Title

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Publication Date

1982-02-01

LBID-485
UC-91

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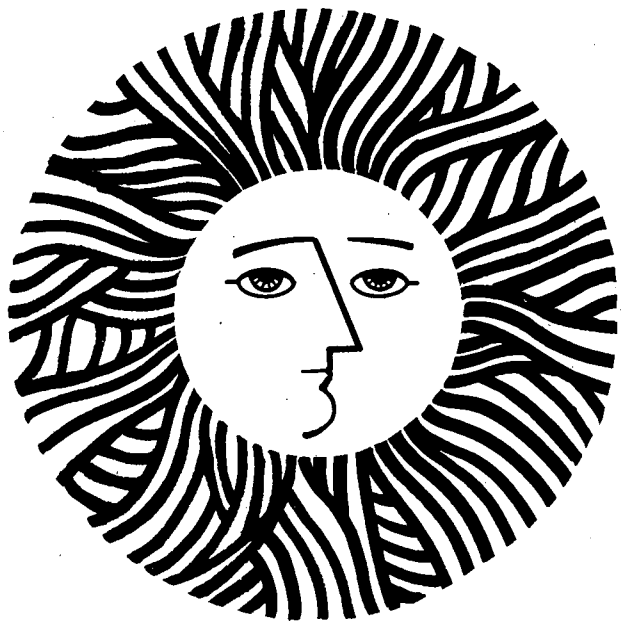
UNIVERSITY OF CALIFORNIA

ENERGY & ENVIRONMENT DIVISION



DISCLAIMER

RAPID FRACTIONATION OF OIL SHALE WASTEWATERS BY REVERSE-PHASE SEPARATION

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This work was prepared for the Department of Energy
under Contract No. W-7405-ENG-48.

Two basic approaches are available to the analyst for the routine quantitation of particular chemical classes or individual compounds in aqueous heterogeneous mixtures. The first approach quantitates solutes by methods of detection that are specific for individual chemical classes or particular compounds; many of these methods involve derivatizations or colorimetric reactions that enable the compounds of interest ("analytes") to be distinguished from myriads of other solutes. Each analyte, however, requires a specific method of detection. The alternative approach employs an initial step that effects a sufficient degree of separation of the analyte from the mixture, and thereby enables the use of routine bulk-property methods as means of detection.

Tremendous qualitative differences can exist between quantitatively identical values obtained by any bulk- or colligative-property method such as chemical oxygen demand (COD), dissolved organic carbon (DOC), or organic nitrogen. In oil shale wastewater treatment, for example, the removal of a large percentage of DOC may be inadequate if the toxicity, color, and odor of the wastewater are strictly associated with the remaining percentage. Identical values for any colligative property obviously can result from solutions of different compounds. The information from nonspecific quantitative data yielded by colligative properties can be greatly amplified by the physical separation or "fractionation" of the sample matrix.

The physical separation of chemical classes is usually accomplished by "isolation" processes such as liquid-liquid partitioning (solvent extraction), ion exchange, or sorption (e.g., onto charcoal or macroreticular resins), and by "concentration" methods such as lyophilization and ultrafiltration (Jolley; Leenheer and Farrier). The isolation schemes usually depend on various sequences of pH adjustment in the acidic, neutral, and basic ranges followed by extraction with water-immiscible solvents and back-extraction into aqueous phases, or passage of the sample through series of exchange resins or sorbents. The fractionated classes are combinations of acidic, neutral, or basic organic compounds. The analytes are then recovered from the various fractions by

selectively removing the water or organic solvent. These procedures risk the chemical or physical alteration or contamination of the sample by introduction of solvents, acids, caustics, and heat. Although these methods are generally capable of effecting extensive separations of many different chemical classes, they are too time consuming for use in routine experiments or for monitoring treatment performance.

We have developed a new fractionation procedure which effects a crude separation of solutes from complex oil shale wastewaters, but which is also simple, rapid, and applicable to other types of aqueous wastes (Daughton et al., 1981a,b, 1982). This method separates a wastewater into polar (hydrophilic) and nonpolar (hydrophobic, lipophilic) fractions by passing the sample through a disposable reverse-phase chromatographic cartridge. We call this approach reverse-phase fractionation (RPF). The method is rapid, and no alteration or contamination of the sample occurs. This crude fractionation step greatly increases the information that can be derived from the subsequent application of colligative detection methods. Moreover, these fractions (especially the hydrophilic fraction) can be subjected to experimental treatments (such as biooxidation or physicochemical treatment) for comparison with parallel treatments of the raw unfractionated water. It is crucial to recognize that the fractions that are generated by a separation scheme are arbitrarily defined because they are strictly dependent on the idiosyncracies of the scheme; operationally, "polarity" is a relative characteristic.

Theory: Chemical Class Fractionation by Reverse-Phase Separation

Reverse-phase chromatography utilizes a stationary phase that is less polar than the mobile phase, which is commonly water modified with organic solvents. After solutes in the mobile phase have been retained by the stationary phase, they can be eluted by further modifying the mobile phase with organic solvent. Solvent "strength" (i.e., capacity to elute sorbates or retained compounds) increases with increasing hydrophobicity of the solvent. A typical reverse-phase stationary material is composed of silica particles whose inherently polar surface silanol groups are covalently bonded to aliphatic moieties (e.g., octadecylsilyl, C-18 groups), which create an immobilized hydrophobic layer around each silica particle.

If an aqueous sample containing organic solutes were passed through reverse-phase material, the composition of the effluent generally would be unchanged. If the stationary phase were pretreated, however, with a water-miscible organic solvent such as methanol, the less-polar solutes would partition into the hydrophobic stationary phase; the more-polar solutes would remain in the aqueous effluent. The methanol serves to "wet" or "activate" the reverse-phase material. The mechanism of retention of organic solutes by the stationary phase has not been delineated. Several mechanisms have been proposed. The most straightforward model assumes that the aliphatic chains simply serve as a stationary liquid phase (Snyder and Kirkland). Activation allows the unlike stationary and mobile phases to contact one another; this contact promotes the partitioning process. The solutes that partition into the activated stationary phase from the mobile aqueous phase can be eluted with an organic solvent of sufficient strength.

The general principal of RPF can be demonstrated by the following experiment. An aqueous mixture of bromophenol blue and p-nitrophenoxide

ion is prepared. Bromophenol blue is a relatively hydrophobic intensely purple sulfonphthalein dye substituted with two dibromophenyl groups. p-Nitrophenoxide, in contrast, is a relatively polar bright yellow ionized phenol. The mixture of these two compounds is deep violet. When this mixture is applied to unactivated C-18 material, the effluent is deep violet and color is not retained by the stationary phase. In contrast, when the cartridge is activated, the lipophilic fraction (LpF) is retained and the hydrophilic fraction (HpF) appears in the aqueous effluent; the effluent is bright yellow and the stationary phase becomes deep purple. A water-miscible organic solvent can easily elute the bromophenol blue from the stationary phase. Complete physical separation of the two solutes is thereby effected.

Similarly, the application of retort water to a cartridge containing unactivated C-18 material produces an effluent that is unchanged. Activation of the cartridge with methanol permits retention of a large percentage of the organic solutes. Raw retort water generally is dark brown and has a pungent tarry smell; these odoriferous characteristics are common to nitrogenous heterocycles. The aqueous effluent from an activated C-18 cartridge, however, smells strongly of ammonia, whose odor had been totally obscured by the intense odor of the heterocycles. If the water is removed from this HpF fraction by drying, ammonia is concomitantly removed, and its absence enables the intense odor of fatty acids to be recognized. The aqueous effluent is colorless or pale yellow; the reverse-phase packing material retains nearly all of the color. A large percentage of the retained compounds can be eluted with organic solvent (e.g., methanol). If the organic solvent is removed from the organic eluate, the residuum (LpF) possesses the characteristic odor and color of raw retort water. A portion of the LpF is irreversibly retained. This is probably a result of sorption of solutes with basic functionalities by surface silanol groups that remained unreacted during the bonding of the silica; these cartridges are packed with material that is not endcapped.

It must be emphasized that RPF is not analogous to liquid chromatography (LC). In LC, the sample is applied as a plug, and the mobile phase carries the components through a highly efficient analytical column. In RPF, the sample and mobile phase are synonymous, and the column has been miniaturized and packed with less efficient chromatographic material. It is therefore essential to ascertain the amount of sample that can be applied before the stationary phase is saturated and the solutes that normally would be retained begin to breakthrough. The analyst should also be aware that the stationary phase can be altered by the partitioning of solutes: the "mutual zone of solubility effect" (Saner et al.). The permanent alteration of the stationary phase may limit reuse of the cartridges for RPF. With mixtures of solutes comprising a wide range of capacity factors (K'), the initial sorption of many solutes with high K' values shifts the selectively away from solutes with lower values.

Chemical Class Fractionation

a. General Procedure.

Miniature reverse-phase cartridges are available from several manufacturers (e.g., C-18 Sep Paks from Waters Assoc., Inc., Milford MA; Chrom-Prep PRP-1 cartridges from Hamilton Co., Reno, NV; Disposable Extraction Columns from J.T. Baker Chemical Co., Phillipsburg, NJ.). We have extensively investigated C-18 Sep Paks because of their ease of use; other cartridges should give comparable

results, although modifications in the protocol would be required. C-18 Sep Pak cartridges contain about 350 mg of packing material (80-μm diameter particle size) held in place in a virgin polyethylene tube by fritted polypropylene discs at the influent and effluent ends.

Activation is achieved by applying 5 mL of methanol to the cartridge followed by rinsing with 20 mL of water; sufficient rinsing is required for removal of residual methanol and minimization of background interferences. The sample is then passed through the cartridge at a sufficiently slow rate (e.g., 5-10 mL/min for retort waters; 100 mL/min can be used successfully on dilute waters). The total quantity applied must be predetermined from breakthrough experiments; these volumes are commonly 2.5-10 mL for retort waters, and up to several liters for cleaner waters. The cartridge should be held vertically to prevent channeling during sample application and elution. The initial milliliter of aqueous effluent (HpF) is discarded because of its dilution by the 400 μL of water that remained from activation of the cartridge. Subsequent HpF can be collected for analysis or for subjection to treatment schemes.

The retained organic compounds can be eluted after the residual unpartitioned 400-μL aqueous sample is rinsed from the cartridge with water. We have observed that some sorbate can be stripped from the stationary phase during the rinse. This could be caused by sample overloading or because the pH and osmolality of the wash water is different from that of the sample. The solvent used for initial elution must be mutually miscible in water and in whatever strong solvent may be subsequently used. Methanol, however, will elute nearly all retort water compounds that have not been irreversibly bound to the stationary phase; less polar solvents will not elute the more polar compounds. Compounds of different polarities can be eluted depending on the strength of the organic solvent. For other types of sample, tetrahydrofuran or dichloromethane may be needed for elution.

The particle size of the C-18 packing material and porosity of the frits give the cartridges the ability to act as depth filters. The particulates in retort water are effectively retained by both the inlet fritted polypropylene disc and by the silica particles. Because organic elution solvents will literally dissolve these retained tarry, oily residues, it is possible to calculate the concentration of suspended hydrophobic solutes by determining the difference in LpF concentrations yielded by the raw sample and by the filtered retort water.

b. Oil and Grease Procedure.

Oil and grease is a broad classification of organic compounds that is arbitrarily defined by the procedure applied. As specified in Standard Methods, it is the group of substances that can be determined quantitatively on the basis of their common solubility in Freon. Oil and grease includes aliphatic hydrocarbons (e.g., paraffins, waxes, and oils), lipids, fatty acids, and soaps. This class of compounds is analogous to the group of lipophilic solutes that compose the LpF of the RPF procedure.

Liquid-liquid partitioning followed by gravimetric quantitation is the standard method for determining the dissolved or suspended oil and grease concentrations of a wastewater sample. This method is fraught with difficulties when applied to oil shale process waters. A stable emulsion forms between the

aqueous and organic extraction phases requiring the addition of acid and large quantities of salt. The high total alkalinity of retort water requires the addition of large amounts of acid which leads to CO_2 offgassing, excessive foam production, and protonation of aliphatic carboxylic acids. The large quantity of additional salts will tend to "salt-out" some of the less hydrophilic solutes that are not oils (i.e., decrease their solubilities in the aqueous phase). In addition, the partitioning process itself is not selective. Polar compounds can be coextracted as organic ligands or ion-pairs, and both polar and nonpolar species can be concentrated at the organic-aqueous interface by surface-active agents, such as carboxylic acids. During the drying step, compounds are lost continuously via volatilization; standardization of volatilization is impossible because the rate of volatilization is substantial and not constant. Finally, quantitation by gravimetric detection is notoriously inaccurate and totally nonselective.

Consequently, we have adapted our general fractionation procedure to the determination of dissolved oil and grease (Fig. 1). The hydrophobic analytes, LpF, are retained by the stationary phase; the HpF is discarded. At this point in the protocol, the general procedure is modified because the method of detection precludes use of a "switchover" solvent of mutual miscibility. The aqueous residuum in the cartridge must therefore be removed by mechanical means, best achieved by lyophilization. The nonpolar compounds (oil and grease) can then be eluted with Freon (1,1,2-trichloro-1,2,2-trifluoroethane) and quantitated via infrared spectroscopy by measuring absorbance of the asymmetric methylene C-H stretch at 2930 cm^{-1} (Fig. 2). True aliphatic oil can be determined by passing the Freon eluent through an activated normal-phase (i.e., dried) silica cartridge. This step removes LpF solutes that contain functional groups and allows aliphatic hydrocarbons to pass through in the effluent for quantitation. A major difficulty with development of the LpF procedure was a high IR background absorbance that was sporadically encountered. This problem has several possible origins which include trace quantities of phthalate plasticizers and residual alkylsilyl bonding reagent. We also found substantial lot-to-lot variation in both background absorbance and apparent partitioning efficiency; this same variability was noted by Saner et al.

Comparison of Methods

The RPF procedure, when used for determining oil and grease of a wastewater sample, has many distinct advantages over the partition-gravimetric procedure. As mentioned above, the RPF procedure obviates the need for liquid-liquid partitioning and gravimetric detection along with the attendant problems of emulsion formation and loss of volatile hydrocarbons during sample concentration. The procedure is simple to perform, minimizes the use of glassware (generally one volumetric flask is required per sample), and minimizes solvent consumption (usually about 20 mL of solvent is required per sample, regardless of sample size, compared with 100 mL of solvent for the partition-gravimetric method). Dilute samples are concentrated directly (i.e., trace enrichment) by the cartridges. This means that the detection limit is restricted only by the sample matrix; large sample sizes can be applied to the cartridges if the solute concentrations are low. The detection limit that we usually observe is 20 mg/L oil (as mineral oil) in the Freon eluent.

The major advantage of the RPF procedure is throughput. An analyst can easily determine oil and grease of the LpF by the RPF procedure for 25-50

samples in an eight-hour working period. The major disadvantage of the method is that it is not known if the compounds in the LpF are the same as the compounds that would be quantitated by the partition-gravimetric method. This is also a limitation of the partition gravimetric method because true fortification/validation experiments cannot be performed.

The reproducibility of the LpF method for determining oil and oil and grease was determined for Oxy-6 and 150-ton retort waters (Table I). The relative standard deviations for both parameters were usually less than 10%. The oil and grease concentrations for Oxy-6 retort water (and presumably for other waters as well) vary from sample to sample because of composition changes over time. Some of these values were obtained from samples withdrawn from different drums over a period of a year.

The oil values of unfiltered samples represent less than a fifth of the total oil and grease concentration. In contrast, the oil values for filtered samples of both Oxy-6 and 150-ton retort waters were not detectable. A possible explanation for these results is that the "oil" in these waters, in contrast to the total oil and grease, is associated exclusively with the particulate fraction.

The LpF method was used to determine oil and grease in seven different retort process waters (Table II). The values were determined for both unfiltered raw sample (i.e., total oil and grease) and for filtered sample (i.e., dissolved oil and grease). The difference between these values represents particulate oil and grease. The samples are arranged in order of decreasing total oil and grease concentrations. This trend is maintained for dissolved oil and grease with the exception of Oxy-6 retort water. Omega-9 and 150-ton contained the lowest and highest LpF concentrations, respectively, differing by over an order of magnitude. The three highest LpF concentrations were found in 150-ton, S-55, and TV. Although the latter two waters were from simulated in-situ projects, all three waters were produced from above-ground retorts. The lower concentrations of oil and grease in the remaining four samples can be explained by noting that these four wastewaters may have been diluted by groundwater during in-situ retorting. It is apparent from these values that the quantity of oil is much lower than originally believed (i.e., 580 mg/L for Omega-9, Farrier et al.; and 3,836 mg/L for 150-ton, Harding et al.), although the effect of long-term storage on the separation of the oil from the water is not known. The above-ground retorts also had lower percentages of total oil and grease that was dissolved (i.e., 53-70% versus 83-101% dissolved oil and grease) even though their absolute dissolved values were generally higher.

The RPF method for determining oil and grease has a further distinct advantage over the partition-gravimetric method in that routine methods such as DOC or COD can be used to quantitate the retained compounds. This can be done indirectly by calculating the difference in values for the unfractionated sample and for the HpF (aqueous effluent). The analyst should be aware, however, that the results can be altered by redefining various parts of the protocol. For example, the pH and osmolality of the sample and rinse water can drastically alter the results.

The quantitation of LpF oil and grease by DOC was done for the seven retort waters (Table III). The relative rankings of the waters for Tables II and III

were the same. By dividing the LpF oil and grease values (i.e., those obtained by the IR procedure) by the LpF DOC values, qualitative information can be obtained about the composition of the LpF. This value, which has arbitrary units, reflects the degree of saturation of the carbon. All of the waters appear to contain about the same types of LpF constituents (i.e., degrees of saturation range from 0.10 to 0.14) except for 150-ton, whose degree of saturation is 0.22.

We have used the RPF procedure routinely for monitoring the performance of our biological and physicochemical treatment research and for generating fractions for further experimental treatment work. Some major conclusions from this work have been summarized (Jones et al. 1982a,b; Sakaji et al. 1982).

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Table I. Reproducibility of RPF Method for Oil Shale Process Waters

test	sample	mean mg/L oil and grease as mineral oil	n	rsd (%)
oil and grease	Oxy-6 RW[a]	204	8	13.2
oil and grease	Oxy-6 RW	262	8	5.8
oil and grease	Oxy-6 RW (filtered)[b]	273	15	5.2
oil	Oxy-6 RW	47.1	9	4.1
oil	Oxy-6 RW	32.9	5	9.8
oil	Oxy-6 RW	27.2	9	9.5
oil	Oxy-6 RW (filtered)	nil	15	-
oil	150-ton[c]	121	10	6.0
oil	150-ton (filtered)	nil	10	-

[a]Oxy-6 retort water; Occidental Oil Shale, Inc., Logan Wash, DeBeque, Co., MIS Retort #6
[b]filtered through 0.8-μm pore diameter polycarbonate membrane
[c]retort water from LETC 150-ton above-ground simulated modified-in-situ retort

Table II. Applicability of RPF Oil and Grease Method to Oil Shale Process Waters

	mg/L oil and grease as mineral oil				
	unfiltered		filtered[a]		% dissolved[b]
sample[c]	mean	range	mean	range	oil and grease
150-ton[d]	641	58	448	37	70
S-55[e]	334	78	178	20	53
TV[f]	276	20	175	18	63
Oxy-6 RW[g]	242	40	219	39	90
Geokinetics[h]	160	24	162	8	101
Oxy-6 GC[i]	86	48	71	35	83
Omega-9[j]	58	33	56	44	97

[a] filtered through 0.8 μm pore diameter polycarbonate membrane
[b] filtered ÷ unfiltered (% of total oil and grease, i.e., unfiltered, which is dissolved)
[c] sample volumes applied to cartridges were 2.5 or 5.0 mL; n = 4 for each sample type
[d] retort water from LETC 150-ton above-ground simulated modified-in-situ retort
[e] retort water from LETC 10-ton above-ground simulated modified-in-situ retort
[f] sour water from a commercial near-term surface retorting process
[g] Oxy-6 retort water; Occidental Oil Shale, Inc., Logan Wash, DeBeque, Co., MIS Retort #6
[h] retort water from Geokinetics, Inc., Utah, a true in-situ process
[i] Oxy-6 gas condensate; Occidental Oil Shale, Inc., Logan Wash, DeBeque, Co., MIS Retort #6
[j] retort water from LETC 1976 Rock Springs, Wy., site 9 true in-situ experiment

Table III. Ranking of Retort Waters by LpF Content as Measured by both DOC and "Oil and Grease"

sample[a]	raw DOC[b]	HpF DOC (mg/L)[c]	LpF DOC (mg/L)[d]	LpF oil & grease[e]	degree of saturation[f]
150-ton[g]	3054	974	2080	448	0.22
Oxy-6 RW[h]	3194	1271	1923	219	0.11
TV[i]	2550	727	1823	175	0.10
S-55[j]	2263	1009	1254	178	0.14
Geokinetics[k]	1646	498	1148	162	0.14
Oxy-6 GC[l]	671	74	597	71	0.12
Omega-9[m]	803	254	549	56	0.10

[a] sample volumes applied to C-18 cartridges were 4.0 mL
[b] filtered through 0.8 µm pore diameter polycarbonate membrane (influent DOC)
[c] C-18 effluent DOC
[d] calculated indirectly; difference between DOC of raw water and HpF
[e] values of filtered samples from Table II
[f] (LpF oil and grease) ÷ (LpF DOC); i.e., extent of hydrogenation
[g] retort water from LETC 150-ton above-ground simulated modified-in-situ retort
[h] retort water from LETC 10-ton above-ground simulated modified-in-situ retort
[i] sour water from a commercial near-term surface retorting process
[j] Oxy-6 retort water; Occidental Oil Shale, Inc., Logan Wash, DeBeque, Co., MIS Retort #6
[k] retort water from Geokinetics, Inc., Utah, a true in-situ process
[l] Oxy-6 gas condensate; Occidental Oil Shale, Inc., Logan Wash, DeBeque, Co., MIS Retort #6
[m] retort water from LETC 1976 Rock Springs, Wy., site 9 true in-situ experiment

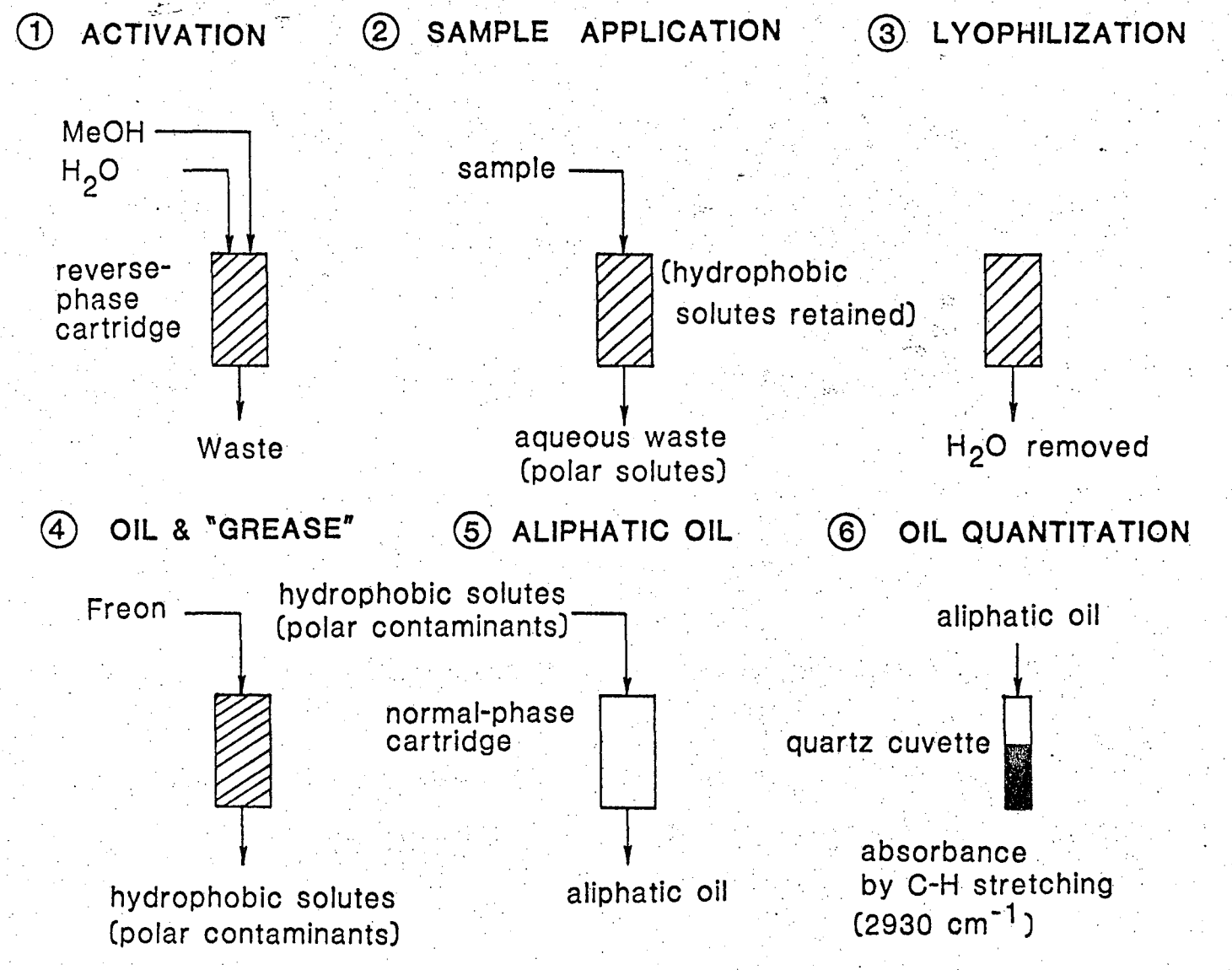

Figure 1. Reverse-Phase Fractionation (RPF) of Aqueous Samples: Separation of Hydrophobic Solutes (HpF) and Aliphatic Oil (LpF)

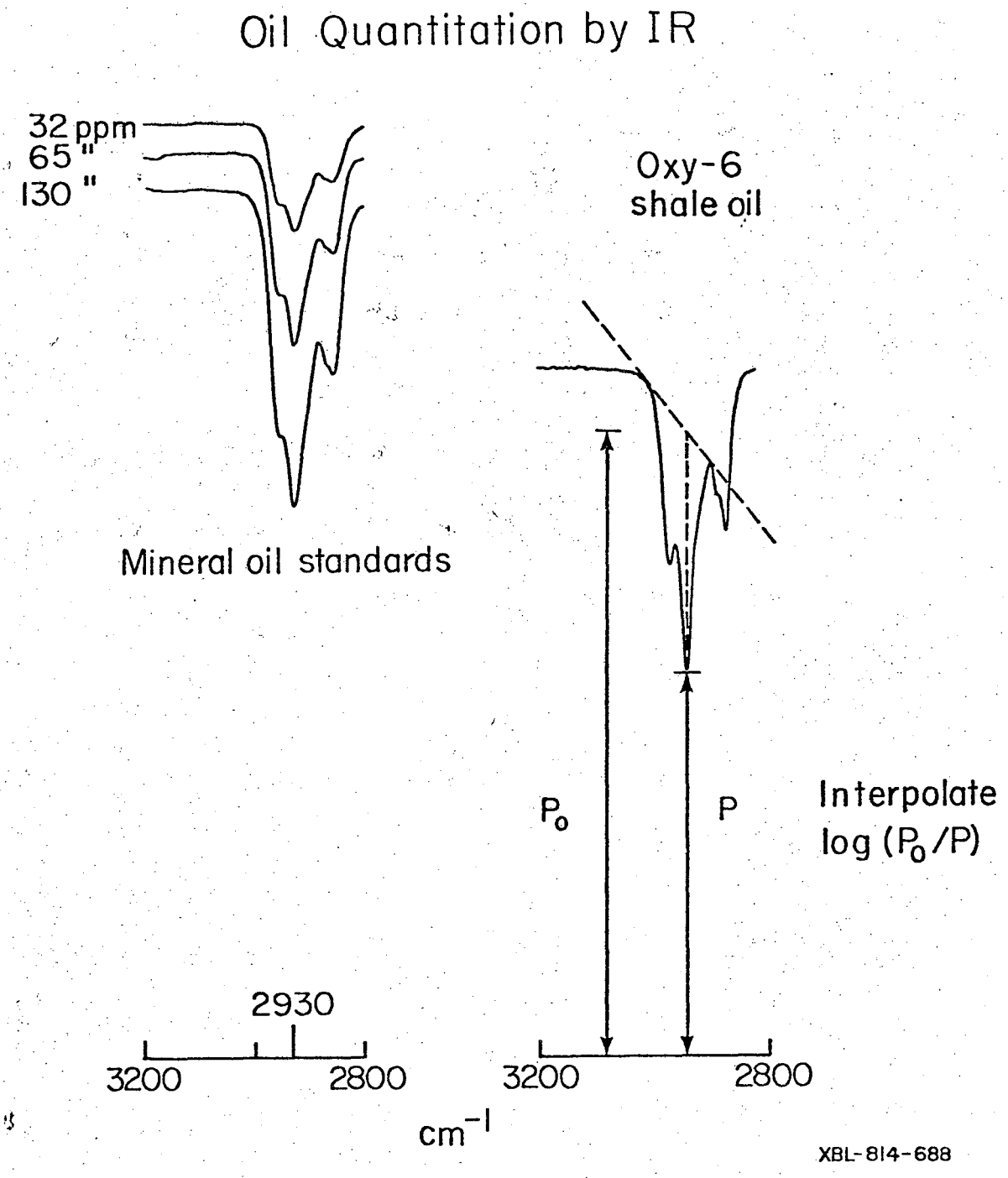

Figure 2. Quantitation of Oil by Tangent-Baseline Method and Infrared Spectroscopy

Glassware & Equipment

- Reverse-phase C-18 cartridges (C-18 Sep-Paks, Waters and Associates, Milford, MA; Part No. 51915)
- Normal-phase Si cartridges (Si Sep-Paks, Waters and Associates; Part No. 51905)
- Positive-displacement pipettes (e.g., 1.0-mL and 2.5-mL from SMI, Inc., Emeryville, CA)
- 10-mL gastight glass syringe with Teflon-tipped plunger and male Luer tip (two)
- Numbered aluminum tags (e.g., for gas chromatography columns; one per cartridge)
- Lyophilization connectors. No. -0- Vikem or silicone solid stopper, 13-gauge stainless-steel tubing with female Luer hub, Teflon male Luer union (Hamilton Co., Reno, NV), and polypropylene male Luer plug (Value Plastics, Inc., Fort Collins, CO) (1 set per cartridge).
  - To construct lyophilizer connection apparatus, insert 4-cm length of 13-gauge stainless steel tubing with a female Luer hub through the narrow end of a No. -0- stopper. Connect the Luer hub to the cartridge end-sleeve with a male Luer union. Seal the open end of the cartridge with a male Luer plug.
- Lyophilization apparatus (manifold, vacuum pump, etc.)
- Volumetric flasks (size and number will depend on organic solute concentration in each sample; generally one per sample)
- Teflon male Luer unions (for coupling C-18 and Si cartridges)
- Infrared scanning spectrophotometer (repetitive scan recommended; matched quartz cuvettes, 1-cm path length)

Glassware Preparation

Acid-wash all glassware
Rinse volumetric flasks with Freon

Reagents

All reagents must be at least Analytical Reagent grade
Tetrahydrofuran (THF)
Methanol (MeOH)
ASTM Type I water
1,1,2-Trichloro-1,2,2-trifluoroethane (Freon)

Procedure

N.B. The maximum number of samples and blanks is limited by the capacity of the lyophilizer.

I. Hydrophilic Fraction (HpF)

A. C-18 Cartridge preparation
(Note: Use cartridges from the same lot for any one experiment)
1. To introduce solvents, connect male Luer tip of gastight syringe to the longer end-sleeve of the C-18 cartridge.

2. Pre-wash each C-18 cartridge with 10 mL of THF.
(Note: Always disconnect the syringe or pipette from the cartridge prior to withdrawing the plunger)
3. To activate each cartridge, apply 5 mL of MeOH, followed by 20 mL of ASTM Type I water, using gastight syringes. Partially remove residual water by forcing approximately 10 cc of air through the cartridge using a gastight syringe.

B. Sample application
1. Withdraw subsample from aqueous sample with an SMI pipette. To introduce sample, connect male Luer tip of SMI pipette to the longer end-sleeve of the C-18 cartridge.
2. Apply sample to a cartridge immediately after activation, at a flow rate of 5-10 mL/min. The SMI pipette tips fit directly inside the cartridge end-sleeve. The cartridge should be held vertically during sample application.

C. Collection of HpF
(Note: This fraction is discarded if only "oil and grease" values are desired; refer to Part II)
1. Discard the initial milliliter of effluent; this fraction is diluted by the residual water occluded by the cartridge.
2. Collect effluent in appropriate container for subsequent analysis or treatment.

II. Lipophilic fraction (LpF): --"Oil and Grease" and "Aliphatic Oil"--

A. Blank preparation
1. Blanks are prepared by following step I. A.
2. Blanks should be prepared in duplicate for each elution volume for oil and grease and aliphatic oil

B. Sample application
1. Follow steps I. A. and I. B.
2. Remove residual sample from cartridge by forcing approximately 10 cc of air through the cartridge using a gastight syringe.
3. Rinse residual occluded sample from cartridge with 1-2 mL of ASTM Type I water (positive displacement pipette). Partially remove residual rinse water by forcing approximately 10 cc of air through the cartridge using a gastight syringe.

C. Sample lyophilization
1. DO NOT MARK CARTRIDGES WITH MARKING PEN! Identify each cartridge with a numbered aluminum tag. Seal both ends of the cartridge with male Luer plugs. Store cartridges at -20° C until lyophilization.
2. Prepare lyophilizer.
3. Start the vacuum pump; operate lyophilizer at less than 0.10 torr.
4. Remove male Luer plug from long end-sleeve of cartridge. Attach each cartridge to lyophilizer manifold (e.g., VirTis 6205-1650, with 0.5-in OD Quickseal valves).
5. Submerge each cartridge in a MeOH-dry ice bath for 30 seconds.
6. Immediately after this freezing step, apply the vacuum; if any loss in vacuum occurs, check for leaks.
7. Lyophilize for about two hours. Lyophilization is complete when the cartridges are at room temperature and no further condensation forms when they are wiped dry.

8. When lyophilization is complete, release the vacuum and turn off the pump.
9. Remove samples from lyophilizer.

D. Elution of LpF
1. For the determination of oil and grease, elute the lyophilized cartridges with 5 mL of Freon; force residual Freon through with 10 cc of air. Elute in the same direction as sample application to avoid washing out non-soluble particulates. Collect eluent and residual in an appropriately-sized volumetric flask. Bring to volume with Freon.
2. For the determination of aliphatic oil, pre-wash the Si cartridges with 10 mL of Freon; force residual Freon through with 10 cc of air and discard. Elute the retained solutes from the C-18 cartridges directly through the Si cartridges (cartridges coupled with male Luer unions) with 5 mL of Freon; force residual Freon through with 10 cc of air. Collect eluent and residual in an appropriately-sized volumetric flask. Bring to volume with Freon.
3. The stoppered volumetric flasks containing the eluates can be stored at 4^{o} C.

E. Sample quantitation
1. Prepare a set of standards using mineral oil or appropriate reference material (e.g., shale oil).
2. For the stock solution, place appropriate volume of oil in a tared 50-mL volumetric flask (if using mineral oil, place 75 µL in flask). Determine the mass of the oil and bring to volume with Freon.
3. For working standards, place 100, 250, 500, or 1000 µL of stock solution (positive displacement pipettes) in 5-mL volumetric flasks, and bring to volume with Freon.
3. Turn on IR spectrophotometer and allow a 20-minute warm-up period.
4. For a Perkin-Elmer model 298 IR spectrophotometer, set the repetitive-scan feature for the range of 3200-2800 cm^{-1}, and set for medium slit width.
5. Fill one matched quartz cuvette with Freon and place in the reference slot.
6. Place sample in second matched cuvette.
7. Scan between 3200 and 2800 cm^{-1} at 4 min/full-range scan.
8. Quantitate the absorbance by tangent base-line measurement of peak height (Fig. 2). Keep peak height below 80 per cent of full-scale by making appropriate dilutions.
9. Interpolate the oil and grease or oil values from the oil standard curve:

$$\text{mg/L oil} = m \times (\log P_o/P \text{ sample} - \log P_o/P \text{ blank}) + b$$

($P_o \approx$ incident energy, $P \approx$ transmitted energy)

a. Determine slope (m) and intercept (b) of the regression equation.
b. Calculate the values of the samples from their log P_o/P values.

10. If absorbance peaks are beyond the range of the standard curve, dilute sample and bring to volume with Freon. Repeat analysis.

11. Report data in the following column format:

A[1]	B	C	D[2]	E[3]
log P_o/P	log P_o/P x dilution factor[4]	B - log P_o/P blank[5]	mg/L oil in volumetric flask	mg/L oil in sample

[1]absorbance determined by tangent base-line measurement of peak height
[2]obtained by interpolation from standard curve of value in column C
[3]transform column D by degree of enrichment or dilution that occurred during elution of samples (i.e., if 2.5 mL of sample was applied to the cartridge and the cartridge was eluted into a 10-mL volumetric flask, multiply the resultant value in D by 4 to determine the true sample concentration, i.e., the value for column E)
[4]if a dilution was required to keep peak within 80 per cent of full-scale, multiply by dilution factor (e.g., if sample was diluted one volume in four volumes total, multiply the values in column A by 4 to obtain value for column B)
[5]blank should be consistent with sample treatment, i.e., if sample was eluted into 10-mL volumetric flask, blank should be eluted into 10-mL volumetric flask.
Typical blank absorbance values for different final volumes:

oil and grease (10 mL)	0.010
oil and grease (5 mL)	0.015
oil (5 mL)	0.023

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This report was done with support from the Department of Energy. Any conclusions or opinions expressed in this report represent solely those of the author(s) and not necessarily those of The Regents of the University of California, the Lawrence Berkeley Laboratory or the Department of Energy.

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