.D. glass capillary column coated with 0.25-μm film thickness of SE-52; the column was programmed from 40 to 100°C at 32°C/min, then programmed at 8°C/min to 300°C, where it was maintained isothermally for 10 min. The 5985 instrument was equipped with a 60 m × 0.25 mm I.D. fused silica capillary column coated with 0.25 μm of SE-54; the column was programmed from 40°C to 300°C at 5°C/min, where it was maintained isothermally for 8 min. Splitless injection systems were used to introduce the sample onto the GC–MS instruments. On the 5982 instrument, the GC column was interfaced to the mass spectrometer via platinum–iridium tubing; the GC column on the 5985 instrument was interfaced directly to the mass spectrometer. A mass range of 50–300 a.m.u. was scanned on the 5982 instrument by computer (HP-5934A) every 1.8 sec. A mass range of 50–400 a.m.u. was scanned on the 5985 instrument every 1.0 sec by computer (HP-2100MX equipped with the HP-7920 Large Disc Drive).

TOC analysis

The total organic carbon (TOC) analyses of the retort waters were performed on a Coulometrics Carbon Analyser by non-aqueous coulometric titration using a silver titrant and ethanolamine.

Mutagenesis assay

Agar-plate mutagenicity assays were carried out essentially as described by Ames *et al.*[16]. The TA98 strain of *Salmonella typhimurium* was used with the S9 fraction from rat liver homogenate (induced with Aroclor 1254) as a metabolic activator. Dimethyl sulfoxide was used as the solvent for all of the standards and HPLC

fractions that were bioassayed. After a 24–36 h incubation of the inoculated plate, revertant colonies of TA98 were counted on a New Brunswick Scientific Company Biotran II automated colony counter.

Materials

Standards. The standards used in the HPLC study were purchased from Aldrich (Milwaukee, WI, U.S.A.) and RFR Corporation (Hope, RI, U.S.A.); 10-azabenzo[*a*]pyrene was donated by Professor M. Lee of Brigham Young University.

Samples. The crude Paraho product oil was sampled on August 24th, 1977, at the Paraho aboveground retort (Anvil Point, CO) from heated 500-barrel tanks and transferred into PTFE-lined stainless steel drums for shipment to our laboratory. The oil's temperature at the initial sampling was 65°C. The crude oil was transported at a temperature of ≈4°C and stored in the laboratory at ≈4°C. Subsampling of the Paraho product oil consisted of transferring it to PTFE-sealed, amber-glass bottles after the sample had been thoroughly mixed at 65°C.

The Paraho retort storage water, separated from shale oil in storage tanks, was sampled on August 26th, 1977; the Paraho condensate water, composed of condensed off-gas, was sampled on October 13th, 1980, at the Paraho aboveground retort (Anvil Points, CO). The Occidental retort water was sampled from a retort water holding tank on March 7th, 1979, at the vertical, modified *in-situ* retort (Room 6) at Logan Wash, CO. The Geokinetics retort water was sampled from a shale oil–retort water separation tank on July 11th, 1978, at the horizontal, true *in-situ* retort at Vernal, UT. The retort waters were collected in PTFE-lined stainless steel drums or PTFE bottles and stored under the same conditions as the Paraho shale oil.

The SRC-II distillate blend was shipped to the laboratory from the SRC pilot plant in Fort Lewis, WA (operated by the Pittsburg & Midway Coal Mining Co.), and stored under the same conditions as the Paraho shale oil. The distillate blend consists of a mix of 2.9 parts middle-distillate cut (175–290°C) and 1 part heavy-distillate cut (290–454°C).

The SRC-I process solvent and solid product samples were collected on June 4th, 1980, at the SRC-I pilot plant (Wilsonville, AL) during steady-state operation and blanketed under nitrogen. The samples were subsequently transferred into PTFE-lined stainless steel drums for shipment to our laboratory, where they were stored at ≈4°C prior to subsampling and analysis. The solid product was crushed and then dissolved in toluene–methanol (3:1 v/v) prior to HPLC.

Chromatographic columns. The μBondapak NH_2-columns and precolumns used in the HPLC analyses and the C_{18}-Sep Pak cartridges used for the C_{18}-partition chromatography were purchased from Waters Assoc. (Milford, MA, U.S.A.). The NH_2-column was a semipreparative column (30 cm × 7 mm). The glass columns (45 × 2.5 cm) and Sephadex LH-20 used in the initial fractionation of the shale oil and SRC-II distillate blend were purchased from Pharmacia (Piscataway, NJ, U.S.A.).

Solvents and glassware. The organic solvents used in the chromatographic analyses were redistilled-in-glass solvents purchased from Burdick & Jackson Labs. Deionized water was passed through a milli-Q system (Millipore) containing two ion-exchange resins and two charcoal filters. All glassware was acid cleaned, except for the glass columns used for the Sephadex LH-20 chromatography which were solvent rinsed with sonication.

RESULTS

HPLC of standards

A number of standards were chromatographed on the NH_2-column using the three-solvent mobile-phase system (Fig. 1). As will be discussed shortly, most of the species in the synfuel samples eluted in three distinct regions: non-polar, moderately polar and polar. Standards such as polynuclear aromatic hydrocarbons and non-polar heterocycles eluted in the non-polar region; aromatic amines and N-heterocycles eluted in the moderately polar region and phenol and mixed-function heterocycles, *e.g.*, aminopyridinol, eluted in the polar region. An important feature of this chromatographic fractionation is that there appears to be an appreciable separation of non-mutagenic phenolic-type compounds from mutagenically active aromatic amines. Phenolic-type compounds are generally major constituents of synfuel samples whereas primary aromatic amines, if present, are minor constituents[7].

Mutagenicity of synfuel samples prior to HPLC

The mutagenic activities of the synfuel samples prior to HPLC fractionation are plotted in Fig. 2. The crude, or unfractionated, oil shale retort waters were generally non-mutagenic; only the Paraho storage water showed any activity. After C_{18}-partition chromatography, however, all of the retort waters exhibited some mutagenicity (Table I). The mutagenicity was confined to the hydrophobic organic fraction; the complementary hydrophilic, or aqueous, fraction exhibited no activity. As Table I illustrates, the hydrophobic organic fraction generally constituted a small percentage of the retort waters' total organics (Paraho condensate, 31.4%; Occidental, 34.8%; and Geokinetics, 34.8%); the only exception being the Paraho storage water (72.4%).

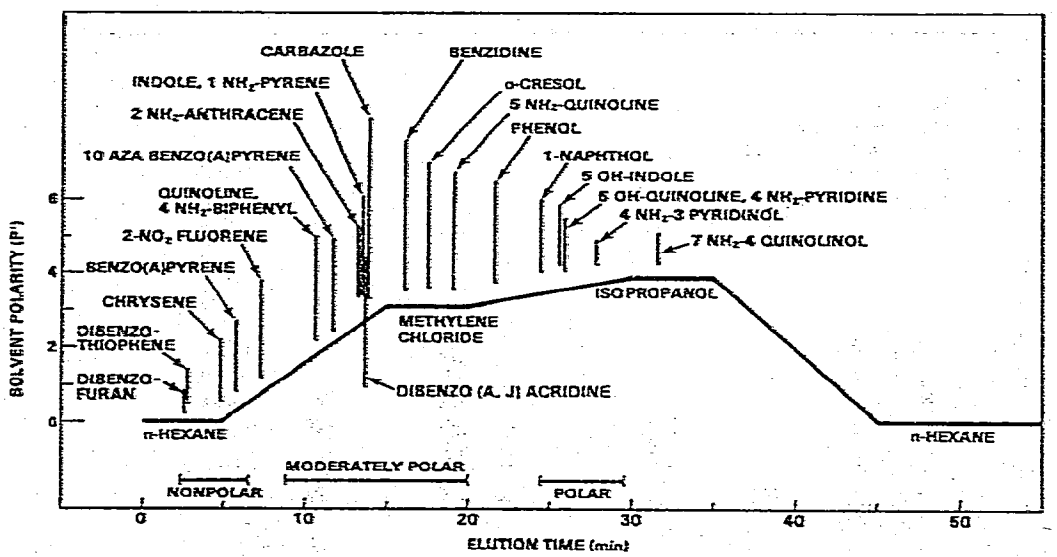

Fig. 1. HPLC of standards. Each standard (1 mg/ml in methanol or chloroform) was chromatographed on a μBondapak NH_2-column using the three-solvent mobile-phase system depicted above at a flow-rate of 5 ml/min. The organic species in the synfuel samples studied typically eluted in three distinct regions of polarity: non-polar, moderately polar and polar.

The hydrophobic organic fraction of the Paraho storage water was much more mutagenic than those of the other waters, including the Paraho condensate water. The storage water was much less mutagenic than the unfractionated Paraho shale oil on the basis of total specific activity (*i.e.*, revertants TA98 per μg dry weight of the total residue in the retort water). On the basis of the specific mutagenic activity of the hydrophobic organic fraction alone (*i.e.*, revertants TA98 per μg dry weight hydrophobic organics), however, the mutagenicity of the Paraho storage water was comparable to that of the Paraho shale oil (1.32 *versus* 1.06 revertants per μg, respectively).

In most cases, the SRC samples were more mutagenic than the shale oil. The SRC-I process solvent, however, exhibited only modest mutagenic activity; it was even less mutagenic than the crude Paraho shale oil. The SRC-I solid product [dissolved in toluene–methanol (3:1 v/v)], on the other hand, exhibited appreciable mutagenicity. Neither SRC-I sample was fractionated by Sephadex LH-20 chromatography prior to HPLC because the samples appeared to be much less complex chemically, compared to the shale oil and SRC-II samples. The SRC-II distillate blend also exhibited appreciable mutagenicity, though slightly less activity than the SRC-I solid product.

Of the Sephadex LH-20 fractions, the non-polar hexane fractions of the shale oil and SRC-II distillate blend showed no mutagenic activity despite the fact that they contained most of the original samples' mass (≈80–95%) (Fig. 2). The moderately polar LH-20 toluene–hexane fractions of the shale oil and distillate blend were moderately active and constituted ≈5% of the original samples. The toluene–hexane fraction of the distillate blend was much more mutagenic than the shale oil counterpart. The polar, LH-20 methanol fractions of the shale oil and distillate blend were the most mutagenic, with the methanol fraction of the SRC-II distillate blend being by far the most active.

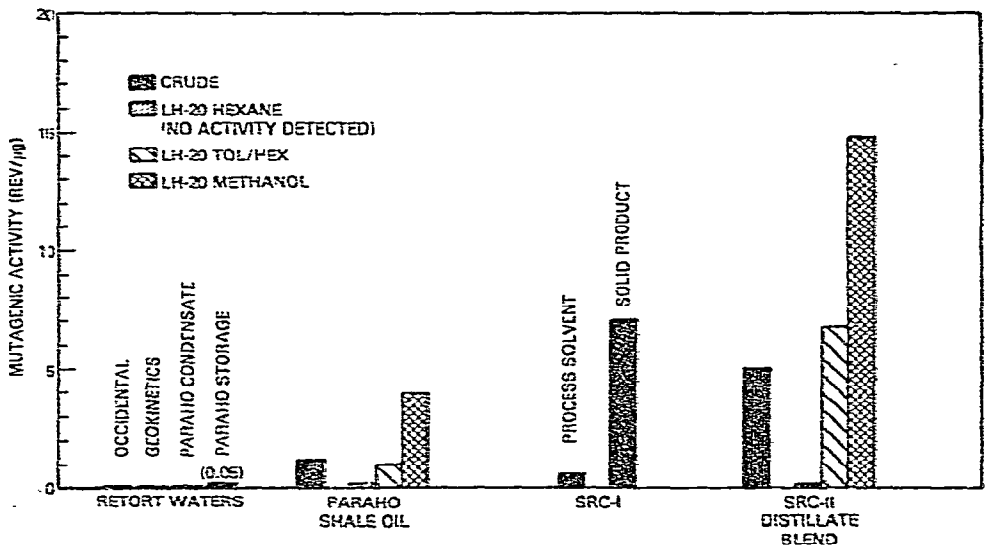

Fig. 2. Mutagenicity of synfuel samples before HPLC fractionation. The unfractionated synfuel samples and their C_{18}-partition or Sephadex LH-20 chromatography fractions were bioassayed using the standard Ames assay with the TA98 strain of *Salmonella typhimurium* and S9 as a metabolic activator.

TABLE I

ORGANIC AND MUTAGENIC PROPERTIES OF OIL SHALE RETORT WATERS

Retort water	*Organic content*		*Mutagenicity*	
	TOC, crude retort water (mg/ml)	*Hydrophobic* fraction (mg/ml)*	*rev/µl***	*rev/µg****
Paraho storage	42.75	30.94 ± 1.64	23.53 (50.65)‡	1.32
Paraho condensate	4.08	1.28 ± 0.13	0	0.05
Occidental	4.23	1.47 ± 0.01	0	0.14
Geokinetics	2.93	1.02 ± 0.15	0	0.13

* Extractable by C_{18}-partition chromatography; expressed as mg dry residue per ml retort water.
** Revertants TA98 per µl crude retort water.
*** Revertants TA98 per µg of hydrophobic organics.
‡ Revertants TA98 per mg total dry residue in crude water.

Mutagenicity of the retort waters' hydrophobic organic fraction after HPLC

After HPLC fractionation, most of the species in the hydrophobic organic fraction of each retort water eluted in the polar HPLC region but some species eluted in the moderately polar HPLC region (Fig. 3). Virtually all of the mutagenic activity was concentrated in the polar HPLC region (Fig. 3). The mutagens of the Paraho storage water chromatographed throughout the polar HPLC region, indicating a fair degree of heterogeneity. In contrast, the mutagens of the Paraho condensate water appeared to be less heterogeneous. The mutagens of the Occidental and Geokinetics retort waters were even less heterogeneous, confined exclusively to one area of the polar HPLC region.

Mutagenicity of SRC-I samples after HPLC

Most of the species in the SRC-I process solvent eluted in the non-polar HPLC region, but some species eluted in the moderately polar and polar HPLC regions (Fig. 4A). However, no mutagenic activity appeared in the non-polar HPLC region. Most of the mutagenic activity was associated with the moderately polar HPLC region, but some activity also appeared in the polar HPLC region as well. Unlike the process solvent, most of the species in the SRC-I solid product chromatographed in the polar HPLC region, but some species eluted in the non-polar and moderately polar HPLC regions as well (Fig. 4B). The mutagenic activity of the solid product was spread from the moderately polar to the polar HPLC regions with most of the activity residing in the polar HPLC region.

Mutagenicity of non-polar shale oil and SRC-II fractions after HPLC

The HPLC properties of the Sephadex LH-20 hexane fractions of the shale oil and SRC-II distillate blend were qualitatively similar. On the basis of UV absorbance and the dry weights of the HPLC fractions, most of the species in the LH-20 hexane fractions eluted in the non-polar region of the HPLC chromatogram (Fig. 5). Some of the species chromatographed in the moderately polar HPLC region. The HPLC chromatograms indicate more chemical heterogeneity in the distillate blend hexane

Fig. 3. HPLC and mutagenic properties of oil shale retort waters' hydrophobic organic fraction: A, Paraho storage water; B, Paraho condensate water; C, Occidental retort water and D, Geokinetics retort water. The mass recovery of each sample after HPLC (per cent dry weight recovered) was: A, 34.8%; B, 31.8%; C, 27.4% and D, 20.3%. Each HPLC fraction was collected and analyzed with the Ames mutagenesis assay; the total and specific mutagenic activities of each HPLC fraction were computed.

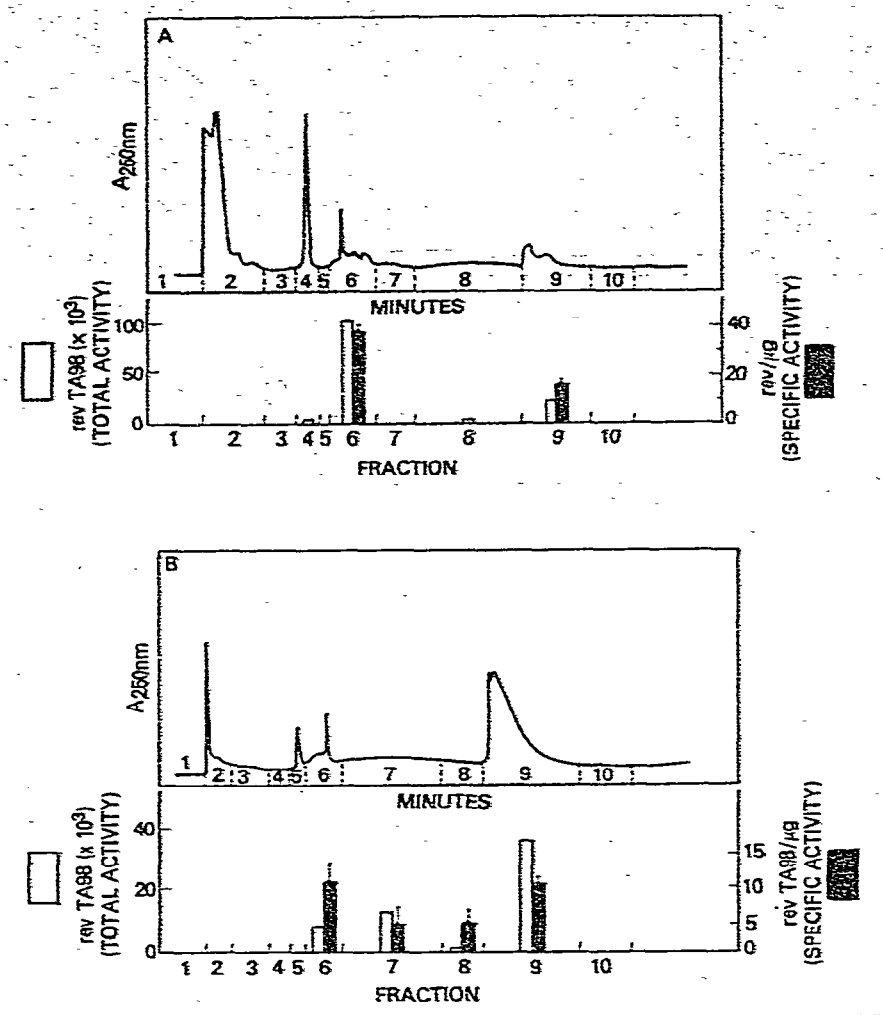

Fig. 4. HPLC and mutagenic properties of SRC-I samples: A, process solvent and B, solid product. The mass recovery of each of the above samples after HPLC (per cent dry weight recovered) was 100%. Each HPLC fraction was collected and bioassayed as described in Figs. 2 and 3; the total and specific mutagenic activities of each HPLC fraction were computed.

fraction compared to the shale oil counterpart.

Based on the Ames assay, the LH-20 hexane fractions of the shale oil and SRC-II distillate blend differed dramatically. The Paraho LH-20 hexane fraction remained mutagenically inactive even after the HPLC fractionation (Fig. 5A). In contrast, HPLC of the LH-20 hexane fraction of the distillate blend unmasked mutagenic activity in the non-polar and moderately polar HPLC regions (Fig. 5B).

Mutagenicity of moderately polar shale oil and SRC-II fractions after HPLC

Most of the species in the Paraho LH-20 toluene–hexane fraction chromatographed in the moderately polar HPLC region, but some material appeared in the non-polar and polar regions (Fig. 6A). The mutagenic activity was spread from the moderately polar to the polar HPLC regions.

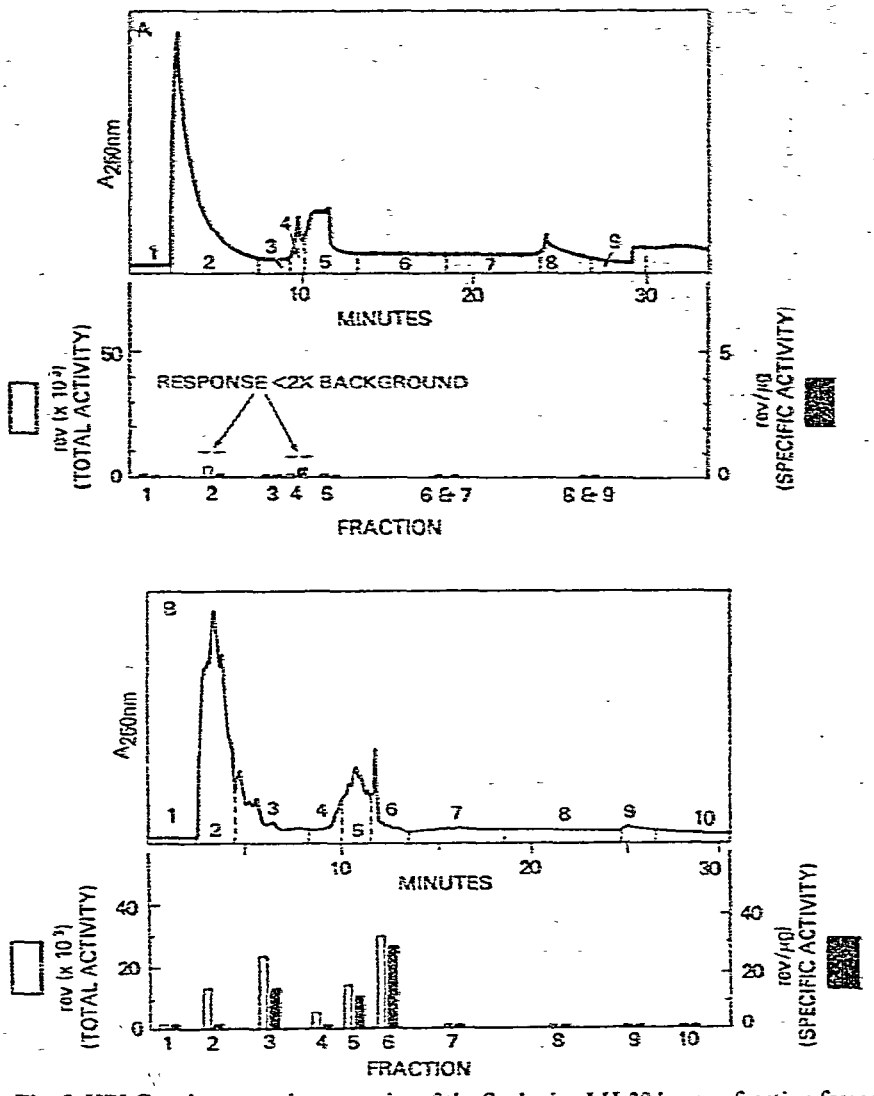

Fig. 5. HPLC and mutagenic properties of the Sephadex LH-20 hexane fraction from: A, Paraho shale oil and B, SRC-II distillate blend. The mass recovery of each sample after HPLC (per cent dry weight recovered) was 100%. Each HPLC fraction was collected and bioassayed as described in Figs. 2 and 3; the total and specific mutagenic activities of each HPLC fraction were computed.

Like the shale oil, most of the species in the LH-20 toluene–hexane fraction of the distillate blend chromatographed in the moderately polar HPLC region (Fig. 6B). However, the HPLC chromatogram of the distillate blend was much more complex than that of the shale oil. Another difference, the mutagenicity in the distillate blend was confined to only one HPLC fraction in the moderately polar HPLC region.

Mutagenicity of polar shale oil and SRC-II fractions after HPLC

Most of the species in the Paraho LH-20 methanol fraction chromatographed in the polar HPLC region; some species appeared in the moderately polar region and nothing appeared in the non-polar region (Fig. 7A). Mutagenic activity was spread from the moderately polar to polar HPLC regions.

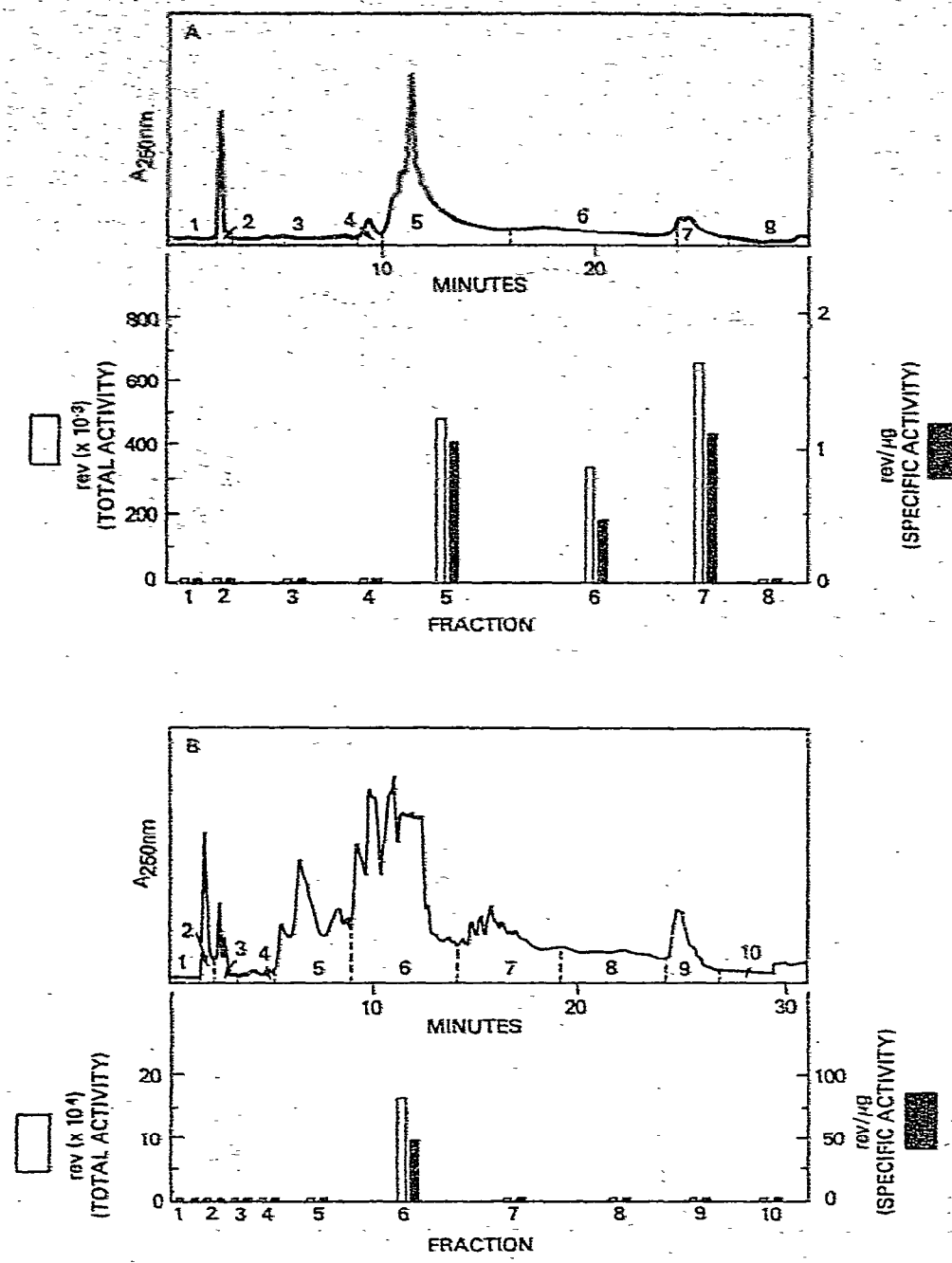

Fig. 6. HPLC and mutagenic properties of the Sephadex LH-20 toluene–hexane fraction from: A, Paraho shale oil and B, SRC-II distillate blend. The mass recovery of each sample after HPLC (per cent dry weight recovered) was 83%. Each HPLC fraction was collected and bioassayed as described in Figs. 2 and 3; the total and specific mutagenic activities of each HPLC fraction were computed.

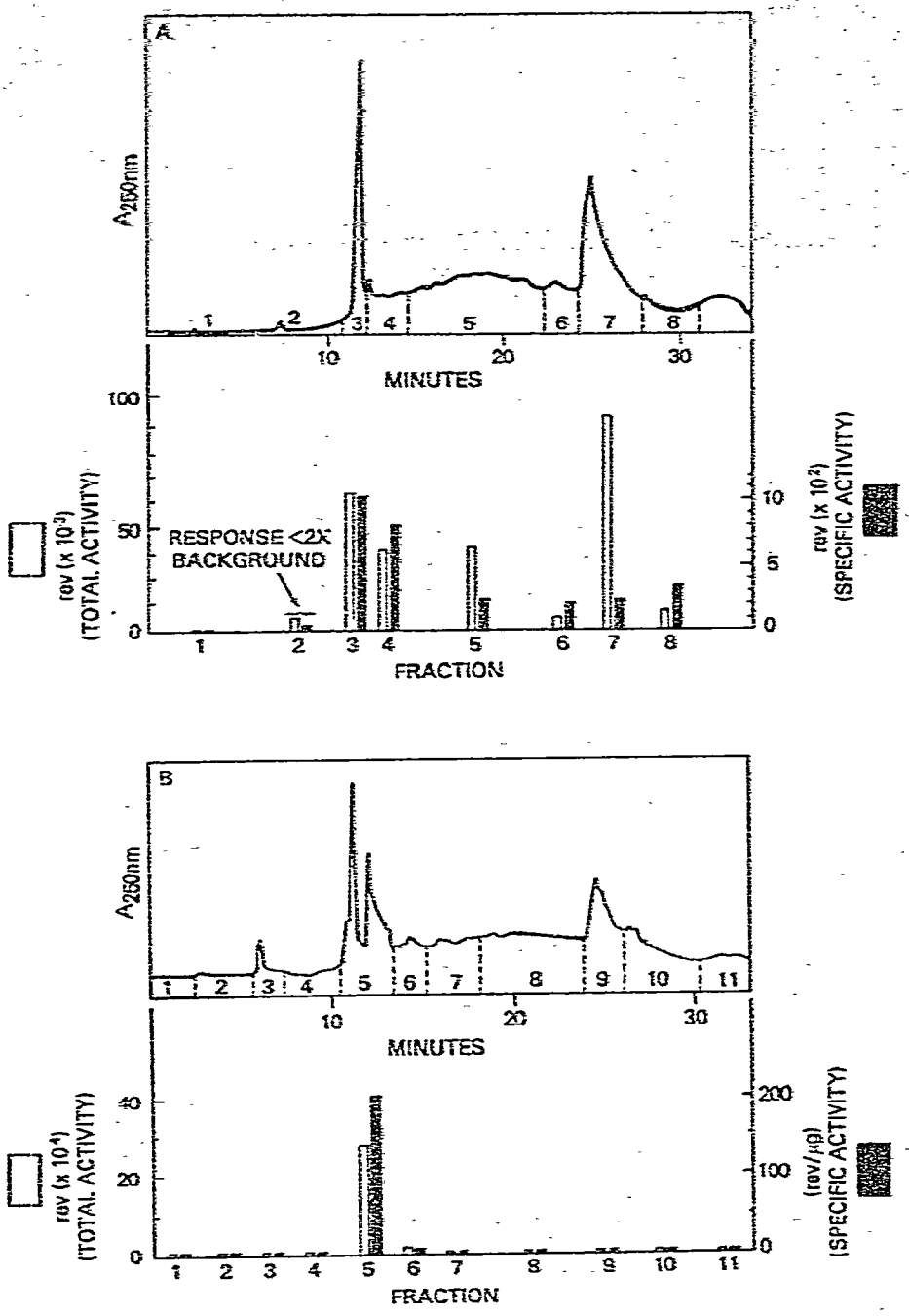

Fig. 7. HPLC and mutagenic properties of the Sephadex LH-20 methanol fraction from: A, Paraho shale oil and B, SRC-II distillate blend. The mass recovery of each sample after HPLC (per cent dry weight recovered) was 100%. Each HPLC fraction was collected and bioassayed as described in Figs. 2 and 3; the total and specific mutagenic activities of each HPLC fraction were computed.

The LH-20 methanol fraction of the distillate blend contained more mass and more heterogeneity in the moderately polar HPLC region than the shale oil counterpart (Fig. 7B). Unlike the shale oil fraction, the mutagenicity of the distillate blend was confined to the moderately polar HPLC region.

Organic characterization of synfuel samples

Qualitative GC–MS analyses revealed that the HPLC fractions of all the synfuel samples were still quite complex. The organic species identified to date are listed in Tables II–IV. The retort waters contained quite a variety of species in the moderately polar HPLC region (Table III) and particularly in the polar HPLC region (Table IV), where most of the organics chromatographed (see Fig. 3). The moderately polar and polar organics of the shale oil and retort waters were qualitatively similar, but the retort waters lacked any of the non-polar organics identified in the shale oil (Table II). Like the shale oil, the SRC-II distillate blend and the SRC-I process solvent contained an abundance of non-polar organics (Table II). Unlike the shale oil, however, the SRC samples also contained low levels of primary aromatic amines, aza-polynuclear aromatic hydrocarbons (Table III) and hydroxylated polynuclear aromatic hydrocarbons (Table IV). Preliminary analyses of the SRC-I solid product indicate that the organic species present are qualitatively similar to those of the SRC-I process solvent, except that they are more extensively oxygenated.

DISCUSSION

Of all the synfuel samples analyzed, the oil shale retort waters were least mutagenic. It is noteworthy that all of the mutagenicity of the retort waters was confined to

TABLE II

NON-POLAR ORGANICS IN SYNFUEL SAMPLES*

*Organic species***	*Shale oil*	*SRC-I process solvent*	*SRC-II distillate blend*
Alkanes	×	×	×
Alkenes	×	×	×
Indans	×	×	×
Indenes	×	×	×
Anisoles	×	×	×
Naphthalenes	×	×	×
Acenapthylenes	×	×	×
Biphenyls	×	×	×
Fluorenes	×	×	×
Anthracenes	×	×	×
Phenanthrenes	×	×	×
Fluoranthenes	×	×	×
Pyrenes	×	×	×
Chrysenes/benzanthracenes	×	×	×
Benzofluoranthene	×	×	×
Benzo[*e*]pyrene	×	×	×
Benzo[*a*]pyrene	×	×	×
Perylene	×	×	×
Dibenzofluoranthene	×	×	×
Dibenzothiophene	×	×	×

* Organics in non-polar HPLC region; no organics detected in retort waters.

** Parent compound plus alkyl homologues.

TABLE III

MODERATELY POLAR ORGANICS IN SYNFUEL SAMPLES*

*Organic species***	*Retort waters*	*Shale oil*	*SRC-I process solvent*	*SRC-II distillate blend*
Pyrroles	×	×		
Pyridines	×	×		×
Anilines	×			×
Indoles	×	×	×	×
Quinolines	×	×	×	×
Isoquinolines	×	×	×	×
Carbazoles		×	×	×
Acridines/phenanthridenes	×	×	×	×
Aminonaphthalenes		T***	×	×
Aminophenanthrenes			×	×
Aminochrysenes				×
Aminopyrenes			×	×
Aminophenylnaphthalenes			×	×
Aminoquinolines			×	×
Aminocarbazoles			×	×
Aminobenzocarbazoles			×	×
Azapyrenes			×	×
Azabenzopyrenes			×	×
Phenazines/benzocinnolines				×

* Organics in moderately polar HPLC region.
** Parent compound plus alkyl homologues.
*** Tentative identification.

TABLE IV

POLAR ORGANICS IN SYNFUEL SAMPLES*

*Organic species***	*Retort waters*	*Shale oil*	*SRC-I process solvent*	*SRC-II distillate blend*
Aliphatic ketones	×			
Benzonitrile	×			
Pyridinols		T***		
Pyridinones	×	T***		
Phenols	×	×	×	×
Fluorenols			×	×
Naphthols		×	×	×
Carboxylic acids	×	×		
Alkylamides	×	×		
Hydroxybiphenyls			×	×
Hydroxypyrenes			×	×
Hydroxybenzopyrene			×	
Dihydroxybiphenyls			×	×

* Organics in polar HPLC region.
** Parent compound plus alkyl homologues.
*** Tentative identification.

the hydrophobic organic fraction. Unlike the other retort waters studied, the hydrophobic organic fraction of the Paraho storage water constituted a large percentage of the water's total organic carbon (see Table I) and exhibited mutagenicity comparable to that of the Paraho shale oil (see Fig. 2 and Table I), suggesting that shale oil and retort water should be separated immediately after oil shale retorting. As a result of the HPLC analyses, it is clear that the mutagens in the hydrophobic organic fraction of all the retort waters are polar and, in some waters, quite heterogeneous.

The Paraho shale oil was significantly more mutagenic than the crude, unfractionated retort waters. The absence of appreciable mutagenicity in the shale oil Sephadex LH-20 hexane fraction indicates that non-polar species do not contribute to the shale oil's mutagenicity, at least at this level of HPLC fractionation. The presence of mutagenicity in the shale oil's moderately polar and polar HPLC regions indicates that the mutagens are chemically heterogeneous.

The SRC-II distillate blend differed from the shale oil in a number of ways. The distillate blend proved to be much more mutagenic than the shale oil. Furthermore, the mutagenic profiles of the distillate blend after HPLC fractionation were quite different than those of the shale oil. Unlike the shale oil, the distillate blend contains non-polar mutagens which are unmasked by HPLC fractionation. Specific chemical characterization of this fraction remains to be done, but on the basis of HPLC retention data of standards, likely candidates for these non-polar mutagens are polynuclear aromatic hydrocarbons and non-polar heterocycles. In contrast to the shale oil, the distillate blend's moderately polar mutagens are confined to a single HPLC fraction in the moderately polar HPLC region, indicating that the distillate blend's mutagens are much less heterogenous than the shale oil's mutagens.

Of the various synfuel samples studied, the SRC-I samples appear to be the most distinct. The process solvent was less mutagenic than the shale oil, whereas the solid product was more mutagenic than the SRC-II distillate blend. On the basis of the HPLC analysis, the process solvent's mutagens are primarily moderately polar, like the SRC-II distillate blend, whereas the solid product's mutagens range from moderately polar to polar, like the shale oil.

Results of earlier studies suggest that primary aromatic amines are major contributors to the mutagenicity of SRC-II coal liquids[6,7,17]. From our current study, it is clear that the mutagenic activity of the SRC-II distillate blend and SRC-I process solvent is largely confined to the moderately polar HPLC region, where primary aromatic amine standards elute. Furthermore, primary aromatic amines and mutagenic aza-polynuclear aromatic hydrocarbons have been identified in the moderately polar HPLC regions of both the SRC samples (see Table III). In contrast to the SRC samples, none of the organic species identified to date in the shale oil are known mutagens. Results of earlier studies suggest that, unlike SRC-II coal liquids, primary aromatic amines probably do not play a central role in the mutagenicity of shale oil[6]. It is possible, nevertheless, that the shale oil contains extremely low levels of the amines which may be responsible for some of the mutagenicity in the moderately polar region of the HPLC chromatograms of the shale oil. Further chemical fractionation and trace analysis techniques may yet uncover low levels of primary aromatic amines which are currently masked by other moderately polar species which are much more abundant.

Finally, HPLC coupled with C_{18}-partition or Sephadex LH-20 partition chromatography promises to be an effective way of fractionating synfuel samples for chemical and biological characterization. C_{18}-partition chromatography appears to

be a quick, efficient way of isolating the mutagenic activity in the retort waters. Sephadex LH-20 chromatography is an inexpensive, fairly effective, preparative-scale method of initially separating the mutagenic species in the moderately polar and polar HPLC regions from the largely non-mutagenic but abundant species in the non-polar HPLC region. As the HPLC chromatograms illustrate, the normal-phase HPLC used in this study fractionated the various synfuel samples considerably. The HPLC fractionation unmasked mutagenicity in the Sephadex LH-20 hexane fraction of the SRC-II distillate blend that would have otherwise gone undetected. On the other hand, the mass recoveries from the HPLC fractionation of the retort waters were poor (30–35%), perhaps due to selective adsorption of polar organics on the NH_2-column. In contrast, the mass recoveries of the shale oil and SRC samples were excellent (see Figs. 4–7). In conclusion, HPLC coupled with partition chromatography appears to be a method of choice for combined chemical and biological characterization studies.

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