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OIL SPILL IDENTIFICATION SYSTEM

Chemistry Branch
U.S. Coast Guard Research and Development Center
Avery Point, Groton, Connecticut 06340

June 1977

Final Report

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This is a complete update of the report entitled "Oil Spill Identification System" of October 1974 (CG-D-41-75, ADA003803)

16. Abstract

A report entitled "Oil Spill Identification System" was issued in October 1974 detailing all aspects of spill identification as conducted by the R&DC Chemistry Branch. Six of "first generation" methods have been revised to incorporate the latest techniques developed by the R&DC. These include sampling, sample handling and transmittal, gas chromatography, fluorescence and infrared spectrophotometry, and thin-layer chromatography. These methods are detailed as they will be used by the Coast Guard operational laboratory.

In addition, back-up techniques of low temperature luminescence and high pressure liquid chromatography are included. An infrared field manual and an infrared field classification manual are included, along with a technique for simulated weathering of oils and section on safety precautions.

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METRIC CONVERSION FACTORS

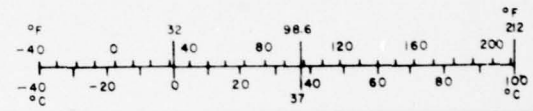
Approximate Conversions to Metric Measures

Symbol	When You Know	Multiply by	To Find	Symbol
	LENGTH			
in	inches	*2.5	centimeters	cm
ft	feet	30	centimeters	cm
yd	yards	0.9	meters	m
mi	miles	1.6	kilometers	km
	AREA			
in^2	square inches	6.5	square centimeters	cm^2
ft^2	square feet	0.09	square meters	m^2
yd^2	square yards	0.8	square meters	m^2
mi^2	square miles	2.6	square kilometers	km^2
	acres	0.4	*hectares*	ha
	MASS (weight)			
oz	ounces	28	grams	g
lb	pounds	0.45	kilograms	kg
	short tons (2000 lb)	0.9	tonnes	t
	VOLUME			
tsp	teaspoons	5	milliliters	ml
Tbsp	tablespoons	15	milliliters	ml
fl oz	fluid ounces	30	milliliters	ml
c	cups	0.24	liters	l
pt	pints	0.47	liters	l
qt	quarts	0.95	liters	l
gal	gallons	3.8	liters	l
ft^3	cubic feet	0.03	cubic meters	m^3
yd^3	cubic yards	0.76	cubic meters	m^3
	TEMPERATURE (exact)			
°F	*Fahrenheit* temperature	5/9 (after subtracting 32)	Celsius temperature	°C

*1 in = 2.54 (exactly). For other exact conversions and more detailed tables, see NBS Misc. Publ. 286, Units of Weights and Measures, Price $2.25, SD Catalog No. C13.10:286.

Approximate Conversions from Metric Measures

Symbol	When You Know	Multiply by	To Find	Symbol
	LENGTH			
mm	millimeters	0.04	inches	in
cm	centimeters	0.4	inches	in
m	meters	3.3	feet	ft
m	meters	1.1	yards	yd
km	kilometers	0.6	miles	mi
	AREA			
cm^2	square centimeters	0.16	square inches	in^2
m^2	square meters	1.2	square yards	yd^2
km^2	square kilometers	0.4	square miles	mi^2
ha	hectares (10,000 m^2)	2.5	acres	
	MASS (weight)			
g	grams	0.035	ounces	oz
kg	kilograms	2.2	pounds	lb
t	tonnes (1000 kg)	1.1	short tons	
	VOLUME			
ml	milliliters	0.03	fluid ounces	fl oz
l	liters	2.1	pints	pt
l	liters	1.06	quarts	qt
l	liters	0.26	gallons	gal
m^3	cubic meters	35	cubic feet	ft^3
m^3	cubic meters	1.3	cubic yards	yd^3
	TEMPERATURE (exact)			
°C	Celsius temperature	9/5 (then add 32)	Fahrenheit temperature	°F



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This report on oil spill identification represents the development efforts of the U. S. Coast Guard R&D Center Chemistry Branch over the period 1973-1977.

The Chemistry Branch Chief was Dr. R. R. Hiltabrand until 1976 when Dr. G. Kleineberg took over the branch.

The Laboratory Identification System Task Manager was Dr. Alan P. Bentz who was responsible for assembling and editing this report.

Below, in alphabetical order, are listed all members of the Chemistry Branch past and present who have contributed to the development of the methods.

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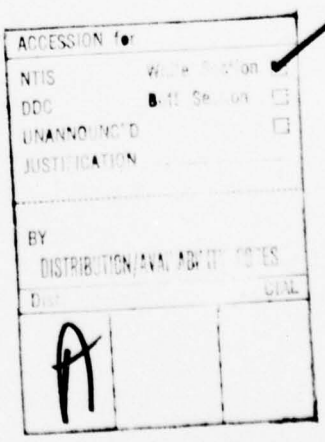

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1.0 INTRODUCTION

The Chemistry Branch of the Coast Guard R&D Center has devoted a major portion of its effort since 1973 on the development of an oil identification system designed to identify the sources of oil spills.

In October 1974 a report was issued entitled "Oil Spill Identification System" (CG-D-41-75; NTIS Accession Number AD A003803) which has achieved wide circulation. The present report is the accumulation of several years work and includes updated versions of six of the original procedures, plus five new ones and a safety section.

2.0 PHILOSOPHY (FOR APPLICATION OF THE METHOD)

The basic Coast Guard identification system for analysis of oil spills consists of four methods: thin-layer chromatography, fluorescence spectrophotometry, infrared spectrophotometry and gas chromatography. The remaining analytical techniques included are either for use as backup methods in extremely difficult cases or for different purposes entirely, as in the field classification of oils.

The reason for the use of multi-method approach, as has been stated many times, is that no single method has been established which is unequivocal in all cases. Any one of the four methods might be adequate in a given case, but each may be sensitive to certain interferences from impurities picked up by the slick. For example, infrared is insensitive to minor fluorescent impurities though sensitive to carbonyl compounds; fluorescence is sensitive to fluorescent compounds and impurities and totally insensitive to many hydrocarbons and carbonyl compounds; gas chromatography requires deasphalting which may give some analytical problems depending on the nature of the impurity, e.g., triglycerides might interfere; TLC may be affected by the substrate from which the sample is extracted.

For a given spill case, any one of the methods might be definitive. Usually, if it is not, the analyst is aware of it because of the anomalous or unusual results which indicate the presence of a contaminant. However, the analyst is much more confident if two analyses agree. If four analyses are conducted and all agree, the evidence is overwhelming that the correct spill source has been identified.

Analysts become dismayed when results of two or more methods disagree as to the source of an oil spill. Fortunately, in most of these cases, the reason for disagreement is apparent from the results, i.e., the experienced analyst can detect interferences in one or more of the methods. He can either discount the affected method or attempt to remove the interfering substances without altering the oil composition. This has been done using column chromatographic techniques or saponification followed by analysis of the unsaponifiable portion.

In still other cases when the analytical methods disagree, the circumstantial evidence of the case may explain an erroneous result, or reinforce a positive result.

Inevitably, there will be cases in which two suspects are nearly identical or the results of different techniques disagree for no discernible reason. These cases may require other experiments or backup analyses. If these additional efforts fail to resolve the issue, the conclusion must be that the source is indeterminate. Our objective is as much not to accuse the wrong party of spilling oil as it is to accuse the spiller.

The fact that an oil sample from a suspect is the closest in a spill set does not automatically make it the spill source. The analyst must use criteria to insure a maximum degree of match, compatible with the extent of weathering before he can assign a match.

3.0 APPLICATION

The methods described herein have been applied to over 130 spill cases at the Coast Guard R&D Center. Virtually no two spill cases are alike and certain technique variations were required, usually in sample preparation.

The most frequently encountered problem was that of insufficient sample from either the spill or occasionally from a source. The result is that microtechniques were developed for the methods. All of these are reported here, except a recently developed micro infrared technique.

The field of oil identification is a fast-moving field with new developments constantly being evolved. This "final" report is by no means the "last word" on the analytical chemistry of oils - rather it is the state of the art as we have developed it to date. The methods give good results. If they are later improved significantly, they will be reported separately; it is unlikely that another single report will be issued which embraces all methods.

Appendices A and B on sampling and transmittal were developed under the Field Oil Identification Project (Task Manager J. Richard Jadamec) and are included here for completeness of the overall "Oil Identification System."

Since Appendix A was written, we have been testing and evaluating a very promising new material (Lipo-pore screen). It is likely that it will provide the sampling method of choice in the future, at which time a separate report will be issued describing the material and its use.

Lipo-pore is a screen which is hydrophobic and oleophilic, and thus, will pass oil without any water going through. The screen can be configured in various ways to different devices. The simplest is a hollow cylinder attached to the top of a deep-drawn steel cup which collects the oil. Collection efficiencies are good enough to collect adequate amounts of sample for analysis from a slick within three minutes. Our analyses indicate that oil samples are neither contaminated nor altered by this collection method, and no water is found in the samples.

Lipo-pore screen was attached to larger sampling devices which are helicopter deployable. This larger device was used successfully to collect the viscous ARGO MERCHANGE oil slick samples in December 1976. Seas were running 10-20 feet with winds to 50 knots. The heavy No. 6 fuel had the consistency of grease after hitting the frigid water. The samples were remarkably free of water - so much so that salt windows for infrared analysis were unaffected by the oil as it came out of the sampler!

Appendix C, on weathering of oils, is new and describes a method used for outdoor weathering of oils. Other accelerated weathering techniques are being developed on a similar scale to simulate weathering with actual spill cases.

Appendix D is a considerably revised gas chromatographic procedure from that of October 1974 and is the basis for a method currently under consideration by ASTM. Significant differences include: a linearizer for the flame photometric detector, automatic capsule injection system (which permits unattended

round-the-clock operation), simultaneous FID/FPD detection from the split effluent of a single capillary (or packed) coulmn with either OV-101 or Dexsil 300 as a liquid phase; a quantitative measure of peak area differences to support visual interpretation.

Appendix E on fluorescence has been updated to include, among other things, the use of corrected spectra, lower concentrations (especially for heavier oils), improved cleaning procedures, specific excitation wavelengths besides 254 nm, and more extensive interpretation procedures to account for weathering.

Appendix F is new and describes low temperature luminescence which includes both fluorescence and phosphorescence as observed from a solid matrix of methylcyclohexane at 77°K. Sharper spectral structure and additional peaks are observed than with room temperature fluorescence to yield more total information.

Appendix G is a revised infrared method with elimination of the multiple internal reflectance technique and considerable revision of the interpretation of spectra.

Appendix H, the infrared field manual, is new since October 1974. It was developed for field use in COTP's with the Perkin-Elmer 727B infrared instrument. It is a detailed step-by-step instruction of all aspects of infrared oil identification.

Appendix I is a new method for classification of spilled oils using a single-beam infrared instrument. It was developed for field use and specifically to distinguish seeps from non-seeps in the Santa Barbara, California, area.

Appendix J is a thin-layer chromatographic method which uses a commercial developing trough for greater reproducibility than the October 1974 procedure.

Appendix K describes a high pressure liquid chromatographic separation of the polar fractions extractable from oil samples.

Since the development of the technique described in Appendix K, a significantly new method has been developed to monitor the effluent and possibly identify the components from their fluorescence spectra. For this technique, two detectors are used: UV absorption at 254 nm followed by fluorescence emission (excitation at 270 nm) using an optical multichannel analyzer (OMA) to obtain real-time spectra. OMA readings are taken continuously. In the future, spectra will be recorded for one second (30 scans), and then transferred in real time to a dedicated data processor, normalized, derivatized, and displayed on a Vidicon tube. The system can be used for both emission and absorption spectra. Ultimate development will include microprocessor control of all the liquid chromatograph functions which will have the capability of selecting the optimum excitation wavelength based on the absorption/excitation curves. Complete details of this technique will be published elsewhere.

Appendix L addresses the safety aspects peculiar to those methods described in this report.

APPENDIX A

OIL SAMPLING PROCEDURES

I. General Oil Sampling Guidelines

A. General. The procedures contained within this instruction are designed to assist the pollution investigative team in obtaining oil spill samples which may be used as evidence in the determination of the source responsible for the oil spill. Although this instruction does not contain a specific sampling procedure for every spill situation that may occur, by following these general guidelines, the investigative team can collect legally valid samples that will assist the responsible operational unit, the field and/or central laboratory in determining the source responsible for the oil spill.

B. Number of Spill and Source Samples Required. It is essential that all possible sources at the site of an oil spill be sampled in order to determine the responsible source. The Coast Guard-developed methods for "fingerprinting" the source of spilled oil are very sensitive and can detect and fingerprint the differences in the same oil stored in different cargo holds of a vessel, different storage tanks within a facility, different bilges aboard a vessel, etc. It is, therefore, necessary that all possible sources of oil be sampled if the fingerprinting techniques currently in use by the Coast Guard can be effectively employed. Furthermore, if the correct source sample is not obtained at the time of the oil spill investigation, it may be impossible to obtain the sample at a later date and will render the analysis of the spill samples useless with respect to determining the source responsible for the oil spill.

It is advisable that three samples of the spilled oil be collected when possible. These three samples should be from the areas where the oil accumulation within the slick is the heaviest, and sampling points should be as far apart as possible. In instances where the oil has washed ashore, samples should be collected from areas of heaviest accumulation. In addition, one sample of water, beach material, or other substrate, dependent on the location of the spilled oil at the time of sampling, which is free of the fresh spill, should be collected to determine if the fingerprint of the spilled oil could have been affected by background oil contamination.

In instances where more than one source is in the immediate area of the spill, and suspected of having the same type of spilled oil, samples should be collected from these sources to show convincingly that only one source was responsible for the oil spill.

C. Amount of Oil. In general the investigator should collect as much oil as possible from the spill site and suspected sources. For a complete fingerprinting analysis by four analytical techniques a minimum volume of 1 milliliter (0.03 oz.) is required. Where the volume or amount of spilled or source product is substantial, the sample collection container should not be filled more than 2/3 full. Procedures for using the preferred[1] Teflon sampling method are presented in Section III.

[1]A new sampling material (Lipo-pore) has outstanding properties for oil collection and is expected to replace Teflon strips for this purpose.

D. Sample Identification. It is critically important that all samples be accurately identified. Figure 2 shows the Oil Sample Identification and Custody Tag. Detailed instructions for filling it out are given in Appendix B on Oil Sample Handling and Transmittal Procedures. This form CG-XXXX should be completed by the pollution investigators at the time of sample collection. It is important that the name of the persons taking and witnessing the actual collection of the sample, the date and time of sample collection, as well as the specific location from where the sample was collected, such as tank number, etc., be entered on Form CG-XXXX. Record all pertinent information in a Sample Collection Log Book.

E. Sample Custody. This aspect in sample collection is covered in detail in Appendix B. Form CG-XXXX, when filled out in accordance with the instructions given in Appendix B, will be the basis for the establishment of a chain of custody. It is very important that a complete record be maintained of sample custody if these samples are eventually used as legal evidence to determine the source responsible for the spill.

F. Sample Contamination. The investigating team should take precautions to prevent the possibility of sample contamination. Sampling equipment should be cleaned and stored in a clean condition as soon as possible after completion of sampling. Disposable gloves should be used as specified in this instruction. Sealed sampling jars and card packs should be opened only when they are needed. Adherence to the procedures given in this instruction will minimize the possibility of sample contamination.

G. Types of Samples. Oil samples obtained at the spill site and from suspected sources may be of many different types. The various types of samples which may be collected, along with some brief notes on their collection, are described below.

(1) Neat Oil - oil taken from fuel tanks, storage tanks, sounding tubes, etc., in which the collected sample is nearly 100 percent oil. Do not fill sample collection jar more than 2/3 full.

(2) Oil and Water - samples drawn from outfalls, overboard discharges, scooped from the water surface, etc. Do not fill more than 2/3 full.

(3) Oil and Sand - Obtain sample of sand with the heaviest accumulation of oil.

(4) Oil and Sawdust - Obtain sample with the heaviest accumulation of oil.

(5) Oil and other absorbent material, e.g. cat litter, etc. - obtain sample with the heaviest accumulation of oil.

(6) Oil-coated vegetation (aquatic or land) - do not attempt to scrape the oil from the vegetation, but place the heaviest coated portions of the vegetation into the sample jar.

(7) Oil-coated Teflon strips as obtained from the Teflon Oil Sampling System.

(8) Scrapings from pilings, sea walls, etc., obtained with a wooden spatula.

(9) Other oil soaked or saturated material - do not attempt to remove the oil from the material, but place the oil saturated material in the sampling jar.

It is essential that the sampling jars supplied with this system be used at all times. These jars are numbered serially and are part of the chain of custody procedures necessary to insure the identity of the collected sample. If a numbered jar is not available, any clean, unused jar with a lid may be used. Line the lid with aluminum foil if available. At no time should the investigator attempt to transfer the sample collected in a non-standard container to the standard containers supplied in the Teflon Oil Sampling Kit. In the event that the non-standard sampling jar is used, extra care must be given to sample identification and custody procedures.

II. Description of the Teflon Oil Sampling Systems

A. Background. Several prototype sampling devices based on different designs have been subjected to laboratory and operational evaluations. The final outcome of these efforts is the Teflon Oil Sampling System, illustrated in Figure 1.

B. General Description. The Teflon Oil Sampling System provides sampling equipment, sample jars, custody tags and other items necessary for sampling oil. The main sampling medium is Teflon. Teflon was selected over other materials which absorb or adsorb oil because it does not impart any contamination to the collected oil, and will not affect the oil fingerprint as obtained by any of the analytical methods currently in use to establish the identity of the responsible spill source. Additionally, when Teflon is used, essentially waterfree oil is collected, thus minimizing the effect of sample alteration through physical, chemical and biological alterations on storage through prolonged contact between small volumes of oil and large volumes of water.

C. The Sampling System. The Teflon Oil Sampling System consists of two carrying cases and an extension handle. Contained within these carrying cases are mailers and sample jar seals for use when samples are being shipped to a laboratory for analysis.

(1) The primary case will be adequate for sampling in approximately 75 percent of all sampling situations. The primary case contains:

(a) Sample jars with tags (CG-XXXX)
(b) Teflon card packs
(c) Disposable gloves
(d) Wooden spatulas
(e) Tweezers
(f) Sample collection log book
(g) Fiber reinforced tape
(h) Forms CG-3639 and CG-3639A (Water Pollution Violation Report and Water Pollution Incident Report Workbook)
(i) Camera and film (to be provided by unit)
(j) Instructions
(k) Other forms, instructions, etc.
(l) Pens, markers, scissors, etc.

(2) The secondary case will be required in the remaining 25 percent of sampling incidents. The extension handle may be required. The secondary case contains:

(a) Disposable sampling head
(b) Sounding tube samplers with string
(c) Sample jars with tags (CG-XXXX)
(d) Teflon card packs
(e) Trash bags
(f) Paper towels
(g) Cleaning equipment and fluid
(h) Miscellaneous

D. Equipment Description and Notes

(1) Oil Sample Jars - Wide mouth, clear glass jars with a 6 oz. capacity will be used to store oil samples. Each jar will be supplied with a metal lid, a Teflon lid liner and a copy of Form CG-XXXX. Each jar, lid and Form CG-XXXX will bear a serial number as described below.

(2) Form CG-XXXX and Serial Numbers - Form CG-XXXX will be as shown in Figure 2. Detailed instructions for the use of this form can be found in Appendix B. The serial numbers used for sample identification and accountability will be a two-digit number followed by a hyphen and a five-digit number. The first two digits are a Coast Guard Unit Identifier Code (UIC). The five-digit number is a serial number with each unit having its own series.

(3) Teflon Card Packs - Each pack contains four 2" x 3" clean Teflon cards.

(4) Disposable Gloves - Disposable plastic gloves are provided to reduce the chances of sample contamination. These gloves are to be used once and discarded.

(5) Wooden Spatulas - The wooden spatulas are merely wooden tongue depressors. They are used to scrape oil from solid surfaces. They are to be discarded after use.

(6) Tweezers - The tweezers are used to hold the Teflon card(s) when the sample can be taken within arm's reach. They must be cleaned after use if they have come in contact with oil.

(7) Tape - One-inch wide fiber reinforced tape is used to seal the lids of the jars to prevent leakage. The tape also is used to secure the tag to the jar.

(8) Disposable Sampling Head - The sampling head is a wooden strip approximately 18" x 1 1/4" x 1/4" with 8 paper clips stapled to it.

(9) Extension Handle. The extension handle is an aluminum handle 10 feet in length with a clamp at one end to hold the sampling head. The handle, together with the sampling head, is called the "rake."

(10) Sounding Tube Sampler - The sounding tube sampler is shaped somewhat like a mushroom anchor with a cup to hold oil. See Figure 3.

(11) Cleaning Equipment and Fluid - Use only the cleaning fluid supplied with the system. The cleaning equipment includes a brush and paper towels.

III. Preparation and Use of the Teflon Oil Sampling System

A. General. The following procedures are based on the use of the Teflon Oil Sampling System and are mandatory when using the kit. Cleanliness, obtaining a sufficient sample, sample identification and establishment of a chain of custody are the primary considerations.

B. Equipment Check. Prior to departing the office or station, inspect the sampling kit to insure that it is clean and that all needed components are in the cases. In the event that the case or its contents are dirty, follow the cleaning procedures outlined in paragraph K below. Discard any disposables which are contaminated. Also check serial numbers to insure that numbers on sample jars and tags correspond.

C. Procedural Outline. The following is a general outline of procedures to be followed in sampling:

(1) Arrive on scene

(2) Examine the situation

(3) Determine the location(s) and method(s) for sampling

(4) Prepare needed equipment

(5) Take samples

(6) Complete log and sample tags

(7) Clean up and secure

More complete explanations of the above will be found in the following paragraphs.

D. Examine the Situation. After arriving at the sampling site, examine the situation to select a suitable staging area(s), select sampling locations, identify areas of spill to be sampled, identify probable sources, etc. Staging areas should be clean and convenient to the sampling site. Field sampling sites should be easily reached and should contain high concentrations of the oil to be sampled. Sampling sites on board vessels, tank farms, etc., will not be as difficult to select as field sites. Potential sources for sampling would include any vessels, barges, pipelines, tank farms, petro-chemical plants and sewage outfalls in the area.

E. Locations and Methods for Sampling. The methods for sampling will depend on the location and condition of the oil to be sampled. Oil to be sampled will be in one of the forms listed in Section I-G. The following methods will handle almost any type of sample which must be taken.

(1) Sampling with the jar. When there is a thick oil slick on the water or when the sample can be collected from a falling stream of liquid containing a large volume of oil, the jar alone will suffice to take the sample. For an oil slick on water simply scoop with the jar. For a falling stream of liquid hold the jar in the stream. Do not fill the jar more than 2/3 full. Replace the lid and lid liner and wipe the jar clean with paper towels.

NOTE: When collecting the sample, if a large volume of water has been collected, invert sample bottle and open cap slightly to allow excess water to flow out. Repeated sampling using only the bottle may now be attempted to increase the volume or amount of collected oil using this technique.

(2) Sampling with Teflon and tweezers. When there is a thin oil slick on water within arm's reach, a sample can be obtained by dipping the Teflon cards slowly through the slick. Hold the cards with the tweezers. Dip them as many times as necessary to obtain a sufficient sample. Place the cards in the jar and replace the lid and liner. Keep the outside of the jar as clean as possible.

(3) Sampling with Teflon strips and rake. The use of Teflon and the rake is essentially the same as Teflon and tweezers. The rake is an extension device for use when the oil slick is not within arm's reach. Four to eight Teflon cards can be used with the rake. Discard the sampling head and clean the handle after use.

(4) Sampling with the spatula. Where oil has covered pilings, sea walls, etc., a sample may be obtained by scraping off oil with the wooden spatula. The oil can be scraped off the spatula onto the inside edge of the jar. In sampling oil on creosote-treated wooden piling, it is important that a sample of the creosote, uncontaminated by the oil spill, be taken and placed in a second sampling jar.

(5) Sounding tube sampler. Tie a length of clean string to the sampler and drop it into the tube to be sampled. Pull it up and pour the oil collected in the cup into a sample jar. Repeat this procedure until the bottom of the jar is covered. After use, clean the sampler and discard the string.

(6) Sampling from a boat. In cases where a sample is to be collected from a slick on open water, the use of the small boat is an essential part of the overall sampling procedure. When a slick is sighted and it is determined that a sample will be taken, it is important that the boat operator approach the slick staying to the downwind side of the slick. Generally, the thickest portion of the slick will be at the head of the slick, which is downwind, whereas the thin region of the slick will be at the tail of the slick or upwind. The boat operator should approach the head of the slick and select that region containing the greatest accumulation of oil before sampling. Samples must be taken as far from the boat's exhaust as possible. If conditions permit, the boat operator should attempt to build up headway, cut his engine and coast into the sampling area.

F. Preparation of Equipment. After determining the method to be employed, select a suitable spot to lay out the equipment. A clean elevated surface such as a table or car hood is preferred to help keep everything clean. Lay out the equipment needed so that it can be reached without going back into the case while sampling. After all equipment is laid out and assembled, put on gloves and open a sample jar and any Teflon packs which will be used. Only one jar and the Teflon which will be used immediately should be opened. Jars and Teflon should be handled only with clean gloves at the start of each sampling attempt.

G. Taking the Samples. The samples should be obtained with the method chosen for the situation. The sample, with Teflon cards used, is to be placed into a sample jar and the lid with lid liner replaced. Care must be taken to prevent oil from getting on the outside of the jar. In particularly messy situations it may be necessary to change gloves before handling the jars. Attempt to keep one glove clean or have an assistant wearing gloves. Wipe the jar clean if necessary.

H. Sample Log and Form CG-XXXX. The importance of properly completing the sample collection log and Form CG-XXXX cannot be over stressed. Follow the procedures of COMDTINST 5922.X2. Seal the lid to the jar with fiber reinforced tape to prevent loosening of the lid. Attach the tag to the jar with the tape.

I. Securing. After all samples are taken and recorded, police any trash. Clean any equipment which requires cleaning following procedures contained in paragraph K. Return all items to their proper cases.

J. Handling of Samples. The fewer people who handle the sample or become involved in the chain of custody the easier it will be to keep proper track of the sample. The investigator should return all samples to the Captain of the Port or the Marine Safety Office as soon as possible. The appropriate authority will establish procedures for handling samples in each office. In transit or storage, samples should be protected from breakage or high heat. Handling of samples is covered more fully in Appendix B.

K. Cleaning Procedures. These procedures are to be followed when cleaning any item of the sampling system is necessary.

(1) Wipe excess oil off the item with a disposable paper towel and discard the towel.

(2) Spray cleaning agent on the areas covered with oil. Scrub sprayed areas vigorously with small nylon brush. Re-spray areas with cleaning agent and allow the cleaning solvent to remain in contact with the oil covered areas for 15 minutes.

(3) After 15 minutes, pressure rinse the sprayed areas with water and wipe dry with a disposable paper towel. Pressure rinse the cleaning brush. Use hot water if it is available.

(4) Repeat cleaning procedure if visible traces of oil are evident.

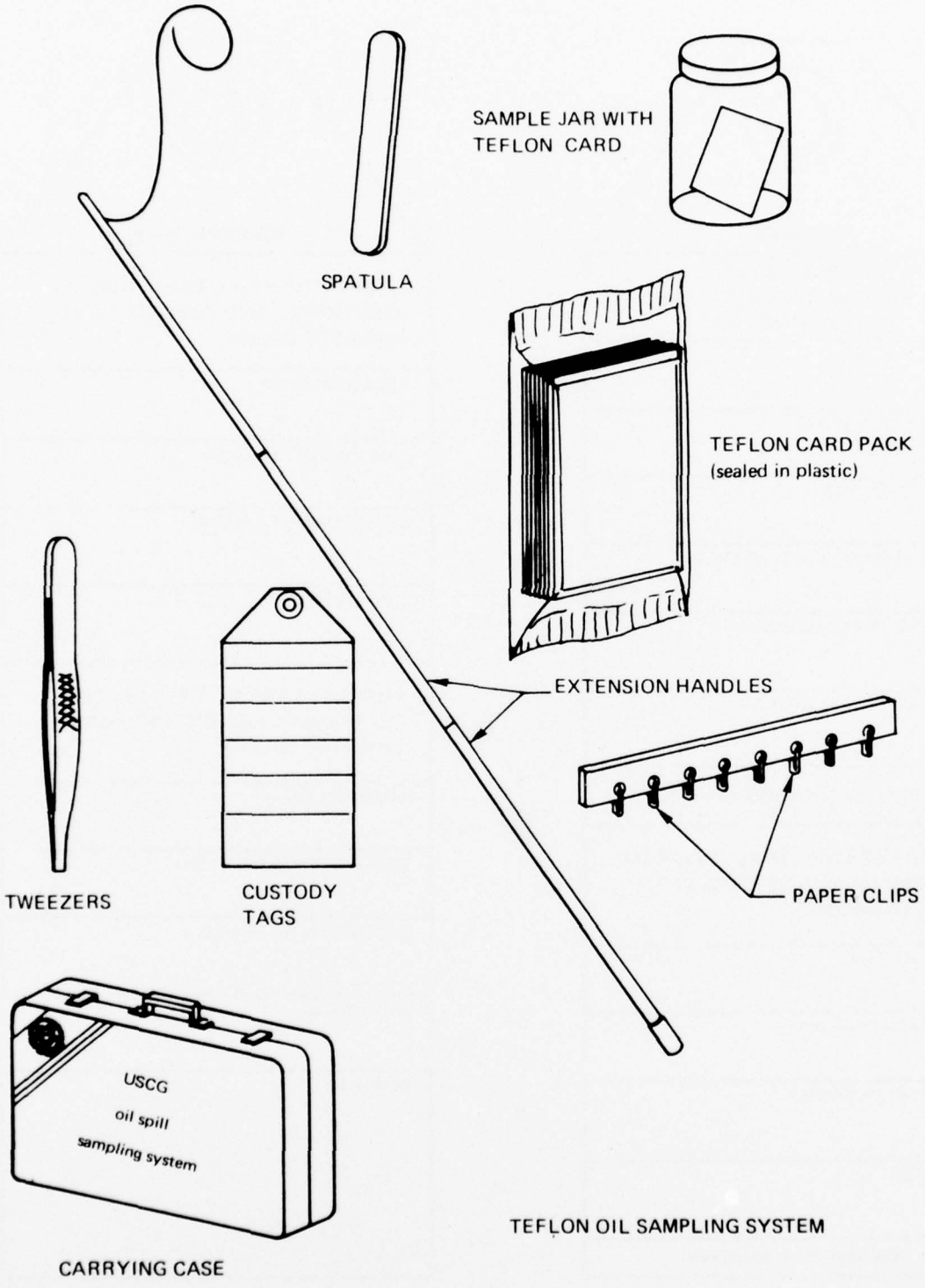

SAMPLE JAR WITH
TEFLON CARD
SPATULA
TEFLON CARD PACK
(sealed in plastic)
EXTENSION HANDLES
TWEEZERS
CUSTODY
TAGS
PAPER CLIPS
USCG
oil spill
sampling system
CARRYING CASE
TEFLON OIL SAMPLING SYSTEM

FIGURE 1

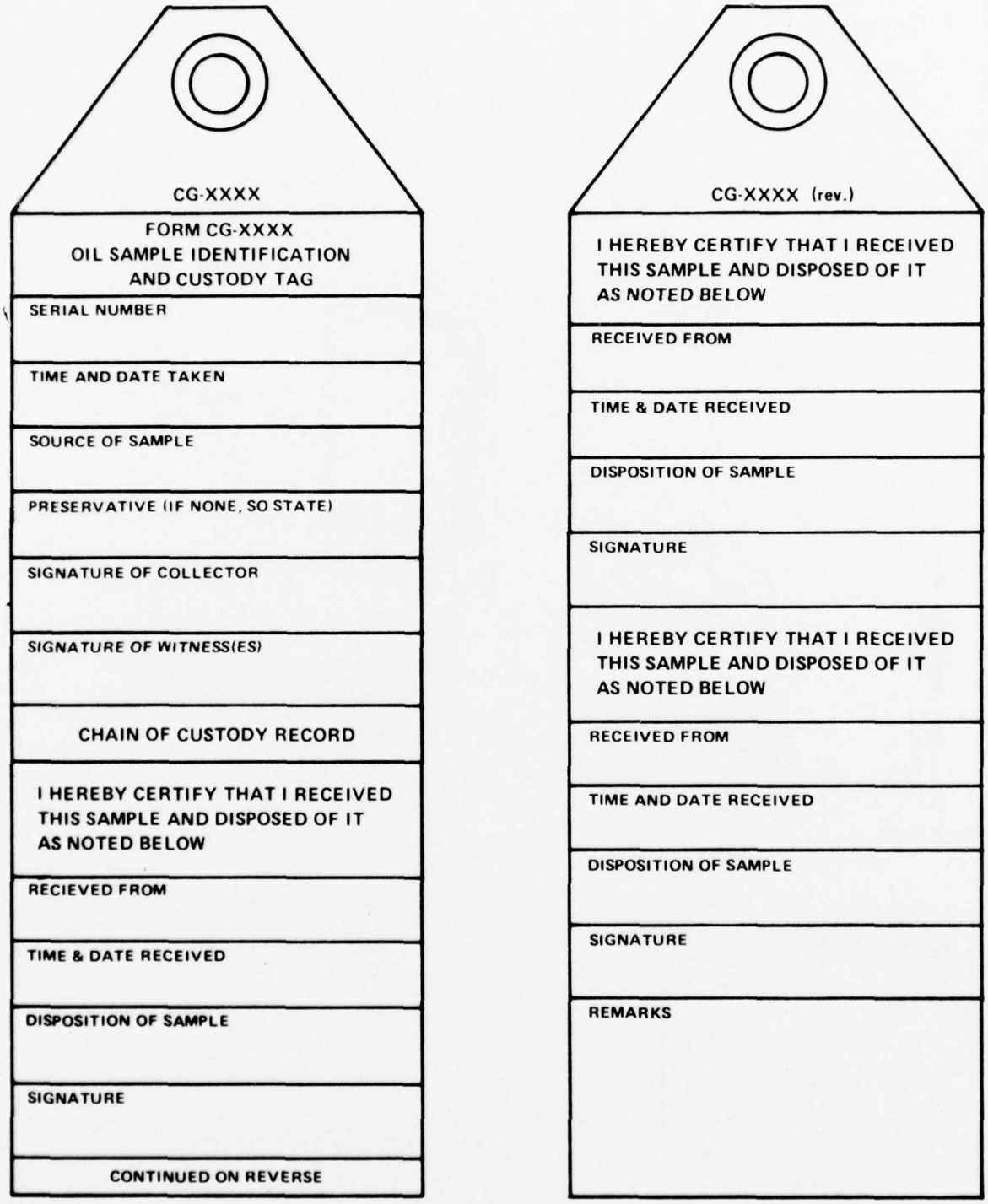


FIGURE 2 - FORM CG-XXXX, OIL SAMPLE IDENTIFICATION AND CUSTODY **TAG**

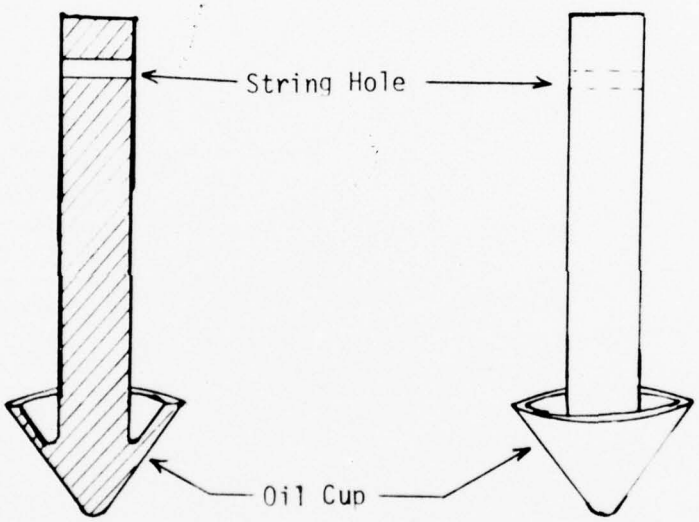


FIGURE 3 - SOUNDING TUBE OIL SAMPLING DEVICE

APPENDIX B

OIL SAMPLE HANDLING AND TRANSMITTAL PROCEDURES

I. CHAIN OF CUSTODY INFORMATION

A. Admissibility of Analyses as Evidence

To be admissible as evidence, samples taken must be proved conclusively to be in an appropriate person's possession until the analyses resulting therefrom have been introduced as evidence. This requires that rigid controls be maintained to establish a chain of custody for the samples from the time of initial sampling until ultimate disposition of the particular case.

B. Possession or Custody

A sample may be considered in a person's "possession" or "custody" if:

1. It is in actual physical possession of an appropriate person - either the individual who collected it or one to whom it has been properly transferred;

2. It is in an area where it can be kept under surveillance by an authorized person; or

3. It is under lock and key where it cannot be tampered with.

C. Storage of Samples

Samples must be kept in such a manner that they cannot be altered either deliberately or accidentally.

Any indication that a sample has been subjected to tampering or physical alteration could disqualify it as evidence for possible legal action. Therefore, the instructions given herein must be followed strictly.

II. FIELD LOG BOOK

A. A field log book will be used for recording field observations pertaining to the collection, handling and transmittal of oil samples.

B. Field observations to be recorded in the field log book should include:

1. Brief description of the oil spill and of the sources sampled, including date, location, extent and/or a diagram, if relevant.

2. Weather and water surface conditions, including wind speed and direction, surface water and air temperatures.

3. Locations within the spill where samples were collected; showing these collection sites on the diagram of the spill site.

4. Description and locations of suspect sources; specific locations aboard vessels where samples were located, e.g. bilge, fuel tank, cargo tank, etc.

5. Notes on the physical characteristics of samples, such as color, odor, apparent viscosity, etc.

6. Observations of dead fish or birds in the spill area.

7. Notation of all sample serial numbers used.

C. Log book entries should be made by the investigative team leader and witnessed by a member of the team. Both individuals should sign the log book entry upon completion of the field investigation.

III. SAMPLE IDENTIFICATION AND CUSTODY IN THE FIELD

A. General. An effective Oil Spill Sampling System requires that a uniform procedure for sample identification and chain of custody be established. The system has been designed to facilitate and insure that proper sample identification and chain of custody procedures can be maintained.

B. Descriptions of Equipment and Forms

1. Sample Jars - Wide-mouth, clear glass jars with a 6 oz. capacity will be used to collect and store oil samples. These jars will be supplied with screw-on lids and Teflon lid liners. The jars and lids will be serialized as explained in Section III-C.

2. Sample Identification and Custody Tag, Form CG-XXXX - The sample identification and custody tag is shown in Figure 1. Detailed instructions for filling out this form are contained in Section III-D,E.

3. Sample Jar Seal, Form CG-YYYY - The sample jar seal is shown in Figure 2. The use of the jar seal is limited to instances when samples are to be shipped to a laboratory for analysis by mail or common carrier. Instructions for their use are presented in Section V.

4. Sample Transmittal Form, Form CG-ZZZZ - The sample transmittal form is shown in Figure 3. Its use is also limited to shipment of samples to a laboratory for analysis by mail or common carrier. Section V has instructions for its use.

5. Other Sampling Equipment - Other items in the Teflon oil sampling system are explained in Appendix A.

C. Numbering of Samples. Each unit included in the Teflon oil sampling system has been issued a two-digit unit identification code (UIC).

Each sample jar, lid, and tag has been marked with a unit identification code followed by a hyphen and a five-digit serial number. The jar, lid and tag with the same number must be used together. Numbers must never be mixed.

D. Sample Identification. Form CG-XXXX has been designed to insure proper sample identification. It is important that the date and time of sample collection as well as the location and/or source from which the sample was collected be identified on this form. The signatures of the persons collecting the sample and witnessing the collection and filling out of this form are mandatory. The sample serial number will be used to cross reference sample identification, chain of custody, and laboratory reports, after the sample has been collected in accordance with Appendix A. The person who has collected the sample must complete the identification portion of the tag and record all pertinent information in the field sampling log. The information required is explained below:

1. Sample Number. Serial numbers for jars and lids will have been entered at the factory. Check to see that the number engraved on the jar and lid are identical. Do not mix the numbers on jars and lids.

2. Date Taken. Self explanatory.

3. Time Taken. Self explanatory.

4. Source of Sample. Be specific, e.g. bilge sample from a particular bilge or tank of a particular vessel; spill sample taken from water 50 feet from end of Bayonne Terminal Pier, Upper New York Harbor, etc.

5 & 6. Sample Collector/Witness(es). Complete legible signature plus rank and title of person collecting sample and person witnessing the collection.

7. Remarks. Specify unusual characteristics that may require special laboratory handling, e.g. nauseous odor, flammability, etc.

A copy of Form CG-XXXX with proper entries is shown in Figure 4.

E. Custody Information. Form CG-XXXX has spaces to record three transfers of sample custody. The number of people in the chain should be kept to a minimum and each custodian must appear in the sequential location on the tag. Form CG-XXXXA (Figure 5) is to be used when additional custody transfer blanks are required. If used, Form CG-XXXXA should be affixed to the jar in the same manner as the original tag. Several scenarios for custody transfer situations which can be expected to occur have been prepared and are presented below. Examples of custody flow charts and filled out CG-XXXX's are included.

1. Situation - Investigator returns sample to COTP with its own lab facilities (Figure 6).

2. Situation - Investigator returns sample to COTP and sends it to a lab facility via mail or common carrier (Figure 7).

3. Situation - Investigator returns sample to COTP and turns sample over to an intermediate custodian who turns sample over to COTP lab technician for analysis (Figure 8).

Some combinations of the above three examples will cover most situations encountered. Maintenance of the chain of custody in the lab and beyond is covered in Section IV.

F. Affixing Tag. Form CG-XXXX is similar to a shipping tag with a string tie attached at the grommet. To attach the tag to the jar, tie the string tightly just below the lid, around the jar. Place a single wrap of tape over the string and around the jar. Overlap the ends of the tape (a minimum of one inch). This will place the grommet end of the tag under the tape as well.

IV. CUSTODY IN FIELD UNIT OFFICE (COTP) OR LAB

A. Security. Samples which are held in a COTP office or a lab must be maintained in a secure space with the chain of custody intact. All samples should be in the custody of one responsible individual who should properly store the samples and insure that only he has access to them. In a COTP with a lab, the sample custodian will probably be the lab technician. In other offices the custodian will probably be the MEP Officer. In either case the custodian should maintain a log showing the samples he has received and their ultimate disposition. This custodian must also appear on the chain of custody tag.

B. Storage of Samples. Samples should be kept in a cool, dark, dry place under lock and key when no actually being worked with. A metal cabinet or locker in an air conditioned room is an adequate location provided the room, the locker or both can be locked and access limited. Ideally, all samples should be stored in a locked refrigerator at a temperature of 35-40F. Only the custodian can have access to the samples without requiring additional entries on the custody tag.

V. SHIPMENT OF SAMPLES

A. Sealing Sample. Before the sample leaves the actual possession of the sampler, the sampler must place a seal on the sample container (Figure 2). This seal must be so placed on the sample container that nothing can be added to or taken from the sample without blocking any of the information on the tag. In order to prevent the removal of the seal and its replacement by someone else, the seal must have the following written on it:

1. The date the sample was sealed. This should be the same date that the sample was collected.

2. The source of the sample.

3. The number assigned to the sample. This number is on the bottle, bottle cap, and chain of custody tag.

4. The signature (legible) of the sampler.

B. Preparation of Shipment

Prior to packaging the sample bottles for shipment, insure that the lids are tightly secured and taped. Place each sample bottle into a polyethylene bag, twisting and sealing the bag opening with tape or twist tie. Place two sample jars in a mailing tube, using the foam circular cut-outs at the bottom and top of each tube and between each sample. See Figure 9. If another shipping container is used, place sufficient packing material beneath, above, and around each sample jar to prevent the possibility of sample breakage while these sample jars are being shipped. Record list of all sample numbers being shipped in the field log book.

C. Shipment of Oil Samples

1. Mail Shipment

a. All types of petroleum samples may be air mailed, with the exception of highly volatile products with flash points below 100°F. If the flash point of the sample is between 20°F and 100°F, it may be sent by land mail. If the flash point of the sample is less than 20°F, then it must be sent by common carrier. Shipment by common carrier is discussed in D below.

b. Before the tubes are mailed, the following steps must be taken.

(1) Insure that the bottle caps are tightly sealed.

(2) Tape the cap to the bottle with two wraps of reinforced tape.

(3) Seal the bottle. (Steps 1, 2 and 3 must be done by the sampler; see Section C.)

(4) At the post office, complete the chain of custody tag which is already attached to the bottle.

(5) Place jars in mailing tube in plastic bag with foam inserts in place and seal the mailing tubes with tape.

(6) Since the chain of custody tags on the bottle are inside a sealed tube, it will be necessary to prepare a Sample Transmittal Form (Figure 3). In the case of common carrier shipment, the Government Bill of Lading (GBL) is used for this purpose. In either case, the result is a piece of paper which lists for the addressee the information he will need to determine what is to be done with the package, without opening the mailing tube and examining the sample tags. All of the entries will be entered on this form, the original and one copy will be attached to the outside of the package, one copy will be retained at the COTP (in

the case file) and the third copy will be air mailed (under separate cover) to the lab which is to be the recipient of the samples. The addressee will return the original once he receives the samples (see Figure 7).

c. Note in the field log book those sample bottles submitted for mailing. Take this book to the post office for the mail clerk's use in preparing the registered mail document.

d. Send the sample shipment "REGISTERED AIR MAIL SPECIAL DELIVERY, RECEIPT REQUESTED" if its flash point is 100°F or greater. Send it "REGISTERED SPECIAL DELIVERY, RECEIPT REQUESTED" if its flash point is between 20°F and 100°F). Affix the sender's copy of the mail receipt to the page in the log book which lists the sample's number. Also attach the receipt signed by the addressee when it is returned to you by the Postal Service.

D. Shipment by Common Carrier

a. Highly volatile products (those with flash points below 20°F) should be shipped via a common carrier such as Railway Express or United Parcel Service. A red warning label (Form S-23) must be affixed to each of four (4) sides of the shipping container.

b. Record the sample numbers and the name of the laboratory receiving the samples in the field log book.

c. Prepare a GBL insuring that the information describing the samples appears on it. All of the items found on the sample Transmittal Form must also appear on the GBL. Again, this allows the addressee to determine what is to be done with the samples without opening the package to look at the sample tags. Similarly, the original and one copy are attached to the outside of the package, while one copy is stapled to the appropriate page in the log book and a third copy is air mailed (under separate cover) to the addressee to inform him that the sample is coming. The copy which accompanies the original will be returned by the addressee, after he receives the samples (see Figure 5).

d. Take the samples to the common carrier's office and obtain a signed copy of the GBL (as a receipt) and see that it is attached to the log book as discussed in the previous paragraph.

E. Documentation for Shipment

All information, certified mail receipts, copies of GBL's, should be entered into the field log book. Forward under separate cover all pertinent information on samples being forwarded indicating operational unit's case number, serial numbers of samples collected under that case number, and manner in which samples were forwarded to the laboratory for analysis.

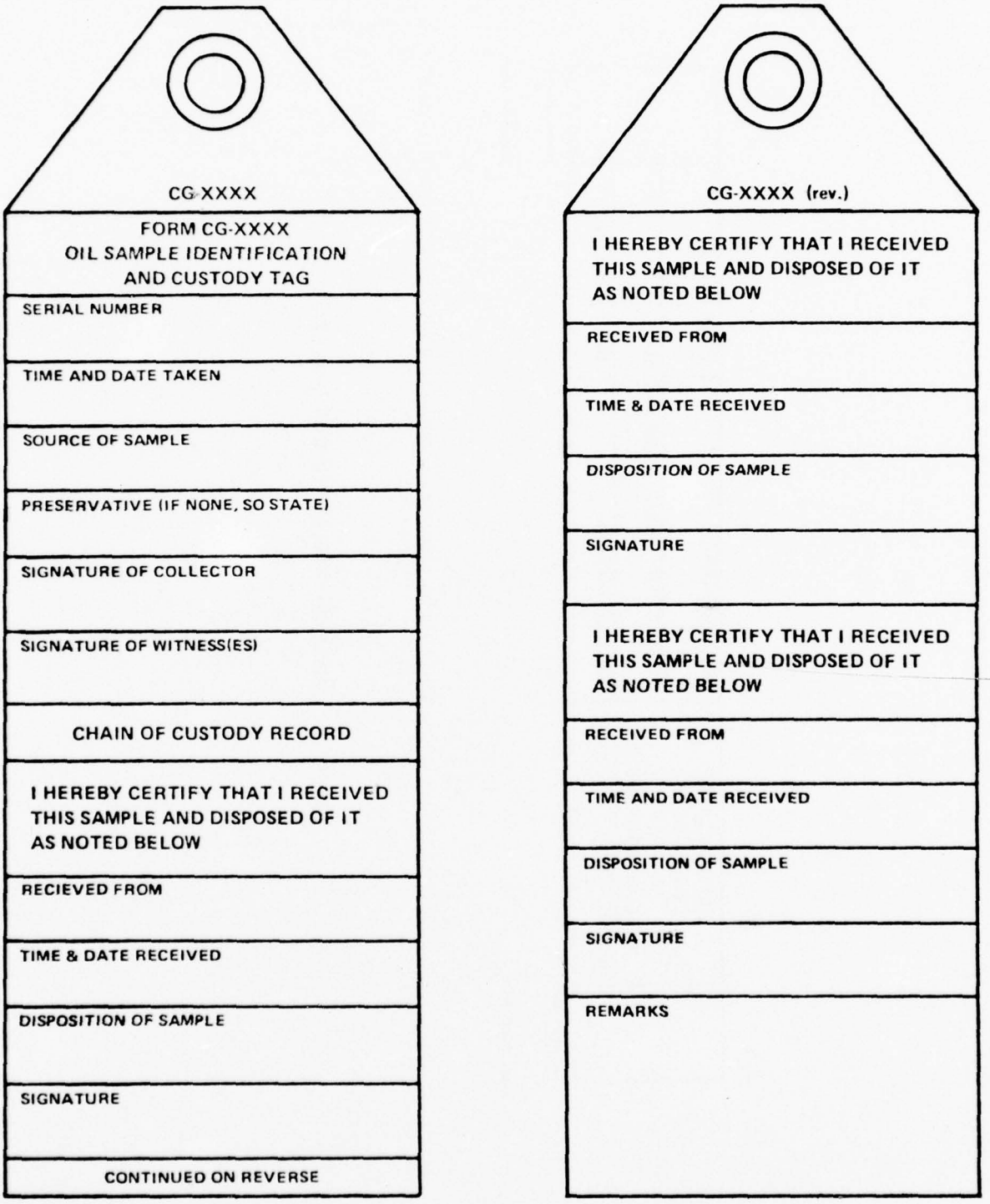


FIGURE 1. FORM CG-XXXX, OIL SAMPLE IDENTIFICATION AND CUSTODY TAG.

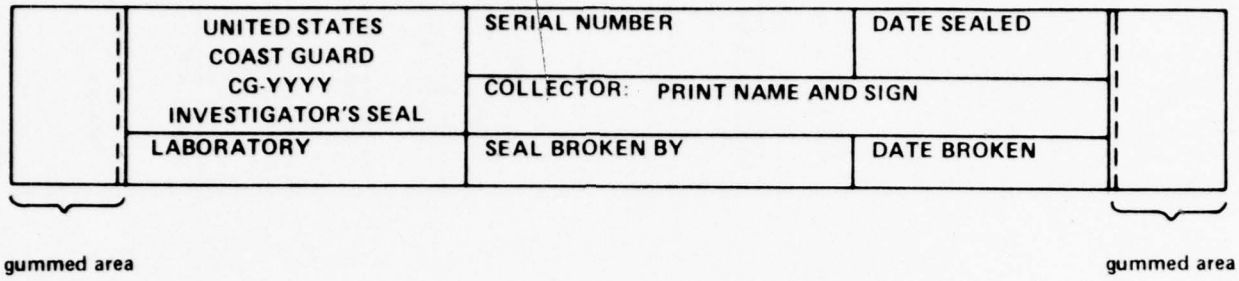


FIGURE 2. FORM CG-YYYY, OIL SAMPLE JAR SEAL.

OIL SAMPLE TRANSMITTAL FORM

To:

From

SAMPLE NO. SOURCE ANALYSIS REQUIRED

TO BE COMPLETED BY DISPATCHING PERSONNEL

Prepared by _____ Date _____ Time _____

Field Notebook (Number & Page) _____ Method of Shipment _____

TO BE COMPLETED BY RECEIVING LABORATORY

Received by _____ Date _____ Time _____
(Signature)

Originating Unit: Prepare in quadruplicate. The original and one copy will accompany sample shipment. Air mail one copy to the addressee (under separate cover). Third copy will be retained by COTP in the case file.

Receiving Unit: After receiving samples, return the original, by mail, to COTP. Retain copy for lab files.

FIGURE 3. FORM CG-ZZZZ, OIL SAMPLE TRANSMITTAL FORM

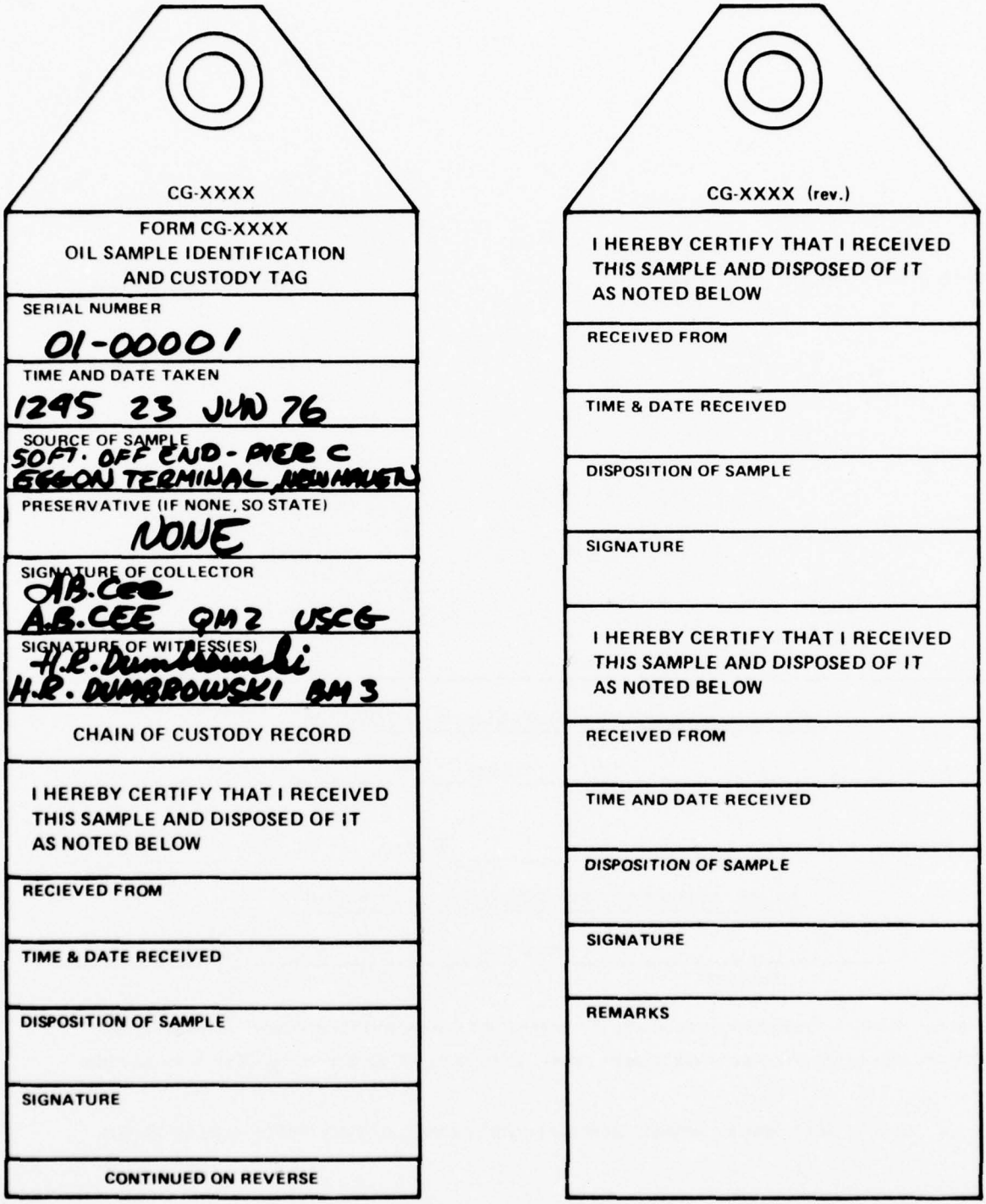


FIGURE 4. FORM CG-XXXX COMPLETED TO SHOW PROPER SAMPLE IDENTIFICATION.

Form CG-XXXXX	OIL SAMPLE IDENTIFICATION AND CUSTODY TAG CONTINUATION

Instructions for use:

1. This form is to be used only in conjunction with Form CG-XXXX and only when Form CG-XXXX has all its custody transfer blocks filled in.

2. This form should be attached to the sample jar or to the Form CG-XXXX and MUST be kept with the sample jar at all times.

Sample Jar Serial Number _____

I hereby certify that I received this sample and disposed of it as noted below.

Received from	Time and date received
Disposition of sample	Signature

I hereby certify that I received this sample and disposed of it as noted below.

Received from	Time and date received
Disposition of sample	Signature

I hereby certify that I received this sample and disposed of it as noted below.

Received from	Time and date received
Disposition of sample	Signature

Figure 5. OIL SAMPLE IDENTIFICATION AND CUSTODY TAG CONTINUATION.

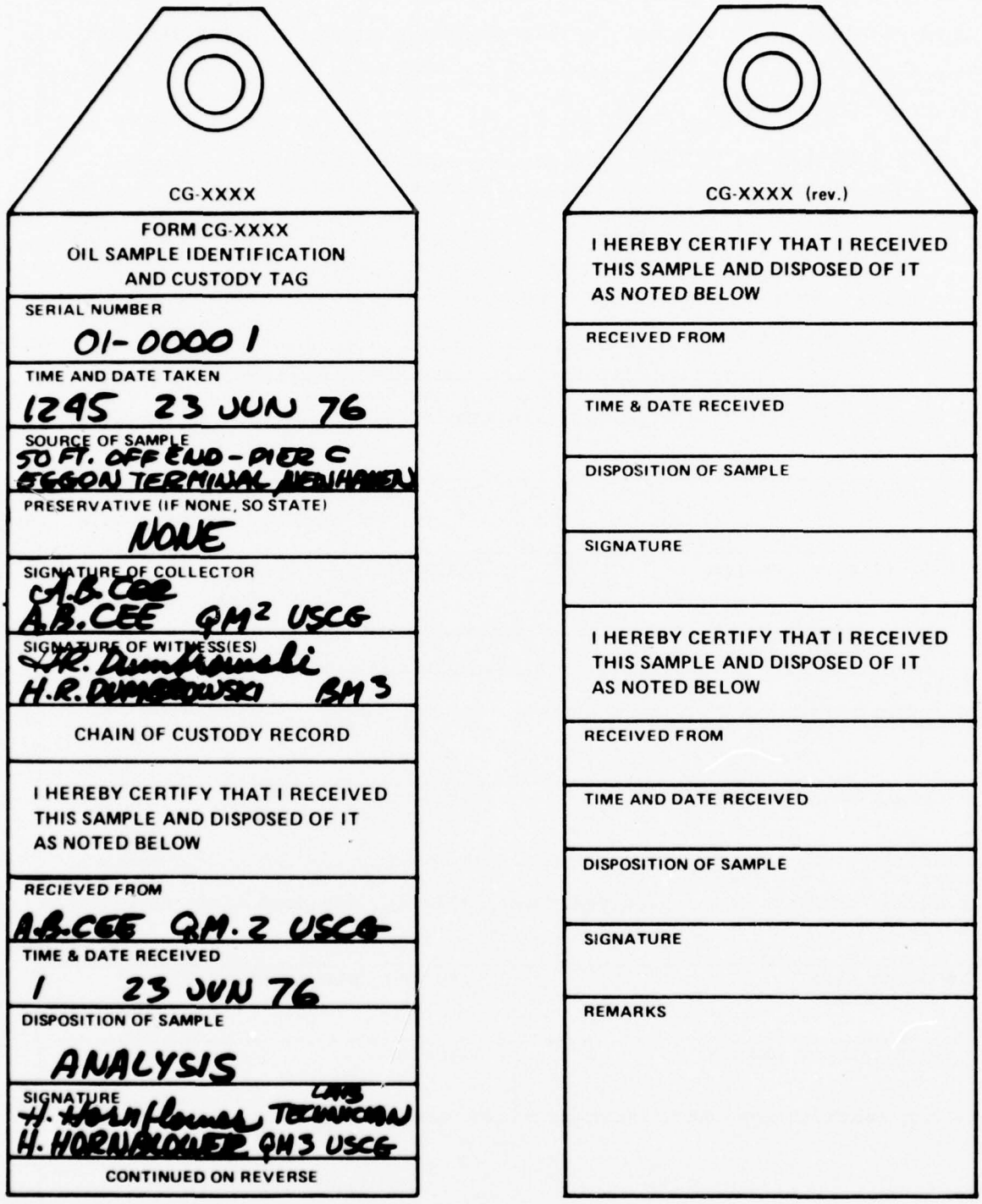


FIGURE 6. FORM CG-XXXX COMPLETED FOR SITUATION WHEN SAMPLE TAKER RETURNS TO COTP AND TURNS SAMPLE OVER TO THE LAB TECHNICIAN FOR ANALYSIS.

CG-XXXX

FORM CG-XXXX
OIL SAMPLE IDENTIFICATION
AND CUSTODY TAG

SERIAL NUMBER
01-00001

TIME AND DATE TAKEN
1245 23 JUN 76

SOURCE OF SAMPLE
50 FT. OFF END - PIER C
EGGON TERMINAL NEWHAVEN

PRESERVATIVE (IF NONE, SO STATE)
NONE

SIGNATURE OF COLLECTOR
A.B. CEE
A.B.CEE QM 2 USCG

SIGNATURE OF WITNESS(ES)
H.R. Dumbrowski
H.R. DUMBROWSKI BM3

CHAIN OF CUSTODY RECORD

I HEREBY CERTIFY THAT I RECEIVED
THIS SAMPLE AND DISPOSED OF IT
AS NOTED BELOW

RECIEVED FROM
N/A

TIME & DATE RECEIVED
1245 23 JUNE 76

DISPOSITION OF SAMPLE
SENT TO NEW YORK
FOR ANALYSIS

SIGNATURE
A.B. Cee
A.B.CEE QM 2 USCG

CONTINUED ON REVERSE

CG-XXXX (rev.)

I HEREBY CERTIFY THAT I RECEIVED
THIS SAMPLE AND DISPOSED OF IT
AS NOTED BELOW

RECEIVED FROM

TIME & DATE RECEIVED

DISPOSITION OF SAMPLE

SIGNATURE

I HEREBY CERTIFY THAT I RECEIVED
THIS SAMPLE AND DISPOSED OF IT
AS NOTED BELOW

RECEIVED FROM

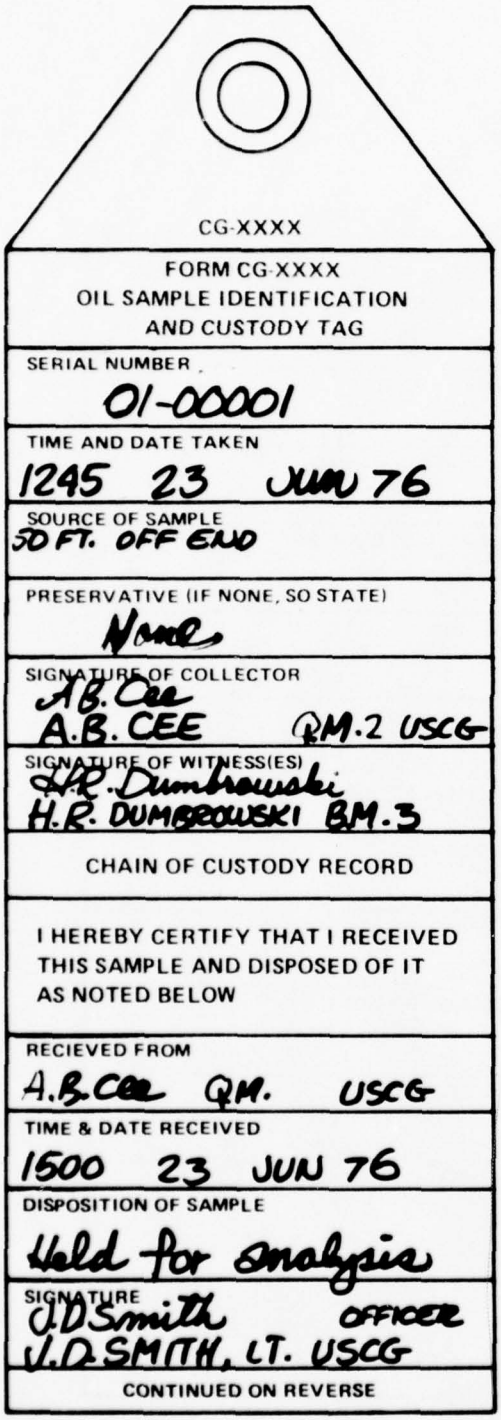
TIME AND DATE RECEIVED

DISPOSITION OF SAMPLE

SIGNATURE

REMARKS

FIGURE 7. FORM CG-XXXX COMPLETED FOR SITUATION WHEN SAMPLE TAKER RETURNS TO COTP WITHOUT LAB AND SENDS SAMPLE TO ANOTHER UNIT WITH LAB FACILITIES.



CG-XXXX

FORM CG-XXXX
OIL SAMPLE IDENTIFICATION
AND CUSTODY TAG

SERIAL NUMBER
01-00001

TIME AND DATE TAKEN
1245 23 JUN 76

SOURCE OF SAMPLE
50 FT. OFF END

PRESERVATIVE (IF NONE, SO STATE)
None

SIGNATURE OF COLLECTOR
A.B. Cee
A.B. CEE QM.2 USCG

SIGNATURE OF WITNESS(ES)
H.R. Dumbrowski
H.R. DUMBROWSKI BM.3

CHAIN OF CUSTODY RECORD

I HEREBY CERTIFY THAT I RECEIVED
THIS SAMPLE AND DISPOSED OF IT
AS NOTED BELOW

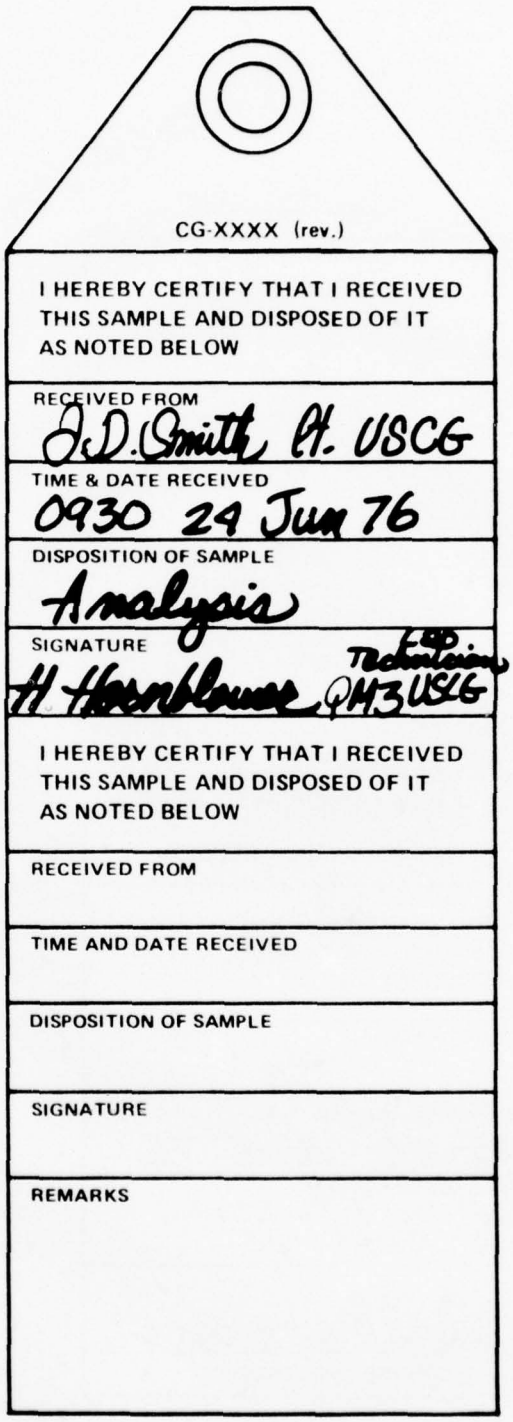
RECIEVED FROM
A.B. Cee QM. USCG

TIME & DATE RECEIVED
1500 23 JUN 76

DISPOSITION OF SAMPLE
Held for Analysis

SIGNATURE
J.D. Smith OFFICER
J.D. SMITH, LT. USCG

CONTINUED ON REVERSE



CG-XXXX (rev.)

I HEREBY CERTIFY THAT I RECEIVED
THIS SAMPLE AND DISPOSED OF IT
AS NOTED BELOW

RECEIVED FROM
J.D. Smith, Lt. USCG

TIME & DATE RECEIVED
0930 24 Jun 76

DISPOSITION OF SAMPLE
Analysis

SIGNATURE
H Hornblower Lab Technician QM3 USCG

I HEREBY CERTIFY THAT I RECEIVED
THIS SAMPLE AND DISPOSED OF IT
AS NOTED BELOW

RECEIVED FROM

TIME AND DATE RECEIVED

DISPOSITION OF SAMPLE

SIGNATURE

REMARKS

FIGURE 8. FORM CG-XXXX COMPLETED FOR SITUATION WHEN SAMPLE TAKER RETURNS TO COTP AND TURNS SAMPLE OVER TO AN INTERMEDIATE CUSTODIAN WHO LATER TURNS THE SAMPLE OVER TO THE LAB TECHNICIAN.

FIGURE 9. CERTIFIED MAIL CONTAINER FOR OIL SAMPLES.

APPENDIX C

SIMULATED WEATHERING OF OILS

I. INTRODUCTION AND BACKGROUND

The identification of the sources of oil spilled in the marine environment requires an extensive knowledge of the effects of "weathering" on the oil. Oil exposed to the natural elements undergoes component changes caused by solution, evaporation, oxidation, biodegradation, etc. To be valid, an analytical technique must either be relatively insensitive to weathering, or it must be able to "recognize" the source of an oil even after weathering.

Numerous systems have been devised to simulate natural weathering conditions. These have varied from elaborate indoor systems, such as those developed by EPA[1] (having recycling sea water, simulated wind, and simulated sunlight), to simple wading pools[2,3] and a system which confines the oil in the actual marine environment.[4]

After trying numerous weathering containers (e.g. crystallizing dishes and battery jars), the R&D Center developed a trough which circulates sea water through its six compartments. Five of the compartments contain different oils which are exposed to natural weathering; the sixth compartment contains sea water used as a control. A screen is placed above the trough to break the force of raindrops and thus avoid contamination caused by splashing oil into adjoining compartments.

II. SCOPE

A suitable weathering trough has been designed in which all types of oil can be artificially weathered under conditions approximating "natural" conditions. An adjunct to the trough is a weather station which is equipped to take the requisite data during a given weathering period. Weathering in the trough can be performed at all times of the year except when ice forms on top of the flowing salt water.

III. SUMMARY OF METHOD

Oil samples are placed in the trough compartments and are sub-sampled at predetermined intervals (daily in 4 day studies and 3 day intervals in two week studies). The corresponding weather data are recorded and the oil samples are sent to the various analytical groups for study.

IV. APPLICATION

The following procedure has been applied to a wide variety of oil types (See Table I for list of oil samples weathered.)

Several oils were weathered in replicate (e.g. studies 1-3, 5 and 7 Table I). Continued weathering studies with replications at various seasons

will reflect those changes due to external weathering conditions.

V. APPARATUS AND MATERIALS

The apparatus consists of:

(1) A weathering trough as shown in Figures 2 and 3

(2) Cleaning materials: CRC Chemical spray, Spic and Span, brushes, sponges, hot water spray

(3) Teflon sampling strips (2" x 3")

(4) Glass jars (8 oz.) with Teflon-lined caps

(5) Weather station, including:

(a) Pyroheliometer

(b) Anemometer

(c) Atmospheric data recorder (temperature, relative humidity and barometric pressure)

(d) Rain gauge

VI. DETAILED PROCEDURE:

A. Oil weathering

1. The trough is thoroughly cleaned as follows:

(a) Drain
(b) Wet with hot water
(c) Spray with CRC chemical spray
(d) Brush walls down with Spic and Span
(e) Repeat steps b-d twice more
(f) Wipe down
(g) Rinse with fresh hot water

2. Turn on sea water pump.

3. Fill the trough with fresh sea water. Caution: The inlet hose should be at the same level as the overflow. If not, a power failure will cause siphoning of water and oil from trough.

4. Place 25 ml samples of oil into compartments 1-5, taking precautions necessary to avoid having the oils flow under the compartment dividers. The sixth compartment is kept clear as a control to note whether any cross-contamination is occurring.

5. Place screen 5" above the surface to break up rain droplets.

6. Weather the prescribed interval (1 day for 4 day study, 3 days for 2 week study).

7. Sample after the proper interval by inserting perforated 2" x 3" Teflon strips through the surface of the oil slick, allowing the oil to adhere to the strip surface. The strips are placed in a glass jar (8 oz.) with a Teflon-lined cap.

8. Sample the control water everytime the oil is sampled.

9. All samples are carefully labelled.

10. The samples are stored in refrigerators at 40-42°F until they are analyzed.

B. Weather Data

Concurrently with our weathered oil studies we record atmospheric data. Temperature, pressure, wind speed and direction, humidity, precipitation, solar insolation, and cloud cover were monitored. A sample environmental data sheet is shown in Figure 1 which indicated the general conditions during each weathering period.

VII. RESULTS AND DISCUSSION

The weathering trough developed at the CG R&D Center has proved to be excellent for its intended purpose. It permits weathering of adequate amounts of oil for laboratory analysis under conditions closely approximating those on open water. The trough is relatively easy to clean and has held up for almost two years without any maintenance other than annual repainting. However, two problems have been encountered: the growth of algae tends to plug the filter and screen in warm weather; hard rainstorms cause splashing of oil from one compartment to a neighboring compartment.

The first problem can be managed by frequent cleaning and backflushing the intake hose with hot water (usually between weathering studies) - weekly during the summer.

Placement of a screen over the trough corrected the rain-caused splashing problems. It did, however diminish the incident radiation (measured in Langleys($g\ cal/cm^3$) by about 25%. This was compensated by placing a similar screen a like distance (5") over the pyroheliometer.

Weather conditions vary during the day and from day to day. Noticeable differences in weathering are expected at different times of year, and between sunny and overcast periods. We arbitrarily chose to use hourly average temperatures and the total radiation flux over the period of weathering.

Weather data measurements are rather precise. Weathering changes in oils are dependent on so many factors that correlations with data measurements are relatively crude. Therefore, we sought to simplify the task of obtaining solar radiation by developing a method for estimating daily solar radiation

rather than laborious manual integration of pyroheliometer data. The intent was to derive intensity tables related to cloud cover based on latitude, the declination of the sun, the seasonal variance of the solar constant (average intensity of the sun above the atmosphere), scattering, absorption and diffraction characteristics of the atmosphere, degrees of precipitation, and localized albedo (fraction of reflected radiation). Average sunlight intensity tables have been generated for the Groton, CT area pertinent to summertime conditions. Tables pertaining to local wintertime conditions are being developed. These tables will permit a reasonable estimate of the daily radiation. Furthermore, when completed, this method of estimating radiation can be transferred to any latitude and longitude. In conjunction with local weather data, this method could be used to compute sunlight intensity during an oil spill and thus enhance the accuracy of oil source identification.

Table II shows the average sunlight intensity in the Groton area during the summer months. The data presented show intensity throughout the days having a 90-100% cloud cover. (Other tables have been developed for bright sunlight and varying degrees of cloud cover). These data are presented as a function of the amount of rainfall and the period of the day. The daylight hours are arbitrarily divided into 16 parts. The first and last periods (1 and 16) are the beginning and end of the day, resp., the middle (periods 8 and 9) shows the most intense radiation as expected for noontime.

Total radiation for a weathering study is the sum of radiation for all daylight periods. For example, a 24 hour weathering study with 90% cloud cover and 0.20" rainfall has an estimated total radiation corresponding to the sum of all 16 periods of the day, i.e., twice the sum of the 0.20" column in Table II. If there were 90% cloud cover for half the weathering time and no cloud cover for the remainder, it would be necessary to use a second table for that portion of the time with 0% cloud cover.

VIII CONCLUSIONS

The R&D Center's oil weathering procedure satisfactorily simulates natural weathering. It provides the necessary samples for studying the effects of weathering on the analytical methods of "oil fingerprinting".

The method was so successful that a second trough has been constructed which will permit weathering seven additional samples.

A new method has been developed for estimating daily solar radiation from estimated cloud cover, rainfall and tabulated radiation values. The technique could be extrapolated to geographical areas where oil spills occur.

REFERENCES

1. John W. Frankenfeld, "Weathering of Oil at Sea", Final Report, Contract No. DOT-CG-23,035-A (Report No. CG-D-7-75), pp 16-22, Sept 1973.

2. Martin E. Scolnick, Arthur C. Scott, Michael Anbar, "Methods of Identifying and Determining Source and Age of Petroleum Found in the Marine Environment," Final Report, Contract No. DOT-CG-22,996-A (Report No. CG-D-61-75), pp 44-47, June 1974.

3. John D. Johnson, Herbert R. Gram, "Discrimination of Waste Oils by Micro Emission Spectrochemical Analysis", Phase I Final Report, Contract No. DOT-CG-33-185A Report No. CG-D-21-75), pp 19-22, June 1974.

4. Mark Ahmadjian, Carl D. Baer, Patricia F. Lynch and Chris W. Brown, "Experimental Conditions for Matching Laboratory and Marine Weathering of Petroleum by Infrared Spectroscopy", Paper 457 26th Pittsburgh Conference, March 1975. (Work done under U.S.C.G. Contract DOT-CG-81-74-1099.)

TABLE I. OIL SAMPLES WEATHERED.

Study 1, 2, 3. March 4, 1974 - March 22, 1974

Gulf 080 Fuel Oil #2
Gulf 020 Low Cost Lubricating Oil
Conoco 020 Motor Oil
Shell 015 Marine Lubricating Oil
Shell 019 Industrial Lubricating Oil

Study 4. March 25, 1974 - March 29, 1974

Getty 002 Fuel Oil #6
Gulf 058 Fuel Oil #5
Union 004 Fuel Oil #4
Conoco 016 Fuel Oil #2
Atlantic Richfield 016 Fuel Oil #1

Study 5. April 1, 1974 - April 5, 1974

Exxon 006 Marine Diesel Fuel
Exxon 024 Fuel Oil #2
Exxon 064 Diesel Fuel Oil #2
Amoco 035 Fuel Oil #2
Atlantic Richfield 022 Fuel Oil #2

Study 6. April 8, 1974 - April 12, 1974

Gulf 034 Fuel Oil #6
Chevron 008 Fuel Oil #4
Sunoco 009 Bunker C Fuel Oil
Exxon 017 Fuel Oil #5
Shell 013 Marine Lubricating Oil

Study 7. April 15, 1974 - April 19, 1974

Exxon 006 Marine Diesel Fuel
Exxon 024 Fuel Oil #2
Atlantic Richfield 016 Fuel Oil #1
Exxon 064 Diesel Fuel Oil #2
Amoco 035 Fuel Oil #2

(continued)

TABLE I (continued)

Study 8. April 23, 1974 - April 26, 1974

Independent 015 Fuel Oil #6
Exxon 008 Fuel Oil #6
Conoco 017 Fuel Oil #6
Exxon 007 Fuel Oil #6
Amoco 025 Fuel Oil #6

Study 9. May 20, 1974 - May 24, 1974

Atlantic Richfield 016 Fuel Oil #1
Amoco 035 Fuel Oil #2
Union 004 Fuel Oil #4
Exxon 017 Fuel Oil #5
Getty 002 Fuel Oil #6

Study 10. May 24, 1974 - June 14, 1974

Chevron 011 Fuel Oil #4
Gulf 031 Fuel Oil #2
Amoco 044 Fuel Oil #4
Exxon 079 Fuel Oil #4
Exxon 011 Fuel Oil #2

Study 11. August 3, 1974 - August 22, 1974

Exxon 016 Fuel Oil #4
Amoco 024 Fuel Oil #5
Amoco 046 Fuel Oil #5
Gulf 032 Fuel Oil #5
Exxon 045 Fuel Oil #4

Study 12. August 24, 1974 - September 12, 1974

Amoco 019 Diesel Lubricating Oil
Amoco 020 Marine Lubricating Oil
Gulf 018 Premium Hydraulic Oil
Amoco 047 Turbine Jet Fuel
Mixture: Amoco 019 Diesel Lubricating Oil
and Atlantic Richfield 013 Diesel Fuel Oil #2

Study 13. February 3, 1975 - February 7, 1975

Exxon 069 Middle East Crude
Gulf 008 Middle East Crude
Conoco 012 Alaskan Crude
Gulf 011 Venezuelan Crude
Exxon 033 Venezuelan Crude

TABLE II. AVERAGE SUNLIGHT INTENSITY WITH 90-100% CLOUD COVER, GROTON, CT, SUMMER WITH MEDIUM OVERCAST AND LIGHT (0-0.10"), MEDIUM (0.10-0.30") AND HEAVY (0.30") RAIN.

	INTENSITY (LANGLEYS)						
Period of day \ Rainfall (in)	0.00	0.05	0.10	0.15	0.20	0.25	0.30
8,9	10.5	9.8	8.9	7.6	6.1	5.0	3.1
7,10	9.2	8.6	7.8	6.6	5.4	4.3	2.7
6,11	8.2	7.6	6.9	5.8	4.8	3.7	2.4
5,12	7.4	6.8	6.2	5.2	4.3	3.2	2.2
4,13	6.7	6.2	5.7	4.8	3.9	2.8	2.0
3,14	6.3	5.8	5.3	4.5	3.6	2.5	1.8
2,15	6.1	5.6	5.1	4.3	3.4	2.3	1.7
1,16	6.0	5.5	5.0	4.2	3.3	2.2	1.6

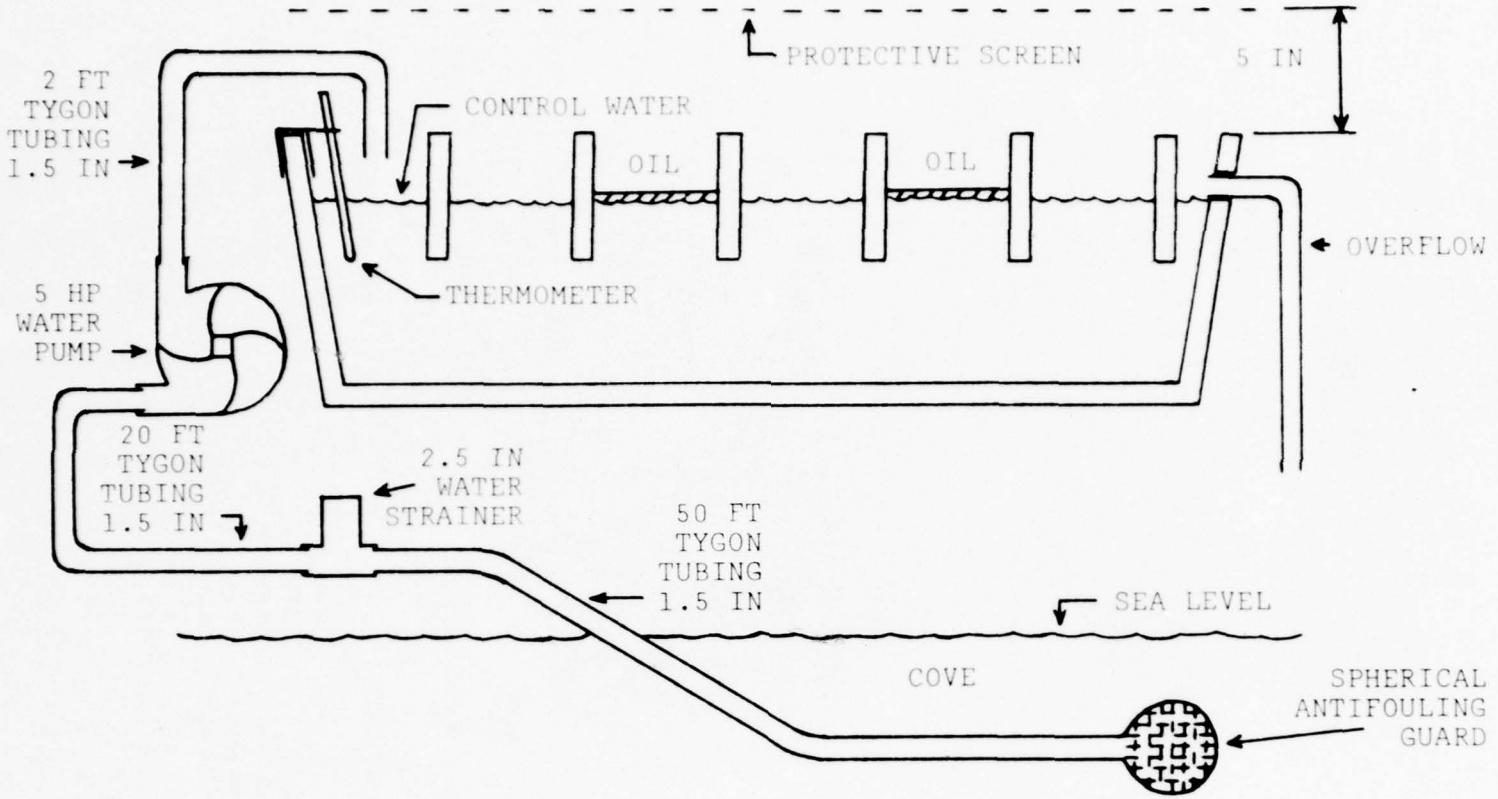
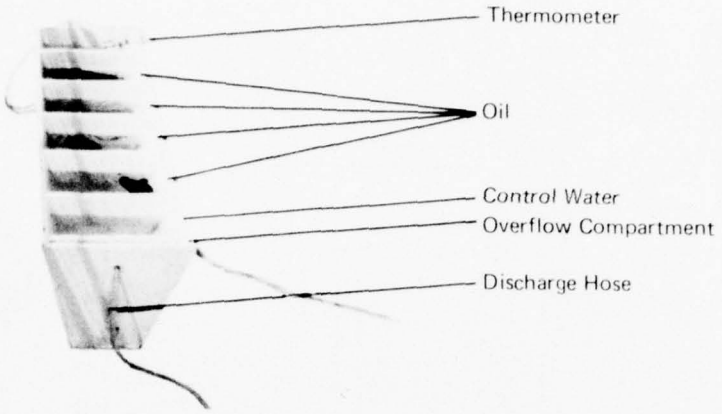
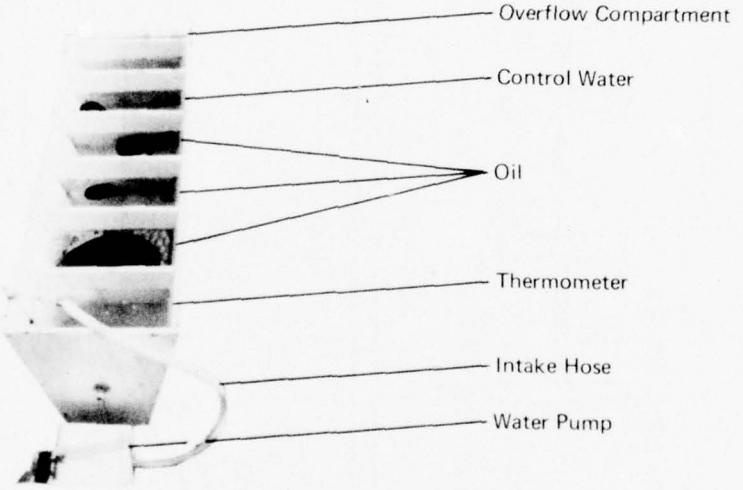


FIGURE 1. SAMPLE ENVIRONMENTAL DATA SHEET.



End View From Outlet Side



End View From Intake Side

DIMENSIONS

Length		6 Ft.
Width	(top)	2 Ft.
	(bottom)	1 Ft.
Depth	(overall)	2 Ft.
	(dividers)	9 In.

MATERIALS OF CONSTRUCTION

Exterior Marine Plywood 1.5 In.
Interior Fiberglassed With
9 Yds. Of Glass Cloth 44 In. Wide
And 1 Gal. Of Polyester Resin
With Hardener.
Entire Trough Painted With White
Oil Resistant Monopoxy Paint.

FIGURE 2. WEATHERING TROUGH.

WEATHERING STUDY No. 10 DATE MAY 24 TO JUNE 14, 74

SAMPLE IDENTIFICATION No. CH 011 PC

DAY No. 3
AVERAGE AIR TEMP. 12.5°C / 54.5°F
AVERAGE SEAWATER TEMP. 13.4°C / 56.2° F
RADIATION FLUX 1213,79 l
PRECIP. AMOUNT 0.11 No. HRS. 3.5

DAY No. 7
AVERAGE AIR TEMP. 12.4°C / 54.3°F
AVERAGE SEAWATER TEMP. 14.8°C / 58.7° F
RADIATION FLUX 2131.94 l
PRECIP. AMOUNT 0.37 No. HRS. 10.5

DAY No. 10
AVERAGE AIR TEMP. 13.0°C / 55.4°F
AVERAGE SEAWATER TEMP. 15.1°C / 59.2° F
RADIATION FLUX 3095.11 l
PRECIP. AMOUNT 1.17 No. HRS. 17.5

DAY No. 14
AVERAGE AIR TEMP. 13.5°C / 56.3°F
AVERAGE SEAWATER TEMP. 15.3°C / 59.6°F
RADIATION FLUX 5129.44 l
PRECIP. AMOUNT 1.17 No. HRS. 17.5

DAY No. 17
AVERAGE AIR TEMP. 14.2°C / 57.6°F
AVERAGE SEAWATER TEMP. 16.7° C No. HRS. 62.1°F
RADIATION FLUX 6539.04 l
PRECIP. AMOUNT 1.17 No. HRS. 17.5

FIGURE 3. WEATHERING TROUGH CONFIGURATION.

APPENDIX D

OIL SPILL IDENTIFICATION BY GAS CHROMATOGRAPHY

1.0 SCOPE

1.1 The recommended gas chromatographic analysis provides a means to "fingerprint" oil samples. Chromatograms of an oil spill sample and potential sources are compared to identify a possible source of the spill.

2.0 SUMMARY OF METHOD

2.1 Using gas chromatographic separation and quantitative detection of eluted components, the analyst obtains a chemical fingerprint for an oil sample. The detection is accomplished with use of two different detectors simultaneously: detection by flame ionization (FID) measures all organic components, and flame photometric detection (FPD) measures the presence of sulfur-containing components. The detector responses are recorded by a strip-chart recorder to give a visual representation (chromatogram) and are simultaneously analyzed by a dedicated data processing system which provides an analysis report of measured peak areas, retention times, relative retention times and other useful information.

2.2 The comparison of chromatograms allows the analyst to determine the similarities and differences between oil samples. With these comparisons and further knowledge of the source of the samples and any other information, the analyst is able to determine the source of a spill.

3.0 APPLICATIONS

3.1 This method is applicable for any petroleum or non-petroleum oil with components having boiling points over 287°C (reference point: n-hexadecane). Light and heavy distillate oils, bunkers, lubricating oils and crude oils can be fingerprinted using this technique. Oils consisting only of low boiling components (boiling point below n-hexadecane) such as gasoline cannot be compared using this method although other gas chromatographic techniques <u>can</u> be used for gasoline; see ASTM D3328-74aT.

3.2 This method may be applied to samples that are neat (unaltered), thin films collected from a water surface, samples scraped from a solid substrate (e.g. sand or vermiculite) and emulsified oil. Samples contaminated by spill control chemicals, by improper sample containers (plastic) or collected in an improper manner may give incorrect results.

4.0 APPARATUS AND MATERIALS

4.1 <u>Instrument</u>

The instruments recommended for use are the Perkin-Elmer Models 900, 910 or 3920 gas chromatographs with flame ionization and flame photometric detectors and dual amplifiers. The detectors are interfaced to a Perkin-Elmer PEP-1 Data Processor. Other equivalent

instruments could be used in place of these. The instrument used should have a pressure regulator (0-15 psig) for carrier gas flow control, detector gas flow controls, a 394 nm bandpass filter for the flame photometric detector, a temperature-programmable oven capable of operating in the range of 75-250°C, a heated-block injection zone, a flow controller with appropriate piping for carrier gas make-up flow, an effluent splitter capable of a 1:2 split ratio, a dual-pen strip-chart recorder having a 0-1 millivolt full-scale deflection range, an output filter and an electronic linearizing circuit for the flame photometric detector.

4.2 Capsule Injection System

The Perkin-Elmer MS-41 and AS-41 sampling accessory systems provide a means to introduce aluminum-encapsulated samples into the chromatograph in a reproducible manner (manually and automatically, respectively). The encapsulated-sample injector system will not introduce the ghost peaks due to septum bleed encountered with normal septum injector systems and is the only automated system which would not require the injection of large volumes of solvent into the column that could affect the column performance.

4.3 Column

4.3.1 A 0.50 mm x 16 m (0.02in.x 50 ft.) support-coated open-tubular (SCOT) column with OV-101 stationary phase is used. (This column is only available commercially from Perkin-Elmer Corp. at this time.)

4.3.2 A similar SCOT column employing Dexsil 300 stationary phase is also used. This liquid phase does not give the separation of pristane and phytane which is informative, but it does allow operation up to 350°C and therefore provides additional information for higher - boiling oils. The Dexsil 300 phase is used only for heavy oils.

4.4 Accessories

Instrument accessories include two foot-switch assemblies to start the detector interfaces and oven programming (not necessary with use of AS-41 injector system), centrifuge capable of 1000 RCF, SGE Model 0.5B micro-syringe (0-0.5 µl), Hamilton syringe cleaner and two-stage gas regulators appropriate for the types of gas cylinders that are used.

4.5 Expendable Materials

Expendable materials include 50 ml glass centrifuge tubes, 50 ml glass Erlenmeyer flasks, Pasteur pipettes, anhydrous magnesium sulfate, pentane (chromatoquality), glass funnels and Schleicher and Schuell No. 576 filter paper (or equivalent). Gases used include helium (high purity grade), nitrogen (prepurified grade), air (zero grade) and hydrogen (prepurified grade) and should be in Matheson size 1A cylinders (or equivalent, approximately 200 cubic feet). Hydrogen and air may also be supplied by laboratory generators. Gas filter-absorbent traps should be installed on all gas lines to the chromatograph as close to the instrument as is possible.

5.0 ANALYTICAL PROCEDURES

5.1 Column

The column is the most important part of the chromatographic system because it is where the separation of the oil components takes place; the separation of the oil components in a specific and repeatable manner is the basis for this technique. New columns are not immediately ready for use and must be conditioned prior to use. The importance of proper conditioning cannot be overemphasized as this initial step can be significant in the column performance. A recommended conditioning procedure is outlined below; conditioning times are minimum times at a given temperature and may be as long as 16 hours in overnight operation.

a. Connect the head of the column to the inlet fitting in the oven, but do not connect the effluent end of the column.

b. Adjust the carrier gas pressure regulator to 2 psig and allow the column to purge itself of entrapped air for several minutes.

c. Set the initial oven temperature at 75°C. Raise the oven temperature at 1°/min to 175°C and hold at final temperature for 6 hours.

d. Raise the oven temperature at 0.5°/min to 225°C for OV-101 (300°C for Dexsil) and hold for 10 hours.

e. Raise the oven temperature at 0.5°/min to 250°C for OV-101 (325°C for Dexsil) and hold for 10 hours.

f. Raise the oven temperature at 0.5°/min to 275°C for OV-101 (350°C for Dexsil) and hold for 16 minutes.

g. Recycle the instrument to an initial temperature of 75°C. Inject 0.2 µl of a light fuel oil and temperature program the oven at 8°/min to 250°C for OV-101 and hold for 32 minutes. Repeat this step one time. (For Dexsil program to 325°C and hold for 16 minutes.)

h. Adjust the carrier gas pressure control and give a 2 ml/min gas flow at 250°C (approximately 2.1 psig).

i. Recycle the instrument to 75°C. Connect the column to the effluent fitting in the oven. Temperature program the instrument at 8°/min to 250°C and hold for 32 minutes. (Program at 8°/min to 325°C and hold for 32 minutes for Dexsil column.) If the recorder should show spurious peaks, an unstable baseline or a column bleed in excess of 10% of recorder full-scale deflection (range 100x, attenuation 1x), then the conditioning sequence should be repeated.

j. A light fuel oil having a mixture of compounds that will give 20-80% full-scale recorder deflection by both detectors is useful for monitoring the performance of the chromatographic system and is used as a standard reference oil. The standard oil should be preserved in a manner which will minimize alterations; store the oil in a sealed container that is kept out of direct sunlight and preferably in a refrigerator. The column should be able to resolve the isoprenoid compounds pristane and phytane from n-heptadecane and n-octadecane respectively; the pristane should be resolved to the extent of 80%

and the phytane to 100%. (The Dexsil column will not resolve pristane and phytane.) A library of chromatograms for a standard oil, run over a period of time, on the same column, is valuable in assessing changes in the column and chromatographic system performance. Replicate samples of this oil should be run at intermittent periods and compared to themselves and to previous chromatograms. Inconsistencies should be noted and evaluated for reduced performance; see Section 6.4.4 for specific steps to be taken in evaluating these changes.

k. A mixture of n-alkanes (n-hexadecane, n-octadecane and n-eicosane),"resolution mixture", is used to provide a quantitative measure of the column performance. Performed on a routine basis, the chromatographer is able to ascertain whether the system is operating within specifications. Details concerning the use of the resolution mixture are in section 6.5.

5.2 Chromatograph

Operation and features of the chromatograph and data processor are given in the manufacturer's instructions. Once the chromatograph has been placed in operation it is recommended that the power be left on except for repairs and long periods of non-use (greater than one week). It is recommended that the oven be left on overnight so that thermal changes of the instrument and column are minimized.

5.2.1 Operating parameters are listed in Table 1. It is vitally important that gas flows to the detectors be optimized. This optimization is accomplished by introducing an organic sulfur-containing compound, namely, thiophene, into the make-up carrier gas. A device called a "carrier gas conditioner" is installed for this purpose and at room temperature provides an adequate amount of the test compound to optimize the performance of both detectors. Optimize the gas flows by first adjusting the air flow with a hydrogen pressure setting of 40 psig, then optimize the hydrogen flow. The recorder pen deflection should not be off scale on range 100x with a maximum attenuation of 32x for either detector. (The adjusted flows will not be the same as recommended by the manufacturer for packed column operation!)

5.3 Data Processor

The data processor is an integral part of this system and is useful to the analyst for checking system performance and for sample matching.

5.3.1 The Sequential Sampling Program is recommended for use with the data processor. This program is necessary for use with the AS-41 injection system and can also be used with the manual injection system; it has the advantage of rapidly resetting the initializing conditions and affixing a name to the printed-out data analysis without having to use the teletype terminal to initiate each chromatographic run.

5.3.2 Printing of relative retention times in the analysis report can be performed by the data processor and is useful in the comparison of data; details for computation of these values are in the manufacturer's instruction manual.

5.3.3 Automatic peak file deletion is necessary after the analysis report is printed out, when operating the system with a AS-41 injector over long periods of unattended operation, otherwise the computer memory will be exceeded. Details for this step are in the manufacturer's instruction manual.

5.3.4 The threshold settings for the data processor are not determined as recommended by the manufacturer because the software program was not specifically designed for oil analysis. An area threshold of 1000 and baseline threshold of 100 are used; these values will ignore many small peaks, but will allow the most repeatable area integrations to be achieved.

5.3.5 The software allows forced base points to be designated. These should be distributed throughout the regions of useful information: C16 - C23 for OV-101 columns, C16 - C28 for Dexsil 300 columns.

5.4 Sample Preparation

Samples are deasphalted prior to analysis to prolong the usefulness of the column. Use the following procedure:

5.4.1 In a 50 ml centrifuge tube, dissolve 1 ml of the oil sample in 40 ml of pentane. Add approximately 1 g of anhydrous magnesium sulfate to the solution and mix well; the magnesium sulfate removes any water present in the sample. Allow the sample to sit for 30 minutes.

5.4.2 Centrifuge the sample at 1000 RCF for 20 minutes; both the magnesium sulfate and the insoluble asphaltenes will be precipitated on the bottom of the tube.

5.4.3 Decant the supernatant liquid through a funnel with filter paper, into a 50 ml Erlenmeyer flask. Strip the pentane from the sample by passing a stream of dry nitrogen gas over the top of the sample. The stripping procedure also removes many of the oil components with boiling points below n-pentadecane. Alternatively, the supernatant liquid can be decanted or carefully withdrawn with a pipet for subsequent removal of pentane. This eliminates the dehydration step with magnesium sulfate and is suitable if the deasphalted samples are not to be analyzed by infrared spectroscopy.

5.4.4 Load 0.2 µl of sample into an aluminum sample capsule with a syringe and seal the capsule. This sample volume is sufficient for most oils having low concentrations of sulfur. Oils having very little or no sulfur components give chromatograms which may be useful for comparison in distinguishing those oils from similar ones with more sulfur present.

5.4.5 Occasionally the analyst will be presented with a sample container having less than 1 ml of oil available for the deasphalting procedure. In such instances the sample can be transferred directly to the sample capsule. When only minute amounts are present, the sample should be dissolved in pentane in the sample container. An aliquot of the pentane solution is transferred to a sample capsule; the aliquot size for a good chromatogram will be dependent on the concentration of the pentane solution. Evaporate the pentane before sealing the capsule.

5.5 Sample Analysis

5.5.1 Adjust gas flows to the specified values (Table 1), zero the amplifiers and ignite the detectors; the manufacturer's instruction manual describes all steps necessary to prepare the instrument for sample analysis.

5.5.2 The first chromatographic run each day should be a blank injection. Program the oven temperature to 250°C at 16°/min and hold at that temperature for at least 16 minutes. Observe the detector response on the strip chart recorder; this signal is referred to as column bleed. At amplifier settings of range 100x and attenuation 8x, the signal should level off at no greater than 5% full-scale pen deflection. If the detector response is less than 5%, recycle the instrument to 75°C. If the detector response is greater than 5%, then maintain the oven at 250°C until the response becomes satisfactory. Should the response continue unsatisfactorily, it may be an indication that the column is no longer useful and needs to be replaced. The purpose of this first run is to check the column for contamination and to observe the column bleed at the maximum oven temperature.

5.5.3 The second chromatographic analysis each day should be the resolution mixture. Perform the analysis by the standard procedure used for oil samples and compare the results to previous runs of the standard oil in the manner described in Section 6. The purpose of this step is to insure that the chromatographic system is functioning properly before beginning the day's work.

5.5.4 Initialize the data processor interfaces as outlined in the manufacturer's instruction manual. Set the chromatograph operating parameters as listed in Table 1. Make sure that the autosampler is in the PEP operation mode.

5.5.5 The chromatograph is now ready for sample analysis. Inject the sample. Start the oven programming, detector interfaces, and strip-chart recorder. The hold-time at the final oven temperature will be dependent upon the composition of each oil; maintain the final oven temperature until all of the components have eluted from the oil and the detector response is the same as that of the blank run. A final hold-time setting of 32 minutes is sufficient for most light-to-medium distillate oils and some crude oils; a setting of 64 minutes is necessary for most heavy oils and some crude oils.

5.5.6 Recycle the instrument to the initial settings when all components have been eluted. The chromatograph will turn on a "ready" light when it can accept another sample. Repeat the analysis steps for other samples.

5.5.7 When the data processor has completed collecting and analyzing detector responses, it will print out an analysis report at the teletype; simultaneously, it can also provide the same information on punched paper tape. After reporting the analysis information, the data can be either stored as a data file or erased from the memory circuits. Eventually the data processor memory capacity will be consumed if all data files are stored and the analyst must erase files from memory to provide more available space. The manufacturer's instruction manual explains how to select file storage and deletion commands, and how to check on the amount of available space in the memory circuits.

TABLE I. STANDARD OPERATING CONDITIONS.

	Liquid Phase	
	OV-101	Dexsil 300
Column length, m (ft)	16 (50')	16 (50')
Column I.D., mm (in)	0.50 (0.02")	0.50 (0.02")
Initial Column temperature, °C	75	75
Initial Hold	0	0
Final Column temperature	250	325
Final Hold	32 (light-medium distillates)	32
	64 (heavy oils, some crude)	
Oven programming rate, C°/min	8	8
Carrier gas flow rate*, ml/min	2	2
Carrier gas make-up flow rate, ml/min	30	30
Injection port temperature, °C	250	250
Detector manifold temperature, °C	275	350
Amplifier range, FID, FPD	100x, 100x	100x, 100x
Amplifier attenuator, FID, FPD	8x, 4x	8x, 4x
Detector gas flows**, ml/min		
Hydrogen: FID, FPD	35, 134	35, 134
Air: FID, FPD	667, 91	667, 91
Output filter	Medium	Medium

*measured at 250°C
**approximate flow rate, detector response must be optimized

5.5.8 Instrument shut-down will normally consist of shutting off the detector flames, closing the valves on the gas cylinders, and bleeding off the hydrogen and air pressures. The oven should be left on at 75°C with normal carrier flow and the make-up carrier gas can be stopped. For extended shutdown periods (in excess of three days) the oven should be shut off, but the power switch left in the "on" position. Unless there is a need to shut all power off (such as making repairs to the chromatograph), the power to the data processor and all interfaces should always be left on.

6.0 INTERPRETATION OF DATA

6.1 Principle of Fingerprinting

Petroleum is a mixture of thousands of different molecules. This complexity is due to the process of formation of the oil; different starting materials and different temperature and pressure conditions during this process result in a varied mixture of oil components. Differences in the composition of oils can be very dramatic. Refined oils are "cuts" of crude oils and can be differentiated from their source oil because their composition is different. When two oils are mixed together they have a new composition. This variability in composition gives each oil a uniqueness which accounts for its characteristic fingerprint. The analyst compares the fingerprints of oils to match those oils with identical compositions.

6.2 Chromatogram Characteristics

6.2.1 The chromatograms have several features which the analyst utilizes for the comparison of samples in much the same way as the criminologist examines a human fingerprint to determine unique characteristics. Sample chromatograms are shown in Figures 1 and 2 for the two column types. The significant point to note is that pristane and phytane are not resolved by the Dexsil 300 column and result in larger-than-normal C17 and C18 peaks.

6.2.2 Retention Time

From a given gas chromatographic column with a given carrier gas flow rate and a given oven temperature program rate, an oil component takes a fixed period of time from the start of the analysis until it is eluted from the column and measured by the detectors. This period of time is called the retention time and is specific for each component. Since precise injection and chromatograph-start synchronization is difficult, retention times may vary slightly. However, relative retention times (retention times relative to the elution time of a specific component) compensate for this and make the direct comparison of retention times easier. If components of two chromatograms have the same relative retention times, then they may be presumed to be the same components.

6.2.3 Detector Response

The response of the detectors is a quantitative measure of the eluted component. Because of variations in sample size encountered in the loading of the sample capsules, the detector response for components with the same relative retention time cannot be directly compared. To compensate for this sample size variability, peaks are generally compared in pairs; the ratio of two peaks in one sample are compared to the ratio of the same two peaks in a second sample (peak ratioing).

6.2.4 Resolved Components

The chromatogram will show a broad hump with peaks along the top (see Figures 1 and 2); these peaks are due to the presence of components of specific molecular structure and in sufficient quantity that the chromatograph is able to separate them from all other components. The separated peaks are termed "resolved" components. There are a number of resolved components which are usually present in oils and may appear as large peaks; these are normal alkanes and are identified in Figure 1 with the prefix "n" and a number corresponding to the number of carbon atoms in the molecule (n-hexadecane is shown as nC_{16}). Isoprenoid hydrocarbons are often present and the most commonly observed are pristane and phytane; the locations of these components are shown in Figure 1.

6.2.5 Unresolved Components

Those components which are not resolved as peaks, but appear as part of the "hump" are termed the unresolved envelope. This part of the chromatogram reflects the general distribution of non-resolvable oil components as to boiling points (time scale) and amounts (response scale).

6.3 Visual Comparison

6.3.1 Visual comparison of chromatograms is a rapid and established method to note similarities and differences in samples. Overlaying of chromatograms for comparison is often difficult due to variations in sample size in the loading of sample capsules. However, visual matching of two chromatograms set one above the other is usually satisfactory. The following sequence should be followed:

a. Set two chromatograms on a flat surface so that one is above the other and the starting point of each chromatogram and each retention time location are vertically aligned. In this configuration, the components with identical retention time values will be exactly above and below each other.

b. Observe the shape of the unresolved envelopes and the relative size of the resolved peaks for components with retention times greater than and equal to n-hexadecane (nC_{16}, approximate retention time of 16 minutes). In many cases there will be significant differences apparent at this point and the two samples can be assumed to be different. Components with retention times less than n-hexadecane cannot be used for comparison purposes because they are subject to alteration due to weathering and sample preparation.

c. Observe the relative peak heights for the same components in the two samples. Begin with the larger peaks with retention times greater than or equal to n-hexadecane. Visually ratio the heights for two peaks and compare this ratio to the ratio of the same peaks in the other sample. If these ratios are different, then the oils can be assumed to be different. If the ratios are similar, then continue the comparison with the smaller peaks.

d. With knowledge of the sample identification, collection location and handling as additional information to supplement the visual comparison, the analyst can compare the spill samples with each other and to the suspect sources for identification of a spill source.

6.4 Quantitative Comparison

6.4.1 The closer the composition of two oils, the more difficult it will be to distinguish differences in composition. Visual comparison often becomes difficult with similar oils and a quantitative comparison will assist the analyst in matching of samples. The quantitative comparison utilizes the peak areas of resolved components measured by the data processor. The quantitative comparison is also useful for samples of very different composition by providing a quantitative reaffirmation of what is observed visually.

6.4.2 Components from two samples are considered to be the same if their relative retention times are the same. The precision for relative retention time matching is ± 0.05 minutes for peaks between n-hexadecane (nC_{16}) and n-pentacosane (nC_{25}) relative to the retention time of n-hexadecane. The data processor can be programmed to include relative retention time values as part of its analysis report (see Figure 3); the manufacturer's instruction manual provides details as to the necessary procedures to include this as part of the analysis report.

6.4.3 Peak area ratios must be compared rather than direct peak areas because of variations in sample size. The ratio of peak areas for two components in the same sample is compared to the ratio of the same two components (as identified by relative retention times) in a second sample. The precision of the ratioed values is dependent on the magnitude of the peak area and the selection of a reference peak for ratios. Only peak areas having magnitudes greater than 0.2 processor area units, with an amplifier setting of range 100x, can be used (the attenuation setting has no effect in the measurement of peak areas by the data processor); peak areas with smaller magnitudes will introduce increased error in the precision of the peak area ratios. The precision of the ratioed values is dependent on the separation of components. The analyst should scrutinize the chromatograms carefully to find a major normal alkane peak in the range of n-hexadecane (nC_{16}) and n-pentacosane (nC_{25}) that is present in all of the samples and that is completely resolved from all other components; look at the base of the peaks and find one with a base which does not have small shoulders or peaks present. The peak that is selected will be the one which is used as the reference peak. Ratio the n-alkane peaks, n-hexadecane through n-pentacosane, as well as pristane and phytane, to the reference peak. Ratios compared in this manner will have a relative standard deviation of ± 5% for FID and ± 6% for FPD analysis for replicate runs of the same oil.

6.4.4 The criteria for comparing peak area ratios that are computed in the manner outlined in section 6.4.3, is as follows:

a. Calculate the difference in percent between the ratios of the same peaks for each potential spill source to each spill sample; calculate the difference between all spill samples.

b. Two peak area ratios are considered the same if they differ in magnitude by no more than 10% (by FID) or 12% (by FPD).

c. Average the percent differences for all peak area ratio comparisons. Two oils are considered the same if the mean difference are within 10% and 12% for FID and FPD analyses respectively.

d. The analyst can now include the mean difference and individual differences between peak areas of samples as part of the analysis report. Thus, an unbiased, quantitative measure can support the visual comparison results.

6.4.5 Routine replicate analyses of a standard oil and subsequent visual and quantitative comparisons are useful for evaluating the chromatographic system performance. Once every two weeks, or whenever the system performance is suspect, the standard oil should be run in triplicate. The retention times for the n-hexadecane relative retention time standard are checked to see if the peak is eluting within the time frame entered in the data processor method; if not, it is an indication that the carrier gas flow rate has changed or that the column performance may have deteriorated. The relative retention times of eluted components should be checked both visually and with the data analysis reports. The peak area ratios should be compared in the same manner as samples are normally compared, as described in Section 6.4.3; the computed results should be within the specified limits; deviations from previous values are indications that some factor(s) in the chromatographic system has changed. A change in peak area ratios between replicate sets could be an indication of reduced column performance or a change in carrier flow rate. A change in relative retention times is an indication of reduced column performance or a change in carrier flow rate. Reduced column performance should be apparent in a visual comparison of the separation of n-heptadecane and pristane and of n-octadecane and phytane; the degree of separation of these components will diminish with column degradation. The comparison of analyses of replicates is important for evaluating the repeatability of the system at a given time; increased variability between runs is an indication of increased column bleed, dirty detectors, electrical power fluctuations or a failure of the chromatographic system. Bake out the column at 250°C for OV-101 or 325°C for Dexsil 300 and inject several 1 µl volumes of Freon; repeat the replicate runs of the standard oil and check the comparisons again. If the variations are still not satisfactory, then clean the detectors and repeat another set of replicate runs and analyses of the standard oil. If the performance is not satisfactory, replace the column. Should the performance of the system still not improve, then get the manufacturer's service representative to check the chromatographic system.

6.4.6 Weathering Effects

A spilled oil is altered by several factors: evaporation, dissolution, photochemical oxidation, and microbial degradation. Of these factors, only the first two will have an effect in altering the composition of most oils collected within 48 hours of spillage. Fortunately, the compositional changes that are normally encountered will be restricted to components with retention times less than n-pentadecane. The sample preparation procedure can also alter the composition of oil sample.

All sample comparisons are restricted to components with retention times greater than, or equal to, that of n-hexadecane; this effectively avoids differences due to weathering and sample preparation.

6.5 Performance of chromatographic system

The performance of the chromatographic system will change with time as a column's performance degrades and as residues buildup in the injector. The performance of the system is quantitatively monitored, by the use of a resolution mixture, to determine whether the system is performing adequately.

6.5.1 The resolution mixture is prepared by adding 100 µl of each normal alkane used (n-hexadecane, n-octadecane and n-eicosane) to 9.5 ml of hexane in a 10 ml volumetric flask. The solution is brought to 10 ml volume with additional hexane. Gentle warming of the solutes may be necessary to carry out their transfer.

6.5.2 Instrumental conditions for analysis are exactly the same as for oil samples. A 0.2 µl injector volume of the resolution mixture is used and will give 40-60% full-scale FID response with an amplifier setting of 800x, approximating the concentration found in many oils.

6.5.3 Resolution(R) of the C16-C18 and C18-C20 pairs is determined. The following measurements are required:

$$R = \frac{\Delta t_{1-2}}{W_{h1} + W_{h2}}$$

Δt_{1-2} = time in minutes between peaks 1 and 2

W_h = width of peak at half-height in minutes

6.5.4 The measurement of W_h is not precise at normal recorder chart speeds. Operate the recorder at a chart speed of 100 mm/min. The data processor can easily be used to determine the W_h value. Measure the peak height (H), retention time (by PEP), peak area(A (by PEP), and W_h for the resolution mix. Determine the correction factor (CF) to compute the W_h value using the following formula:

$$CF = W_h \times \frac{H}{A}$$

After the correction factor is determined, the resolution of two peaks can be determined at normal recorder chart speeds using peak height measurements and PEP output. The formula for calculating the resolution is:

$$R_{1-2} = \frac{\Delta t_{1-2}}{\left(\frac{A1}{H1} + \frac{A2}{H2}\right) CF}$$

6.5.5 The resolution for a well performing system using the OV-101 SCOT column will give values of 12.5 for the 16-18 pair and 10.5 for the 18-20 pair. (Values have not yet been determined for the Dexsil 300 column, but should both be approximately 12.5). Columns should be replaced, injectors cleaned and/or all instrumental conditions checked if resolution values are 75% or less of the values for a well performing system.

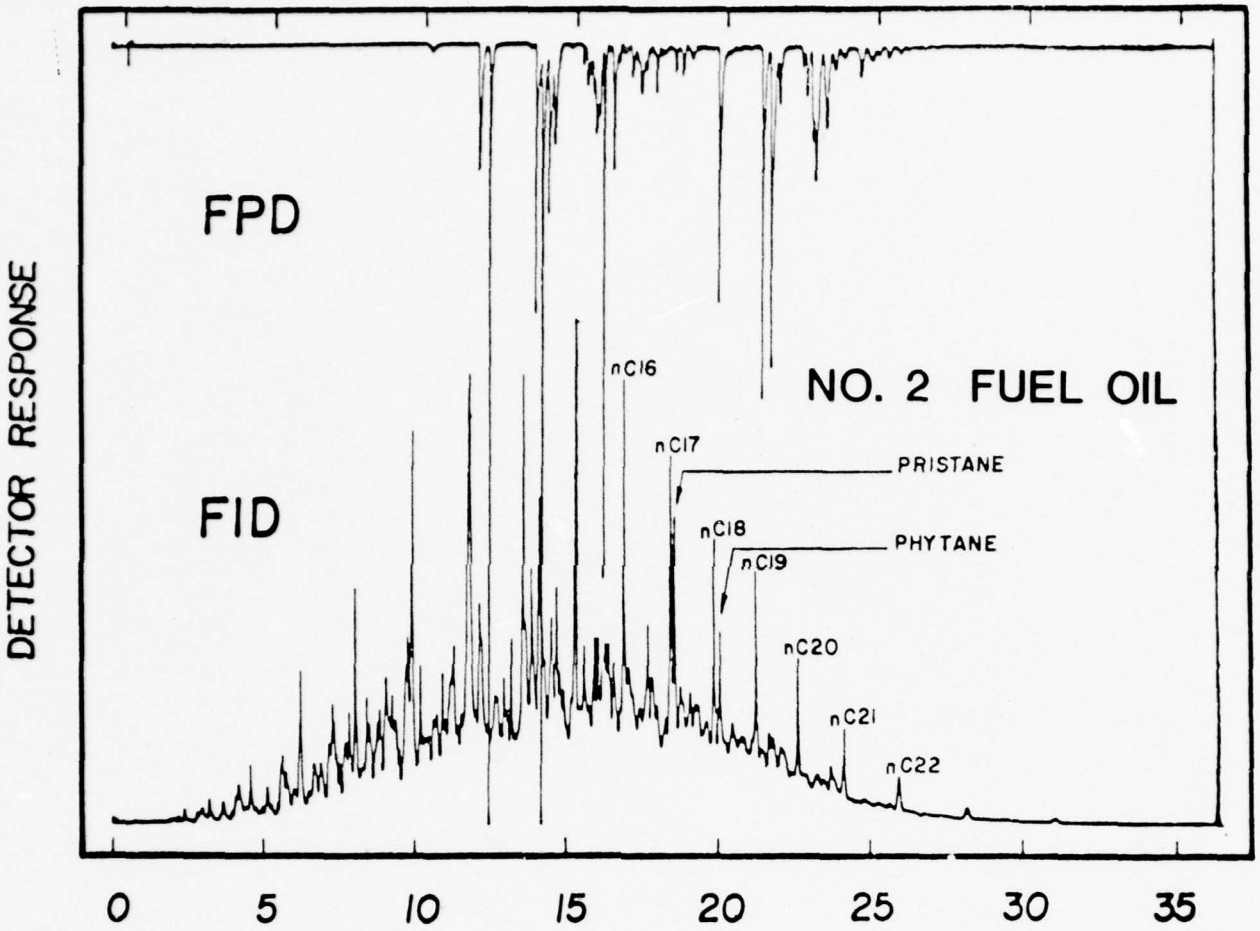


FIGURE 1. NO. 2 FUEL OIL ON OV-101 COLUMN.

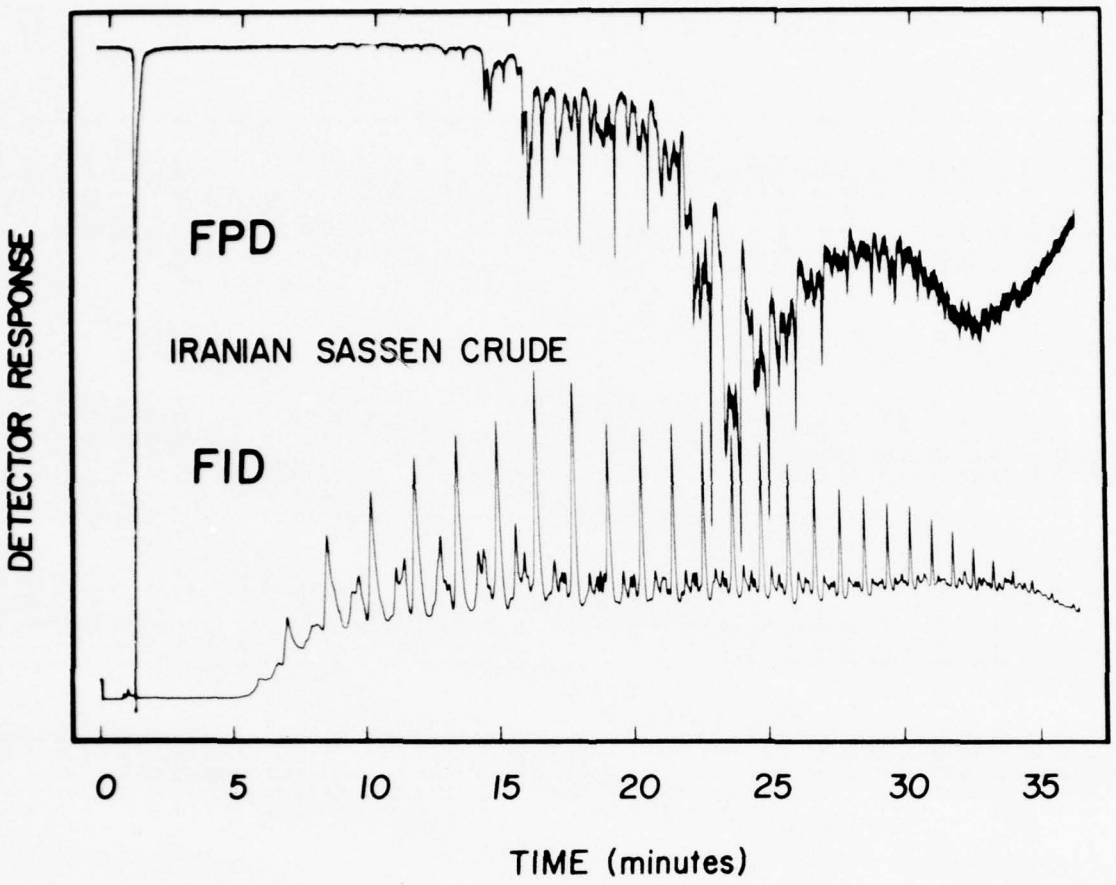


FIGURE 2. IRANIAN SASSEN CRUDE OIL ON DEXSIL 300 COLUMN.

THRESHOLDS 100 100

RUN 9 NØ 2 FØ RUN 1 4-5-76

TIME	AREA	RRT	RF	C		NAME
17.18	.5720	.959,	1.0000,	3.7208,	!	
17.32	.5457	.967,	1.0000,	3.5496,	!	
17.53	.1843	.979,	1.0000,	1.1988,	!	
17.74	.1488	.991,	1.0000,	.9682,	!	
17.90	1.8149	1.000,	1.0000,	11.8040,	!	C16
18.40	.3642	1.027,	1.0000,	2.3690,	!	
18.69	.8516	1.044,	1.0000,	5.5388,	!	
18.90	.4045	1.055,	1.0000,	2.6312,	!	
19.02	.0665	1.062,	1.0000,	.4326,	!	
19.16	.0956	1.070,	1.0000,	.6218,	!	
19.29	.0962	1.077,	1.0000,	.6258,	!	
19.47	1.3440	1.087,	1.0000,	8.7416,	!	C17
19.60	1.0846	1.094,	1.0000,	7.0548,	!	PRISTANE
19.84	.1776	1.108,	1.0000,	1.1552,	!	
20.18	.6532	1.127,	1.0000,	4.2488,	!	
20.43	.4828	1.141,	1.0000,	3.1400,	!	
20.73	.2541	1.158,	1.0000,	1.6530,	!	
20.96	1.0866	1.170,	1.0000,	7.0672,	!	C18
21.15	.5917	1.181,	1.0000,	3.8488,	!	PHYTANE
21.38	.0196	1.194,	1.0000,	.1276,	!	
21.60	.2122	1.206,	1.0000,	1.3806,	!	
21.88	.2759	1.222,	1.0000,	1.7946,	!	
22.00	.2023	1.229,	1.0000,	1.3160,	!	
22.16	.0451	1.237,	1.0000,	.2934,	!	
22.40	1.0952	1.251,	1.0000,	7.1232,	!	C19
22.63	.0936	1.264,	1.0000,	.6090,	!	
22.88	.0214	1.278,	1.0000,	.1392,	!	
23.01	.0335	1.285,	1.0000,	.2180,	!	
23.09	.0482	1.289,	1.0000,	.3134,	!	
23.32	.0474	1.302,	1.0000,	.3086,	!	
23.47	.1377	1.311,	1.0000,	.8956,	!	
23.66	.0892	1.321,	1.0000,	.5804,	!	
23.91	.6563	1.335,	1.0000,	4.2684,	!	C20
24.57	.1386	1.372,	1.0000,	.9018,	!	
24.99	.0771	1.396,	1.0000,	.5016,	!	
25.17	.0520	1.406,	1.0000,	.3382,	!	
25.29	.1043	1.412,	1.0000,	.6786,	!	
25.68	.4665	1.434,	1.0000,	3.0342,	!	C21
25.97	.1180	1.450,	1.0000,	.7676,	!	
26.45	.0775	1.477,	1.0000,	.5040,	!	
27.85	.2974	1.555,	1.0000,	1.9348,	!	C22
28.86	.0403	1.612,	1.0000,	.2622,	!	
30.62	.1254	1.710,	1.0000,	.8160,	!	
34.18	.0506	1.909,	1.0000,	.3294,	!	

FIGURE 3. TYPICAL DATA PROCESSOR ANALYSIS REPORT.

APPENDIX E

OIL SPILL IDENTIFCATION BY FLUORESCENCE SPECTROSCOPY

1. Scope

1.1 The recommended fluorescence method provides a means of fingerprinting oil by spectral characteristics and thereby matching a waterborne crude or refined petroleum oil sample to a suspect source oil sample.

1.2 This method can be applied to any neat oil, waterborne oil, or sample of oil-soaked material whether the sample is weathered (environmentally or artificially) or unweathered.

2. Summary of Method

2.1 The neat, weathered or unweathered, petroleum crude or refined oil sample is prepared for fluorescence analysis by diluting a known weight of oil in a low-actinic glass volumetric flask to volume using spectroquality cyclohexane as the solvent. It is recommended that the initial oil concentration be 100 ppm by weight. This is an acceptable working concentration at 254 nm (nanometers) excitation for all light distillate fuel oils, light crudes, and lubricating oils. For heavy crude oils, cut or residual number four, five and six fuel oils, the concentration should be adjusted to 20 ppm by weight through a serial dilution.

The prepared sample is then transferred to a 1 cm square fluorescence-free quartz cell using a disposable Pasteur pipet. The fluorescence emission monochromator is then manually scanned over the emission spectrum of the oil at a fixed excitation wavelength of 254 nm to locate the major fluorescence emission response. The major fluorescence peak is then adjusted to 95$\pm$2% of full-scale by adjusting instrument amplifier gain settings. The solution is then replaced with a fresh solution of the same sample (see NOTE 1) and the fluorescence emission spectrum of the oil is recorded from 280 nm to 500 nm. A typical fluorescence emission spectrum is shown in Figure 1.

NOTE 1: The solution is replaced with a fresh solution to prevent the possibility of errors in the recorded spectrum of the oil through photodecomposition of the sample by prolonged exposure of the sample to high intensity ultraviolet light.

2.2 Identification of the waterborne oil sample is made by direct comparison of the sample's spectrum with the spectra from suspected source samples over the spectral range from 280 nm to 500 nm. In many instances the emission spectrum, with excitation at 254 nm, is adequate to match an oil. In other instances, more information is required for verification. This can be obtained by running emission

spectra using other excitation wavelengths, e.g., 290, 270 and/or 235 nm. Solvent blanks should be run with the same gain settings. Alternatively, excitation spectra of the solutions can be used as "fingerprints", detecting at wavelengths corresponding to prominent peaks from the emission spectrum excited at 254 nm. If desired, these excitation spectra can be used to select further appropriate excitation wavelengths for additional emission scans. In the case of cut or residual fuel oils and heavy crudes, both the excitation and emission monochromators can be scanned simultaneously (synchronous scanning technique) with an offset of 25 nm between the two monochromators to record the fluorescence fingerprint of the oil. It is recommended that in performing a simultaneous scan that the excitation monochromator be set at 250 nm, and the emission monochromator be set at 275 nm and that the scan be run to a final reading of 500 nm and 525 nm on the excitation and emission monochromators respectively.

NOTE 2: If another excitation wavelength is used, it should be remembered that the Raman shift in cyclohexane is 2880 cm^{-1}. If it is desired to eliminate the Raman peak from the spectrum to avoid confusion with the fluorescence, the recorded emission spectrum should be scanned from an emission wavelength which is approximately 30 nm longer in wavelength than the excitation wavelength. (At an excitation wavelength of 254 nm the Raman peak is at 274 nm whereas with an excitation wavelength of 290 nm it is at 316 nm, etc.). Additionally, the emission spectral region used as the fingerprint should be a minimum of 220 nm in length.

NOTE 3: In the verification of sample identification it is important that the signals be normalized to 95±2% of the recorder scale in all cases. The normalization to 95±2% of scale was chosen to allow maximum display of spectral features to assist in rapid comparative identification of oil spectra.

NOTE 4: In the recording of all spectra for a given set of samples, it is important that the excitation and emission slit widths be kept constant. This will eliminate the probability of spectral changes, for a given oil, which would result from slit-width variations.

3. Significance

3.1 This method provides a means for the rapid identification of waterborne petroleum oil samples and other oil samples which may be obtained from beaches, boats, debris, etc. This method is also applicable to weathered and unweathered neat oil samples.

3.2 The unknown oil is identified through the comparison of the fluorescence spectrum of the oil with the spectra (obtained at similar

excitation wavelength and slit settings) of known suspected source samples. A substantial match between the entire spectrum of the unknown and that of the suspected source sample indicates a possible common source.

NOTE 5: Under certain conditions where the waterborne oil sample has undergone substantial environmental weathering (severe weather conditions and/or exposure for periods in excess of 24 hours), a lack of parallelism between certain spectral regions will exist. When comparing the fluorescence fingerprints of a weathered and non-weathered petroleum oil sample, the fluorescence envelope of the spilled oil will be narrower than that of the unweathered oil. The lighter fuel oils show variation in the relative intensities of major and/or minor peaks as great as ± 10% (in intensity) relative to the major peak for prolonged or severe environmental weathering (Figure 2). Additionally, the amount of spectral structure on the final downslope of the fluorescence envelope increases with prolonged weathering. In the case of heavy fuel and crude oils the structure on the initial slope of the fluorescence envelope increases, whereas the downward slope tends to show a decrease in both structure and intensity (Figures 3 and 4). In cases of prolonged and severe weathering, artificial weathering of source samples will probably be necessary to permit comparison of spill and source samples. The oil should be placed in a thin film on water in a beaker for at least eight hours in sunlight, and the spectra of the weathered oil samples examined to observe the trend in spectral changes resulting from weathering.

4. Apparatus

4.1 Fluorescence spectrophotometer (or spectrofluorometer) - an instrument recording in the spectral range of 220 to at least 600 nm for both excitation and emission responses and capable of meeting the specifications stated in Table I.

4.2 Excitation source - a 150 watt xenon lamp.

4.3 Fluorescence cell - standard cells, made from fluorescence-free fused silica with a path length of 10 mm and a height of 45 mm.

4.4 Recorder - strip chart or X-Y recorder. A wavelength marker capability is desirable but not mandatory. Recorder response, less than one second for full-scale.

4.5 Cell Filling Device - disposable Pasteur pipet having a 2-5 ml capacity.

4.6 Volumetric Flasks - low-actinic glass, stoppered volumetric flasks (50 and 100 ml).

4.7 Micro-pipet - micro-pipet having a 10-50 microliter capacity.

4.8 Analytical balance - analytical balance having a precision of ±0.1 mg.

5. Calibration of Spectrophotometer

5.1 The spectrophotometer (i.e., the emission and excitation monochromators) is adjusted and calibrated using a low pressure mercury lamp (or similar line source). Refer to ASTM E-275 for the approved calibration method.

6. Reagents and materials

6.1 Purity of reagents - spectroquality grade reagents should be used in all instances unless otherwise stated. It is intended that all reagents shall conform to the specifications of the Committee on Analytical Reagents of the American Chemical Society, where such specifications are available.

6.2 Cyclohexane - Spectroquality grade Matheson Coleman/Bell (MCB CX2285), or its equivalent should be used. Cyclohexane is dispensed throughout the procedure from a 500 ml Teflon wash bottle. To check on the suitability of the solvent, the fluorescence from the solvent blanks should be checked periodically since different lots have been found to vary in content of fluorescent impurities.

6.3 Aluminum weighing pans - 57 mm diameter, 18 mm deep.

6.4 Teflon strips - 1" X 3" pieces, 0.010" thickness.

7. Definitions of Collected Samples

7.1 Neat oil samples - neat oil samples are defined as samples which contain only oil (no water), i.e., these are generally samples collected from suspected sources. A spill sample may be considered to be a neat oil sample if there exists no visible sign of water and the sample does not appear to be emulsified.

7.2 Oil on water - oil on water samples are defined as samples containing a visible layer of oil on water. This oil layer may or may not be emulsified.

7.3 Sample of oil on foam, sand, debris, etc. - samples are defined as those which have no visible amount of oil present, but contain oil-soaked material from which no sample of oil can easily be removed.

8. Preparation of Neat Oil Samples

8.1 Remove and weigh out 0.0078±0.0001 gm of oil onto a clean aluminum weighing pan using a micro-pipet. Transfer weighed oil sample into a clean 100 ml low-actinic glass volumetric flask by creasing the aluminum pan and washing the oil directly into the volumetric flask using spectroquality cyclohexane dispensed from a Teflon wash bottle. Dilute the solution up to volume (100 ml) and shake vigorously several times and allow the prepared solution to stand for 30 minutes prior to performing the analysis to insure that all oil dissolves. This solution is 100 ppm; dilute serially to prepare 20 ppm oil solution.

NOTE 6: It is preferable that the prepared solutions be used the same day. Do not use solutions that have been standing for periods in excess of six hours unless they have been refrigerated. In no case should solutions more than 2 days old be used.

9. Preparation of Oil on Water Samples

9.1 Samples containing only a thin film of oil on water may be concentrated in the following manner. Dip or pass a Teflon strip through the oil layer, then allow the oil to drip into a clean aluminum weighing pan. Continue this process until enough oil has been recovered to use the micro-pipet, then continue as stated in 8.1.

9.2 Emulsified samples should be prepared in the following manner. Place approximately 25 cc of the emulsified oil in a 50 cc centrifuge tube. Centrifuge this material at a relative centrifugal force

$$rpm = 265 \sqrt{rcf/d}$$ (See ASTM Method D1796-98)

rcf = relative centrifugal force

d = diameter of swing

of 1000 in/min^2 for ten minutes. Prepare the sample as described in 8.1 or 9.1 depending on the volume of oil recovered.

10. Preparation of Oil-Soaked Samples

10.1 Place a portion of the oil-soaked material in the center of a clean aluminum weighing pan. Fold the sides of the aluminum weighing pan over the material and then squeeze out the oil into a clean aluminum pan. Continue squeezing the fabricated tube until enough oil has been collected to use the micro-pipet and then continue as stated in 8.1.

10.2 In the event that enough oil cannot be obtained by the procedure stated in 10.1, it is recommended that a pentane extraction procedure (refer to Appendix D, Section 5.4) be used to prepare the sample. Once the sample has been prepared, continue as stated in 8.1.

NOTE 7: In the event that this procedure is used (i.e., the pentane extraction), it is important that all samples contained in this set be prepared in a similar manner to prevent the possibility of spectral errors being introduced by the use of different sample preparation techniques.

11. Cleaning Glassware

11.1 All glassware used in this procedure should be cleaned in the following manner. Volumetric flasks and cells should be first rinsed repeatedly (three times each) with spectroquality acetone and then with spectroquality cyclohexane. Prior to the use of glassware and cells throughout this procedure, they should be rinsed again with spectroquality cyclohexane.

11.2 When working with heavy oils, a cleaning procedure using organic solvents may not be sufficient. Heavy oils build up a residue on cells which solvent cleaning will not remove. If the solvent blank shows significant impurities, a residual film on the cell, rather than an impure solvent, may be the cause. Soak the cells in concentrated nitric acid for one hour. Observe proper safety precautions by using adequate eye and hand protection. Empty contents and rinse the cells repeatedly with distilled water.

12. Recording the Fluorescence Emission Spectrum

12.1 Fill a clean fluorescence cell with the oil solution using the Pasteur pipet. Gently wipe the outside of the cell with lens paper, removing any fingerprints or oil smears and place the cell in the sample compartment.

12.2 Set the slit width of the excitation monochromator at a maximum setting of 34 nm, if necessary, for intensity. (Presently a 10 nm slit is used with a 28,800 lines/inch grating.)

NOTE 8: Commercial instruments have a wide variation in available excitation slit settings, some being fixed and others adjustable. In general, a maximum excitation slit width setting of 34 nm and a minimum setting of 5 nm should be used (10 nm is most commonly used).

12.3 Set the emission slit width to a maximum of 2.5 nm and a minimum of 1 nm.

NOTE 9: Do not change either the excitation or emission slit width settings during the analysis of a given set of samples.

12.4 Manually scan over the fluorescence emission range from 280 nm to 500 nm and determine the wavelength of the major fluorescence response. Adjust the major response to 95±2% of the recorder full-scale using amplifier gain controls.

12.5 Remove the sample cell, discard the solution and place a fresh solution of the sample in the cell. Replace the cell in the sample compartment.

12.6 Set the emission monochromator at 280 nm and scan the emission spectrum from 280 to 500 nm at an emission monochromator scan speed of preferably 25 or 30 nm per minute (not to exceed 60 nm

per minute which may be required on some older instruments) and a chart speed (if a strip chart recorder is used) not to exceed 60 mm per minute.

13. Interpretation of Spectra

13.1 The spectrum of the unknown sample is compared as an overlay with the spectra of the suspect samples. If two spectra substantially match (i.e., the two spectra are exact overlays to within the error indicated in 13.4), the samples may be surmised to have originated from a common source. Additional data from further independent analyses (e.g., IR, GC, TLC) are necessary to confirm this inference.

13.2 Approximately five features are to be noted when comparing the oil spectra: 1) general shape, 2) number of peaks, 3) wavelengths corresponding to the peaks, 4) ratios of the peak intensities, 5) contour of the upward/downward sloping sides of the curve. The correlations of these features among spectra will become apparent with practice.

13.3 Weathering may cause changes in the fluorescence emission signature of an oil. These changes and the magnitude of these changes are dependent on the oil involved. These changes are discussed in Section 3.2, Note 5.

13.4 As discussed in Section 3.2, Note 5, changes will occur in the fluorescence fingerprint due to natural weathering processes. The magnitude of these changes is dependent on the criteria cited in Section 3.2, Note 5. In general a positive match of a weathered oil's fluorescence fingerprint to that of a non-weathered oil's fingerprint requires that the two spectra are within $\pm$4% of each other* over the spectral range of 280-500 nm. As discussed in Section 3.2, Note 5, the probable alterations in these fingerprints as a result of weathering processes must be considered, i.e., a change on the short wavelength side of the fluorescent envelope must be accompanied by a change on the long wavelength side of the fluorescent envelope.

13.5 Further verification of the match utilizing this fluorescence technique may be made at a 20 ppm concentration level for those spectra which appear similar. The recordings of these spectra are obtained by following the procedure given in Section 12. Additionally, the excitation spectra of these solutions at the 20 ppm level may be obtained for further confirmation of the spectral matching of two oils applying the above procedures on matching. Refer to Section 14 on procedure for recording the excitation spectrum.

14. Recording the Excitation Spectrum

14.1 Place the cell containing the 20 ppm solution in the sample compartment.

*In intensity relative to the major peak.

14.2 Set the emission monochromator at the wavelength of a major fluorescence response as observed when excited at the 254 nm wavelength.

14.3 Set the emission monochromator at a slit width of 10 nm.

14.4 Set the excitation monochromator at a slit width not greater than 2.5 nm or less than 1 nm.

14.5 Manually scan over the excitation spectrum from 220 nm to the wavelength setting of the emission monochromator to determine the wavelength that has a major response. Adjust the major response to 95±2% of recorder full-scale using amplifier gain controls.

14.6 Remove the sample cell, discard the solution and refill the cell with a fresh solution of the same sample. Replace the cell in a sample compartment.

14.7 Set the excitation monochromator at 220 nm and scan the excitation spectrum from 220 nm to the wavelength setting of the emission monochromator using an excitation monochromator scan speed of 25 or 30 nm per minute (not to exceed 60 nm per minute) and a chart speed (if strip chart recorder is used) not to exceed 60 mm per minute.

14.8 Interpret spectra as discussed in Section 13.

TABLE I. SPECIFICATIONS FOR FLUORESCENCE SPECTROPHOTOMETERS.

Wavelength Reproducibility	
Excitation monochromator	Better than ±2 nm
Emission monochromator	Better than ±2 nm
Gratings	
Excitation monochromator	Minimum of 600 lines per mm blazed at 300 nm
Emission monochromator	Minimum of 600 lines per mm blazed at 300 nm or 500 nm
Photomultiplier Tube	
Either S-20 (e.g., Hamamatsu R446-UR or S-5) or S-5 (RCA 1P28 or Hamamatsu R106) response	
Resolution	
Excitation monochromator	2.5 nm or better
Emission monochromator	2.5 nm or better

NOTE 11: Commercial instrumentation is not uniform in design. The differences in available slits, gratings and photomultiplier tube selections will produce variations in the recorded fluorescence spectra. Therefore, the comparison of spectra can only be made for spectra recorded on a particular instrument and cannot be compared from instrument to instrument with the possible exception of spectrally corrected spectrofluorometers.

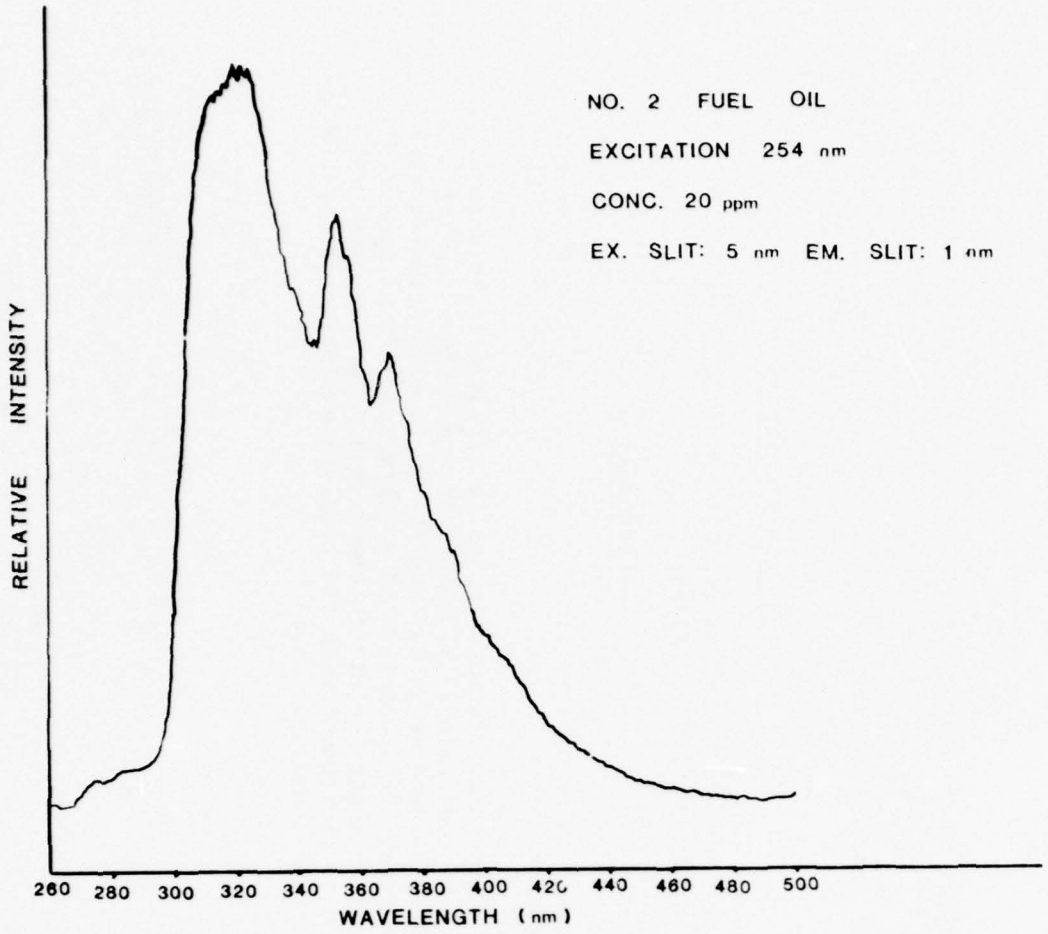


FIGURE 1. FLUORESCENCE EMISSION SPECTRUM OF A NO. 2 FUEL OIL (CORRECTED FARRAND MARK I).

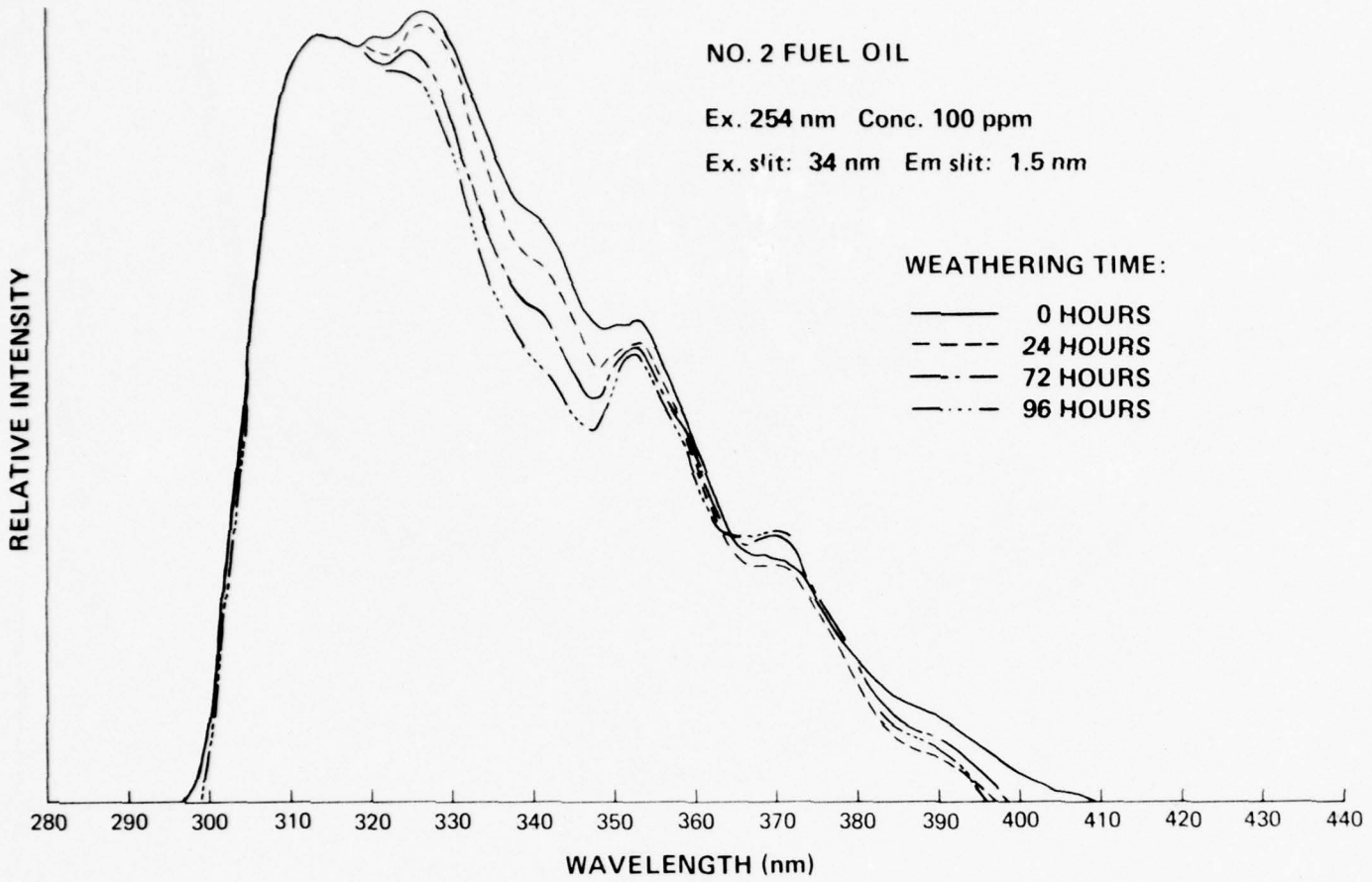


FIGURE 2. FLUORESCENCE EMISSION SPECTRA OF A WEATHERED NO. 2 FUEL OIL (UNCORRECTED P-E MPF-3).

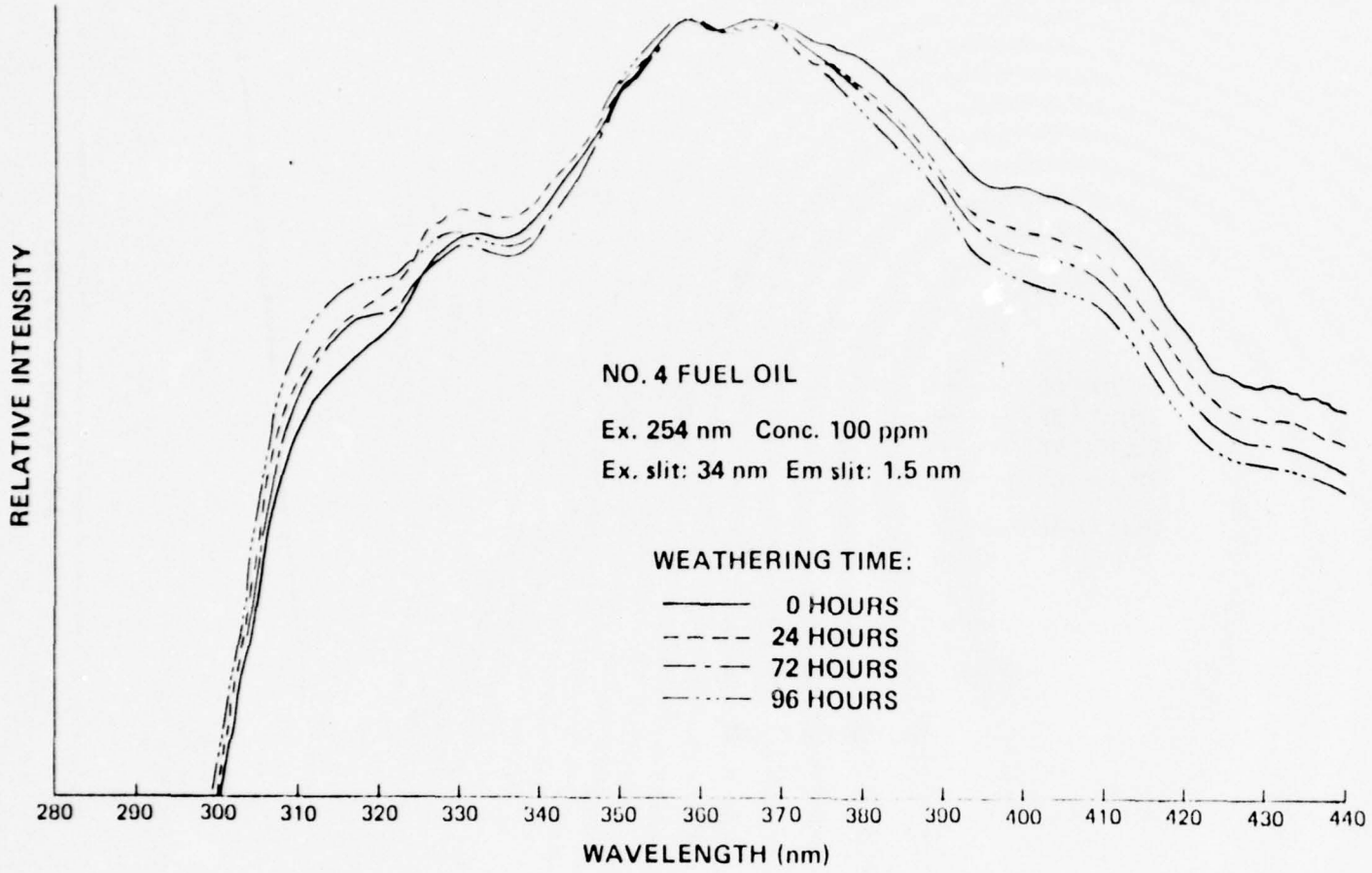


FIGURE 3. FLUORESCENCE EMISSION SPECTRA OF A WEATHERED NO. 4 FUEL OIL (UNCORRECTED P-E MPF-3).

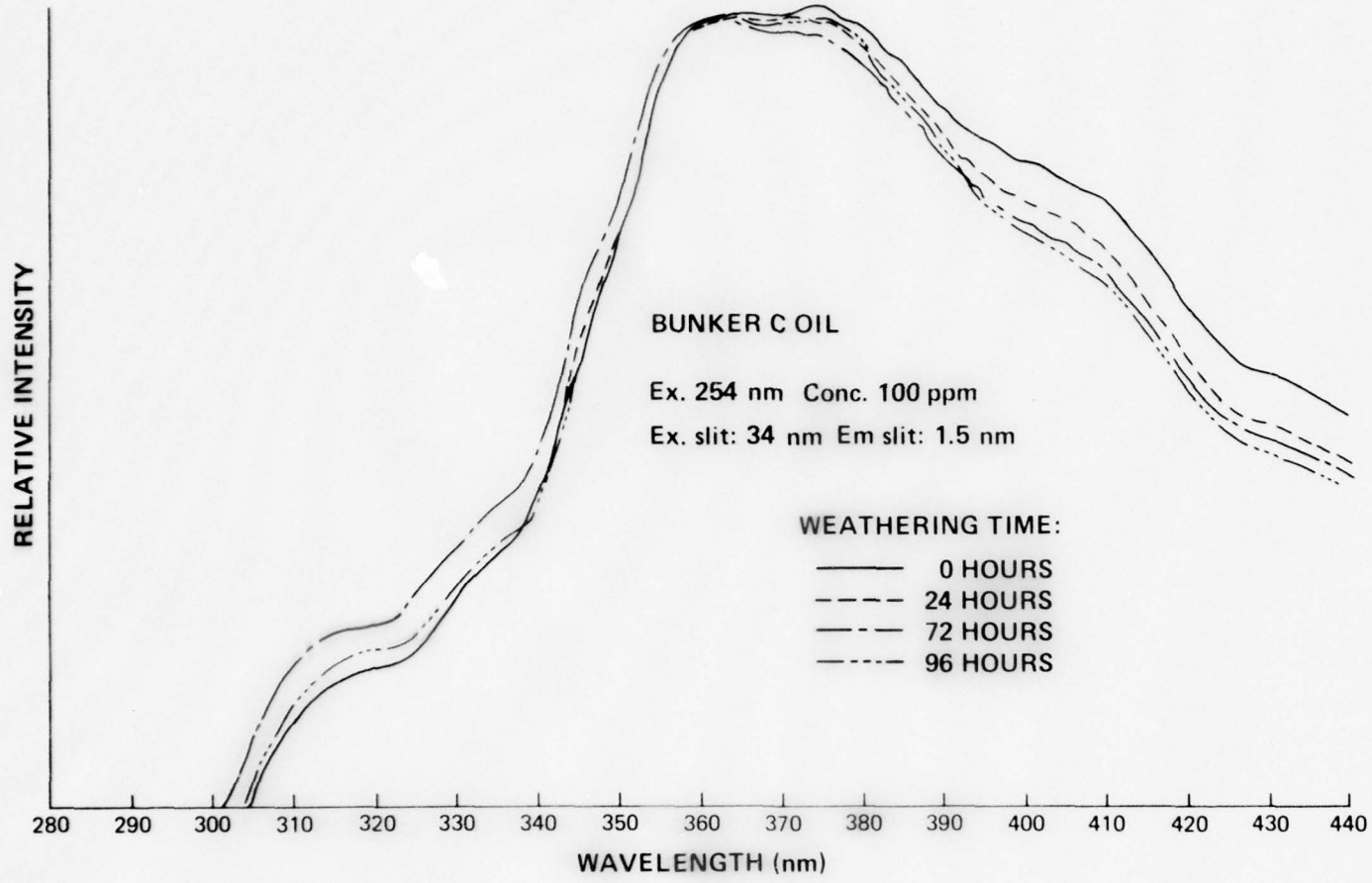


FIGURE 4. FLUORESCENCE EMISSION SPECTRA OF A WEATHERED BUNKER C OIL (UNCORRECTED P-E MPF-3).

APPENDIX F

OIL IDENTIFICATION BY LOW TEMPERATURE LUMINESCENCE

1.0 SCOPE

1.1 Luminescence is the emission of absorbed radiation as light, either as fluorescence or phosphorescence. Most fluorescence occurs at longer wavelengths than the longest wavelength of absorption. Its time delay is approximately on the order of 10^{-8} seconds. Phosphorescence, which is from a different type of excited state than fluorescence is at longer wavelengths than fluorescence; its time delay is within the range of 10^{-4} - 10 seconds. Low temperature luminescence is conducted in a rigid matrix at temperatures below ambient, usually liquid nitrogen temperature (77°K). The low temperature luminescence oil analysis procedure provides emission and/or excitation spectra which are used to match spilled petroleum oil to a suspected source.

1.2 The procedure can be used to fingerprint any neat (unweathered) oil. It can fingerprint weathered oil found in or upon the water or any other type of substrate for weathering periods of one or two days for light oils or up to one week for heavy oils.

1.3 By slowly cooling the dilute oil solution (in methylcyclohexane) to form a clear rigid material (crystallographically classified as a "glass") at 77°K, emission intensity increases, much sharper spectral structure is obtained and phosphorescence, not seen at room temperature, as well as fluorescence is observed, yielding additional information (Figures 1 and 2).

2.0 THEORY

For organic molecules, light is absorbed in the ultraviolet and/or visible region of the electromagnetic spectrum resulting in transitions from the lowest (ground) electronic state to various excited electronic states.

An excited molecule may return to the ground state by radiating light, or by radiationless transitions (aided by molecular collisions) which dissipate the energy as heat. If all the absorbed light is re-emitted as fluorescence, the efficiency is 100%. (Some molecules do not fluoresce at all because of the high efficiency of the internal radiationless processes.)

Fluorescence is the emission corresponding to the transition of an electron from the lowest excited electronic state, into which appreciable absorption occurs, to various vibrational levels of the ground electronic state. Fluorescence is usually not observed from higher excited states corresponding to shorter wavelength absorption bands due to the efficiency of radiationless transitions from higher excited states to the lowest excited state. Most fluorescence emission occurs at longer wavelengths than the longest wavelength absorption band.

A molecule in an excited state can also undergo another kind of radiationless transition, i.e., crossing to another type of excited state at lower energy. Light may also be emitted from this state, giving rise to phosphorescence emission. Since this state is lower in energy than the type of excited state associated with fluorescence the phosphorescence will occur at longer wavelengths than the corresponding fluorescence. At room temperature, phosphorescence is usually quenched by competing radiationless transitions. As a result, phosphorescence is usually measured only at low temperatures in a rigid matrix.

A rigid matrix reduces radiationless transitions due to molecular collisions and low temperature reduces the diffusion of impurities, especially oxygen, which quench the luminescence. Therefore, the efficiencies of both fluorescence and phosphorescence generally increase at low temperatures.

The rigidity of the matrix is also important in increasing spectral sharpness since fewer sites are available to the solute molecules and interaction with the matrix is reduced. Methylcyclohexane is recommended for low temperature luminescence analysis because it freezes to a clear organic "glass" rather than a "snow" and therefore greater spectral reproducibility is obtained. Solvent purity is also more important at low temperature because of the higher sensitivity in detecting impurities, which might not be observed at room temperature.

3.0 SUMMARY OF METHOD

3.1 The oil sample is prepared for analysis by diluting a known weight of oil in a low-actinic glass volumetric flask to volume using spectroquality methylcyclohexane as the solvent. It is recommended that the initial oil concentration be 10 ppm by weight. The prepared sample is then transferred to a clean fluorescence-free quartz sample tube using a Pasteur pipet. The sample tube is then placed in its holder and immersed in liquid nitrogen contained within a fluorescence-free quartz Dewar which is set in the low temperature attachment of the fluorescence spectrophotometer. The excitation wavelength is then set at 254 nanometers (nm) and the emission monochromator is manually scanned over the spectral range of interest to locate the major emission response. The major peak is then adjusted to 92 $\pm$ 2% of full scale with the instrument gain settings. The solution is then replaced with a fresh solution of the same sample (see Note 1) and the luminescence emission spectrum of the oil is recorded from 280 nm to 700 nm.

3.2 Identification of the oil sample is made by direct comparison of the spectra of the spill sample(s) with the spectra from suspect source samples over the spectral range from 280 nm to 700 nm. If it is not possible to ascertain a match on the basis of the emission spectra excited at 254 nm, other emission spectra can be obtained

using different excitation wavelengths (e.g. 290 or 330 nm) to aid in the identification.

3.3 Excitation spectra can also be obtained for additional fingerprint information. To obtain the excitation spectrum, select the wavelength of a major peak with sharp spectral structure from the spectrum obtained in 3.1 (excitation at 254 nm). Set the emission monochromator at this selected wavelength and manually scan the excitation monochromator from 220 nm to the emission detection wavelength to locate the major excitation response. The major peak is then adjusted to 92 $\pm$ 2% of full scale and the spectrum is recorded.

NOTE 1: There is a possibility of introducing errors into the recorded spectrum of an oil through photodecomposition of the sample by prolonged exposure to high intensity ultraviolet light. This is prevented by replacing the solution in the sample chamber with a fresh solution between spectral analyses.

NOTE 2: A check on the reproducibility of the spectra obtained should be made at least once during each spill case. This is effected by repeating the spectral scan on the same piece of chart paper with a fresh aliquot of the solution. If good reproducibility (within $\pm$ 2% of full scale at peaks - see Figure 3) is not achieved, the probable cause is too rapid a freezing rate (see Section 9.4).

NOTE 3: Solvent blanks must be run to ensure that impurities, if present, are not causing a distortion of the true spectra of the oils. Also, any scatter or background fluorescence which may be present due to the Dewar and/or sample tube can be accounted for by examining spectra of solvent blanks. A blank should be run for each wavelength used and for each change in slit width effected.

In addition to the scattering from the Dewar and sample tube, a scattering peak characteristic of the solvent (Raman peak) may be observed in the solvent blank; for methylcyclohexane the Raman peak lies 2870 cm^{-1} to the red (towards longer wavelengths) of the excitation wavelength. This peak must not be confused with the fluorescence spectrum of the oil and should be considered part of the solvent blank and subtracted out before interpreting the spectra.

NOTE 4: The importance of keeping the excitation and emission slit widths constant when recording all spectra for an oil spill case cannot be overemphasized. Variations in slit width for a given oil will probably cause changes in the observed spectra.

4.0 APPLICATIONS

4.1 This procedure is applicable to all petroleum oil types including weathered oils from real world spills. The high discrim-

inating ability has been demonstrated by differentiation of natural seeps from closely similar production oils originating within the same geographical area.

5.0 APPARATUS AND MATERIALS

5.1 Fluorescence spectrophotometer (spectrofluorometer) - an instrument recording in the spectral range of at least 220 to 700 nm for both excitation and emission responses. To date the majority of the data compiled have been obtained with a double monochromator instrument, the Baird-Atomic Fluorispec SF-100. Other double monochromator instruments as well as suitable single monochromator instruments capable of meeting the specifications stated in Table 1 could be used.

NOTE 5: Double monochromator spectrofluorometers are especially desirable since they reduce scattered light. Single monochromator instruments may require the use of narrow bandpass interference and/or cut-off filters. Even with these, a single monochromator instrument may still exhibit considerable scatter.

It is important in the sample preparation not to use fluorescent solvents or those containing fluorescent impurities or any chlorinated solvent which might quench the oil fluorescence. Also, avoid detergents, oil cleaning agents and de-emulsifiers which are often fluorescent.

5.2 Excitation source - a 150-watt xenon arc lamp has been found satisfactory.

5.3 Recorder - an X-Y recorder compatible with the instrumentation used.

5.4 Low Temperature Attachment - necessary for execution of low temperature luminescence measurements. Must be compatible with instrumentation used. Also permits phosphorescence measurements when coupled with a rotating-shutter (chopper) assembly or using a pulsed light source.

5.5 Dewar and Sample Tubes - to hold liquid nitrogen and sample solution. They must be compatible with the low temperature attachment. Both Dewar and sample tubes must be constructed of high quality fluorescence-free quartz in such a manner as to minimize light scattering.

5.6 Storage Dewar - a commercially available container for storing the required supply of liquid nitrogen. A ten-liter Dewar is recommended as it is both a convenient size in terms of handling and holds an adequate quantity for a minimum of one week's work.

5.7 Transfer Dewar - the storage Dewar is too large a container [illegible] to pour liquid nitrogen into the sample Dewar in the instrument.

Therefore, an intermediate-size (0.5 - 1.0 liter) Dewar to be utilized for liquid nitrogen transfer between the storage and sample Dewars is needed.

5.8 Analytical balance - An analytical balance having a precision of $\pm$ 0.01 mg.

5.9 Micro-pipet - a micro-pipet to transfer oil from container to balance; capacity 10-50 µl.

5.10 Expendables - expendables include spectroquality methylcyclohexane, recorder chart paper, micro-pipet tips, weighing pans, low-actinic glass-stoppered volumetric flasks, liquid nitrogen, Pasteur pipets and bulbs, plastic funnels for liquid N_2 , glass funnels for sample and solvent transfer, Pyrex glass wool, extra recorder pens and Teflon strips.

6.0 DEFINITIONS OF COLLECTED SAMPLES

6.1 Neat oil samples - Neat oil samples are those collected from suspect sources which contain oil only, or collected samples containing a visible layer of oil on water or other substrate (where the oil may be easily removed without removing other material such as water, sand, dirt, etc.).

6.2 Emulsified oil samples - Oil-water emulsions which must be extracted using methylcyclohexane.

6.3 Oil-soaked material - Samples present on sand, vegetation, dirt, water, etc., which are not easily removable and will require an extraction procedure.

7.0 PREPARATION OF NEAT OIL SAMPLES

7.1 Remove oil from sample container using a micro-pipet and weigh out 0.00070 $\pm$ 0.00003 gm onto a clean aluminum weighing pan. Wash the oil directly into a 100 ml low-actinic glass volumetric flask with spectroquality methylcyclohexane dispensed from a clean beaker. Dilute the solution to volume with spectroquality methylcyclohexane and shake well to mix.

NOTE 6: When washing the oil into the flask from the weighing pan use a funnel so that none of the sample is accidentally spilled. Then rinse down the funnel into the flask.

NOTE 7: It is important to dispense the methylcyclohexane from a beaker or a glass wash bottle. Teflon wash bottles were found to contaminate the solvent and affect the low temperature blank.

7.2 Oil samples present on substrate which can easily be removed should be transferred to a weighing pan with a micro-pipet. Samples containing a thin film of oil on water may be concentrated in the following manner: Pass the Teflon strip through the oil layer and then allow the oil to drip off of the strip and onto a clean aluminum weighing pan. Continue this process until enough oil has been recovered to prepare a solution as stated in 6.1.

8.0 PREPARATION OF SAMPLE FROM OIL-SOAKED MATERIAL

8.1 Place a portion of the oil-soaked material in the center of a clean aluminum weighing pan. Fold the sides of the aluminum weighing pan over the material and then squeeze out the oil into a clean weighing pan. Continue squeezing until enough oil has been collected to use the micro-pipet and then prepare a solution as stated in 6.1.

8.2 If the procedure stated in 8.1 does not yield enough oil, a solvent extraction should be attempted. This is effected by shaking oil-soaked material with spectroquality methylcyclohexane in a clean stoppered separatory funnel until the oil is removed from the material. The solvent can then be stripped from the sample by passing a stream of dry nitrogen over the sample. Once the solvent has been removed, continue as stated in 6.1.

NOTE 8: Ensure that all samples in a spill set have been prepared in exactly the same manner (preferably at the same time) to prevent spectral errors from being introduced.

9.0 CLEANING GLASSWARE

9.1 Volumetric flasks and other glassware used in this procedure should be rinsed five times with spectroquality methylcyclohexane. Sample tubes should be rinsed three times with spectroquality methylcyclohexane followed by three rinsings with the sample solution next to be analyzed. When working with heavy oils, a cleaning procedure using organic solvents may not be sufficient. Soak the tubes in concentrated nitric acid for one hour. Empty contents and rinse the tubes repeatedly with distilled water. Observe proper safety precautions by using adequate eye and hand protection. The sample Dewar should be rinsed with spectroquality methylcyclohexane at any time when it appears to be necessary. The Dewar should then be dried with dry nitrogen gas or, if this is not available, be allowed to air dry.

9.2 When working with heavy oils, a cleaning procedure using organic solvents may not be sufficient. In that case, soak the cells in concentrated nitric acid for one hour. Empty the contents and rinse the cells repeatedly with distilled water.

NOTE 9: When a second sample of the same solution is to be analyzed, it is only necessary to rinse out the sample tube with the solution itself.

10.0 RECORDING THE LUMINESCENCE EMISSION SPECTRUM

10.1 Fill the transfer Dewar with liquid nitrogen from the storage Dewar, passing it through a plastic funnel filled with Pyrex

glass wool (to remove ice crystals). Then fill the sample Dewar from the transfer Dewar once again filtering it through glass wool. Avoid spilling liquid nitrogen on the instrument to protect it from possible damage.

10.2 Set the slit width of the excitation monochromator at the narrowest width possible which will allow for a good signal response (preferably corresponding to a bandwidth of 6 nm). Set the emission slit width narrower than the excitation slit width (preferably corresponding to a bandwidth of 4 nm). These settings should be optimized for the spill, rather than suspect sample, since the spill sample may luminesce more weakly due to weathering changes.

NOTE 10: Wider slits may be used, if necessary; however, it is important that blanks be run since impurities are more apt to affect the emission spectra at low temperature with wider slits.

NOTE 11: During the analysis of a given set of samples it is imperative that neither excitation nor emission slit widths be changed.

10.3 Fill a clean sample tube with the oil solution using a clean Pasteur pipet. Gently wipe the outside of the sample tube with lens paper to remove any fingerprints or oil smears.

10.4 The sample tube containing the oil sample should be placed into the holder (usually the Dewar cap) and slowly lowered into the Dewar. Lower it at such a rate as to cause minimal boiling of the liquid nitrogen. The freezing process should take between thirty and forty-five seconds.

10.5 Open the shutter to the photomultiplier tube and manually scan over the luminescence emission to determine the major luminescence response. Adjust the gain controls to make the major emission response 92 $\pm$ 2% of the recorder full scale. Always make sure, by comparing with solvent blanks, that the peak maximized is an oil luminescence peak and not a scatter or Raman peak.

10.6 Remove the sample tube, discard the solution and place a fresh solution of the same sample in the tube. Replace any evaporated liquid nitrogen in the sample Dewar, if necessary, and then put the sample tube back into the Dewar as described in 10.4.

10.7 Set the emission monochromator at the shortest wavelength which the scatter will allow (i.e., when the pen returns on scale). Scan the emission spectrum to 700 nm at an emission monochromator scan speed not to exceed 60 nm/minute. Before or during the scan it may be necessary to insert an ultraviolet-transmitting clear filter with a cut-off which will not pass the exciting light. This is to prevent exciting light, e.g., 254 nm which appears in the second order at twice the excitation wavelength (508 nm), from distorting the oil fluorescence. For the Baird-Atomic instrument, at 254 nm excitation, the

Corning 0-56 filter is suitable. For other instruments and excitation wavelengths, the absorption spectrum of the cut-off filter must be known and compared with the solvent blank with and without the filter in place. The fluorescence spectrum of the oil must also be run without the cut-off filter in place to determine that there is no distortion at the short wavelength end of the spectrum.

11.0 INTERPRETING THE EMISSION SPECTRA

11.1 The spectrum of the spill sample is compared as an overlay with the spectra of the suspect samples. Two samples may be considered to have originated from a common source if their spectra match to within $\pm$ 4% of full scale over the spectral range from 300 to 600 nm with the exception of some weathered oils (see 11.3). Heavy oils (No. 4 Fuel oil and heavier) do not weather appreciably; up to one week they can be matched within $\pm$ 4%. Light oils (No. 2 Fuel oil, diesels, etc.) change materially on weathering. After two days the matching criteria must be altered to $\pm$ 6%; after one week to $\pm$ 10%.

11.2 When comparing oil spectra there are several features to be checked: (1) general shape, (2) number of peaks, (3) wavelengths of peak occurrence, (4) peak intensity ratios, (5) contour of the upward and downward slopes of the curve. Confidence in correlating these features between spectra will be obtained with experience.

11.3 Weathering may cause changes in the luminescence emission signature of an oil. The changes and the magnitude of these changes vary with different oils. To date, limited weathering data have been accumulated by the low temperature luminescence procedure. Two examples of weathering changes are shown in Figures 4 and 5 for two different types of crude oils weathered under natural environmental conditions for periods of two days and one week. For light oils weathered longer than two days and for heavy oils weathered longer than one week, it may be necessary to weather suspect samples artificially. (Artificial weathering methods are still under development. One method is to place an oil in a thin film on water in a beaker for a least 8 hours in sunlight and then to examine the spectrum of this oil to observe the trend in spectral changes resulting from weathering.).

12.0 RECORDING THE EXCITATION SPECTRUM

12.1 Place the sample tube containing the oil solution in the sample Dewar.

12.2 Set the emission monochromator at the wavelength of a major luminescence peak as observed from the emission spectrum obtained when exciting at 254 nm.

12.3 In general, reverse the slit width for the excitation and emission monochromators from the way they were set for the emission spectra.

12.4 Manually scan over the excitation spectral range to determine

the major response location. Adjust the gain to make the major excitation response 92 ± 2% of the recorder full scale.

12.5 Remove the sample tube, discard the solution and place a fresh solution of the same sample in the tube. Replace any lost liquid nitrogen in the sample Dewar, if necessary, and then put the sample tube back into the Dewar.

12.6 Set the excitation monochromator at 220 nm. Scan the excitation spectrum from 220 nm to the wavelength setting of the emission monochromator at a scan speed not to exceed 60 nm/minute.

12.7 Repeat, as stated in 12.5 and 12.6 one or more times as necessary for reproducibility check.

12.8 Interpret spectra as discussed in Section 11.

12.9 Repeat from beginning (12.1), if necessary, to record excitation spectra detecting at other major luminescence emission peaks.

TABLE 1
Specifications for Suitable Spectrofluorometers

Wavelength Reproducibility	
Excitation monochromator	Better than ± 2 nm
Emission monochromator	Better than ± 2 nm
Gratings	
Excitation monochromator	Minimum of 600 lines per mm blazed at 300 nm
Emission monochromator	Minimum of 600 lines per mm blazed at 450-500 nm
Photomultiplier Tube	
Either S-10 (e.g., Hamamatsu R136) or S-20 (Hamamatsu R818) response	
Resolution	
Excitation monochromator	Better than 2 nm
Emission monochromator	Better than 2 nm

NOTE 12: As previously stated, most of the data accumulated to date were generated on the Baird-Atomic Fluorispec SF-100 which is uncorrected. (By uncorrected, we mean that the source-monochromator combination

does not give uniform excitation light intensity as a function of wavelength and that the monochromator-detector combination is not flat in spectral response. (This means that peak-ratios for emission (or excitation) spectra are not the true ratios.) Commercial instrumentation is not uniform in design. Differences in available slits, gratings and photomultiplier tube selections will produce variations in the luminescence spectra. Therefore, the comparision of spectra can only be made for spectra recorded on the same instrument, with the possible exception of spectrally corrected spectrofluorometers which may allow for comparison of spectra run on different instruments. There are several spectrally corrected spectrofluorometers now available. Unless solutions are sufficiently dilute (i.e., less than 0.05 O.D. at the excitation wavelength), even spectra run on a spectrally corrected spectrofluorometer may not be truly corrected. The spectra may be distorted due to geometric effects arising from the absorption of exciting light or the reabsorption of emitted light commonly called inner-filter effects. This is a limitation only if spectra run on different makes of corrected spectrofluorometers are being compared. More concentrated solutions will also be subject to spectral distortions due to solute-solute interactions such as excited dimer formation which lead to partial quenching of the emission and possible loss of structure. The choice of 10 ppm was a compromise between minimizing geometric and quenching effects and maximizing signal-to-noise. (A concentration of twenty ppm may also be used, either to minimize the effect of the solvent blank, or to permit direct comparison with room temperature measurements.)

13.0 SAFETY PRECAUTIONS

13.1 Liquid nitrogen (N_2) (boiling point = 77.3°K or -195.7°C) is non-flammable and non-toxic. However, liquid N_2 must be handled with care both because of its very low temperature and because in a confined space the pressure buildup, due to the evaporating N_2,, could lead to an explosion. Also, if stored uncovered or poorly covered it will not only pick up water vapor as ice, but it will also be slowly converted to liquid air (boiling point = 85°K) which is reactive, and which in turn may become enriched with oxygen (boiling point = 90.2°K) to form a very hazardous mixture.

13.2 Safety glasses should be worn when pouring liquid nitrogen to prevent getting it in one's eyes in the event that it bubbles up suddenly.

13.3 Never handle liquid N_2 carelessly. A few drops of liquid N_2 on the skin will evaporate before doing any harm, but if any becomes entrapped between your hand and glove or your foot and shoe it might cause painful frostbite. When transferring large amounts of liquid N_2 , use of asbestos gloves is recommended.

13.4 Always store liquid nitrogen <u>loosely</u> stoppered so that the positive pressure of the N_2 boil-off will prevent the formation of liquid air.

13.5 Glass Dewars are commonly used (transfer and sample Dewars)

for containing small quantities of liquid nitrogen. Since they are evacuated and may implode violently if dropped, shattering the glass into small pieces, they should be wrapped (except for optical surfaces) with strong tape (preferably cloth adhesive) before use.

13.6 Never store Pyrex glass or quartz Dewars in areas where helium gas is used. Helium diffuses through these materials and thereby destroys the vacuum.

13.7 If it is necessary to dispose of liquid N_2 in order to clean and/or dry a Dewar, pour the liquid N_2 slowly onto the floor or the ground. Never pour it into sinks, as it may crack the sink and/or the pipes.

13.8 Xenon lamps generate ozone, which is harmful to health. Therefore, it is imperative that the fumes be exhausted when operating in a confined area.

13.9 Xenon lamps also emit ultraviolet-rich light. Do not under any circumstances look directly at the lamp as damage to the eyes could result. When changing a xenon lamp, remove the lamp from the protective case only when ready to install it and place the used lamp in the case immediately. Always wear safety glasses and heavy gloves when handling lamps (see 13.11).

13.10 Care should be taken when handling methylcyclohexane, or any other petroleum solvent, so as not to allow unnecessary contact with the skin or any contact with the eyes. Irritation may occur by the solvent acting directly on the skin. Working within a fume hood is advisable when rinsing glassware or transferring oil solutions, in order to minimize exposure to methylcyclohexane or other organic solvents. Methylcyclohexane itself is relatively non-toxic, but all organic solvents should be handled with care.

13.11 Safety glasses should be worn at all times while working in the laboratory. This would prevent accidental eye damage from solvent and/or liquid nitrogen. It is especially important to wear safety glasses while handling a xenon lamp, as it is under extremely high pressure and may explode violently. In the field, changing xenon lamps should be left to a qualified service operator.

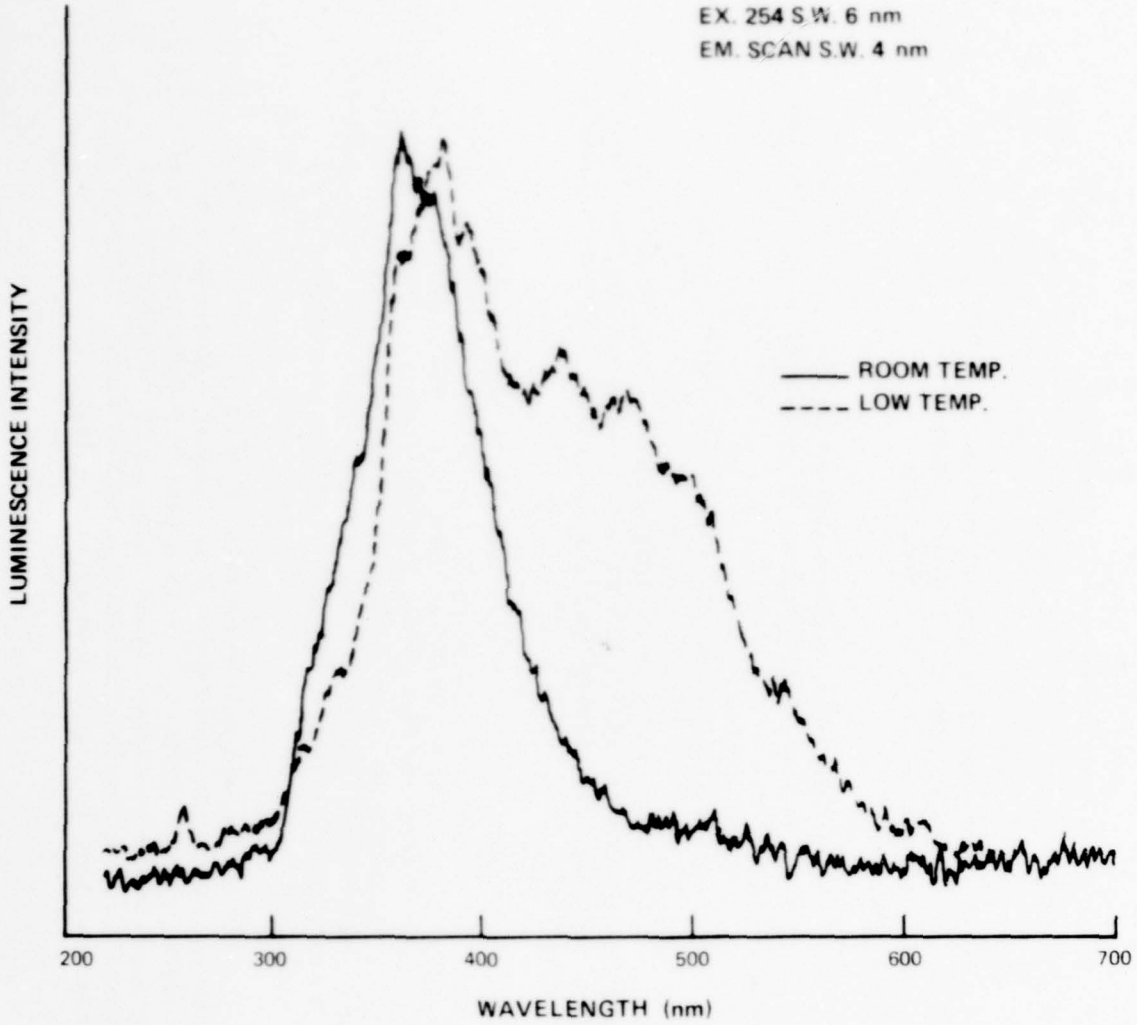


FIGURE 1. LUBRICATING OIL (MARINE DIESEL) ROOM VS. LOW TEMP.

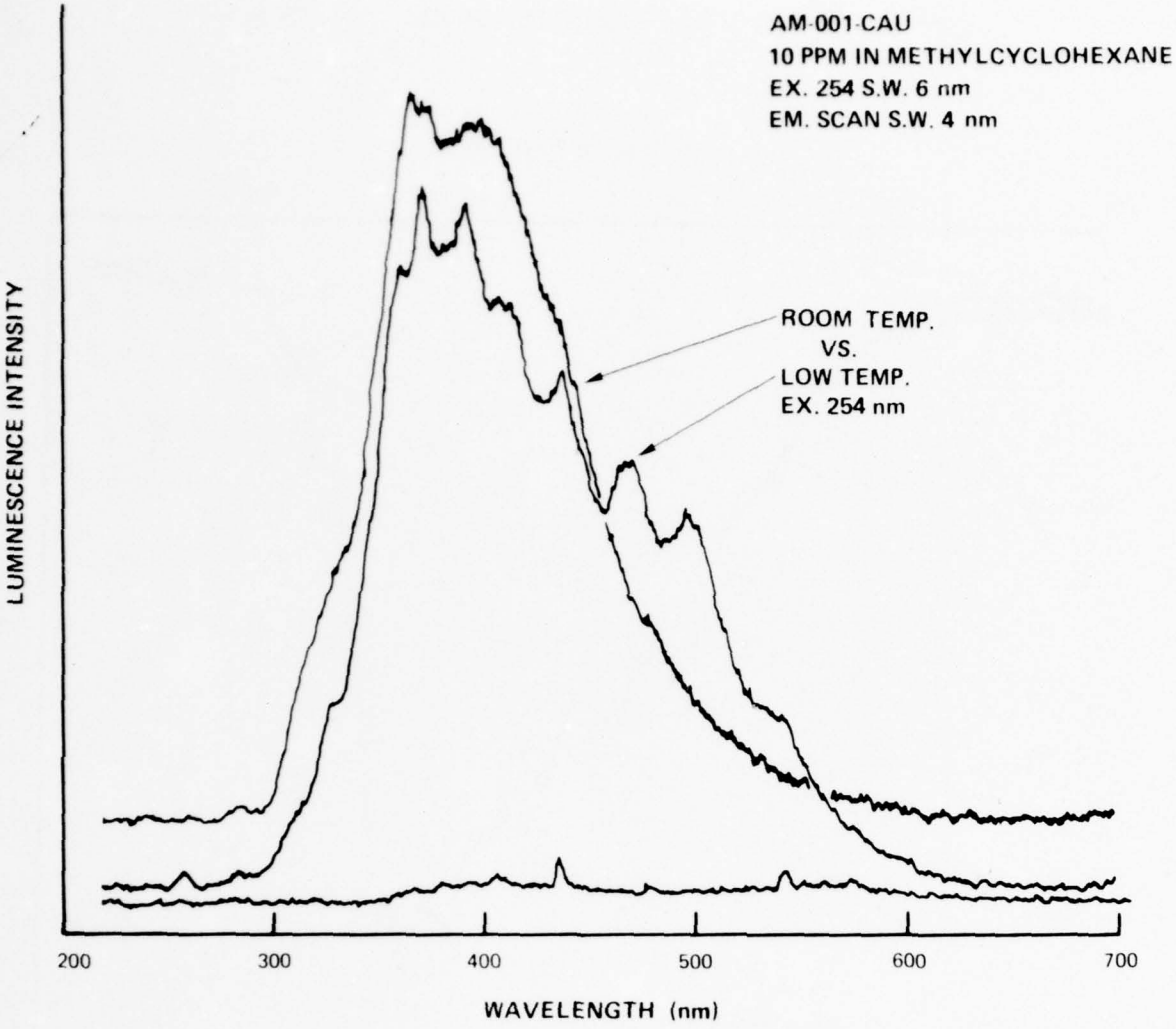


FIGURE 2. LIGHT CRUDE (AUSTRALIA).

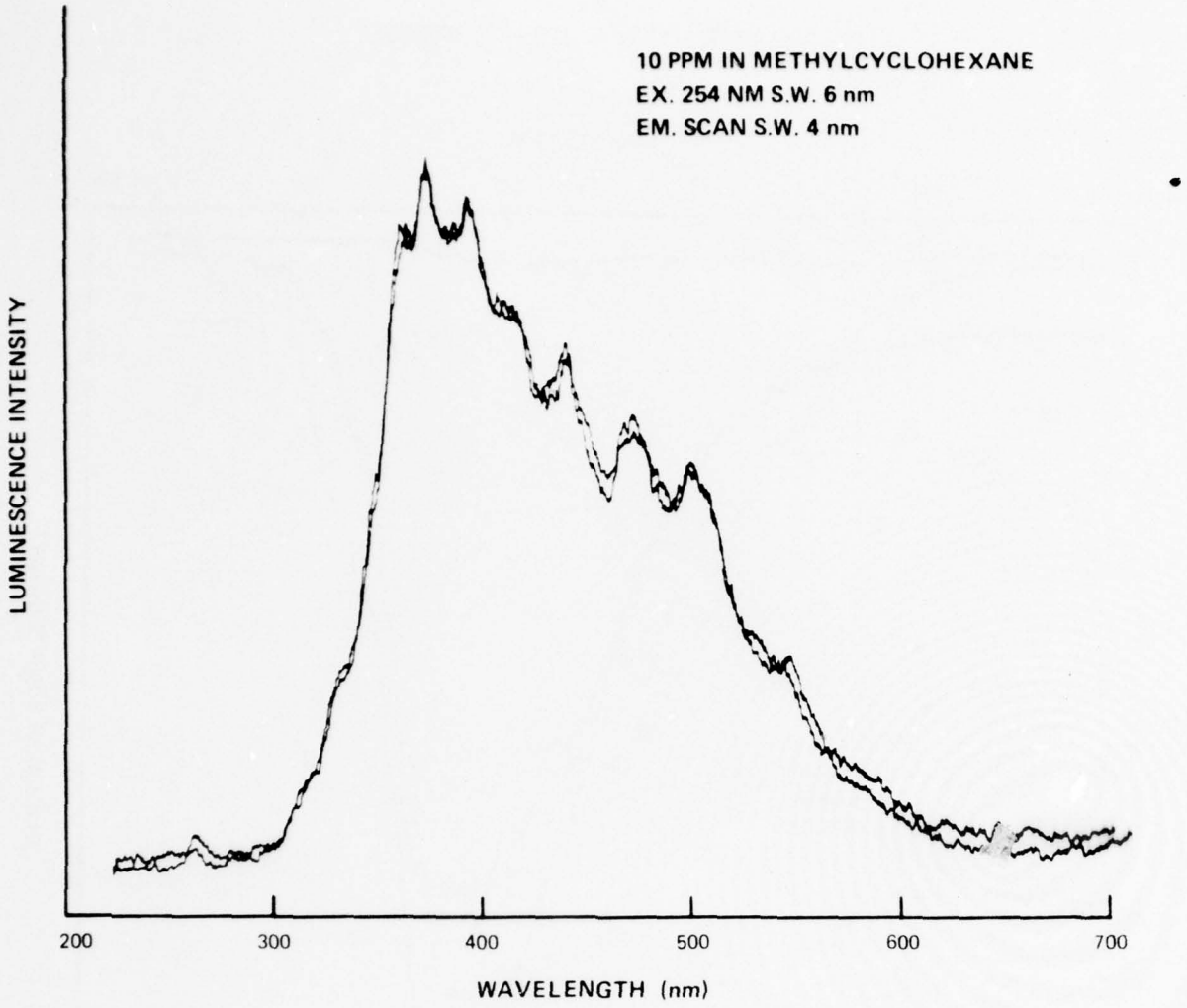


FIGURE 3. REPRODUCIBILITY LIGHT CRUDE (AUSTRALIA).

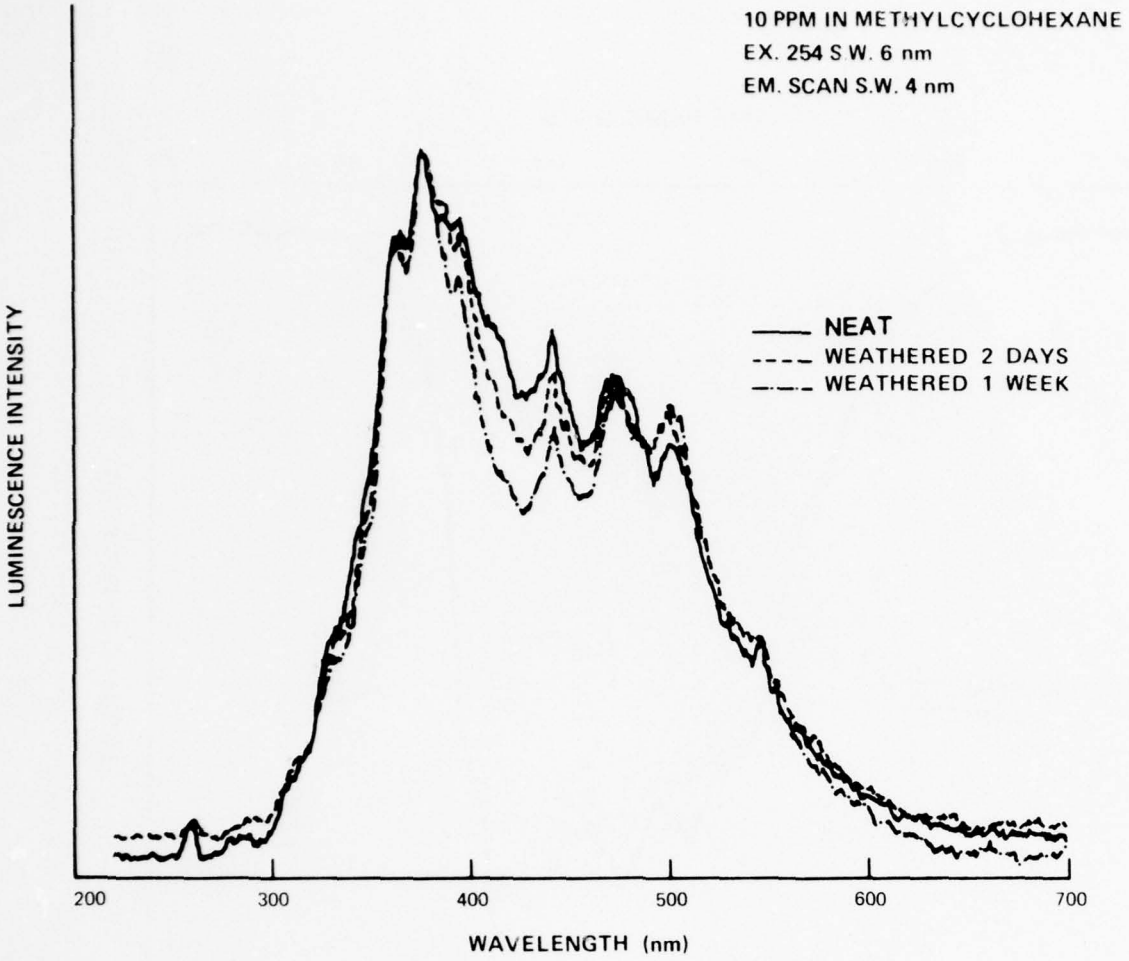


FIGURE 4. CRUDE (LOUISIANA).

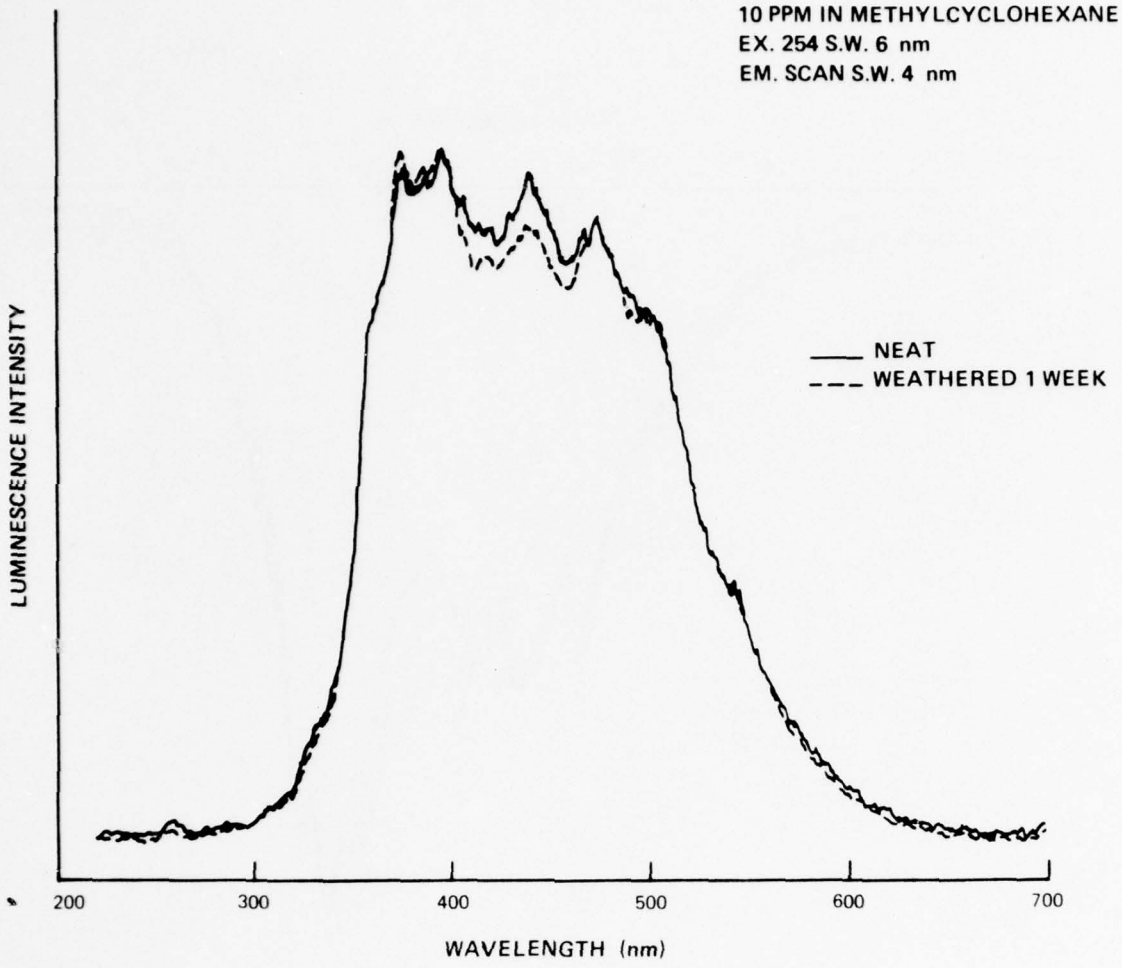


FIGURE 5. CRUDE ECUADOR.

APPENDIX G

Oil Spill Identification by Infrared Spectroscopy

1. Scope. The recommended infrared spectroscopic procedure provides a means of "fingerprinting" petroleum oil spills. This procedure consists of matching the infrared spectral characteristics of a spilled oil sample with a suspected source.

2. Summary of Method.

2.1 The initial step is to ascertain the exact technique required to record the infrared spectrum of an oil since: (a) the technique is dictated by the physical state of the sample; (b) spectra to be matched must be generated by the same technique. The technique involves the choice of cell, cell window material, sample thickness, etc.

2.2 Transmission spectra are obtained in liquid cells. The liquid cell not only has the advantage of confining the volatile components, thus preventing evaporation during the scan, but also insures a uniform oil thickness. These cells cannot be used for samples containing water (unless silver bromide windows are used), heavy, viscous oils or for small quantities of oils (less than 100 µl). The heavy viscous oil can be analyzed as a "smear" in a sealed demountable liquid cell.

3. Applications.

3.1 The infrared spectrum of any petroleum or non-petroleum oil provides abundant information as to its molecular composition. However, the primary application of the infrared identification method is to match a spilled oil sample to a sample from a potential source.

3.2 When an oil spill has been on the water for extended periods, or if the spill is a very light petroleum distillate, losses due to evaporation, dissolution and oxidation alter the spectrum sufficiently to make matching difficult. In such cases, the potential source oils can be artificially weathered by placing a portion of the suspect oil sample on water in a beaker. The sample thickness should not exceed 0.5 mm if reasonable weathering is to occur. The water should be natural or synthetic sea water if the spill occurred in the marine enviroment. The oil is sampled at various time intervals and analyzed to see if the extent of its weathering is comparable to that of the spill.

3.3 The infrared method may be applied to samples that are neat (undilute) oil, thin films of oil on water, oil coated on sand or other solids, or to oil emulsified in water.

3.4 Table I summarizes the method and its applications.

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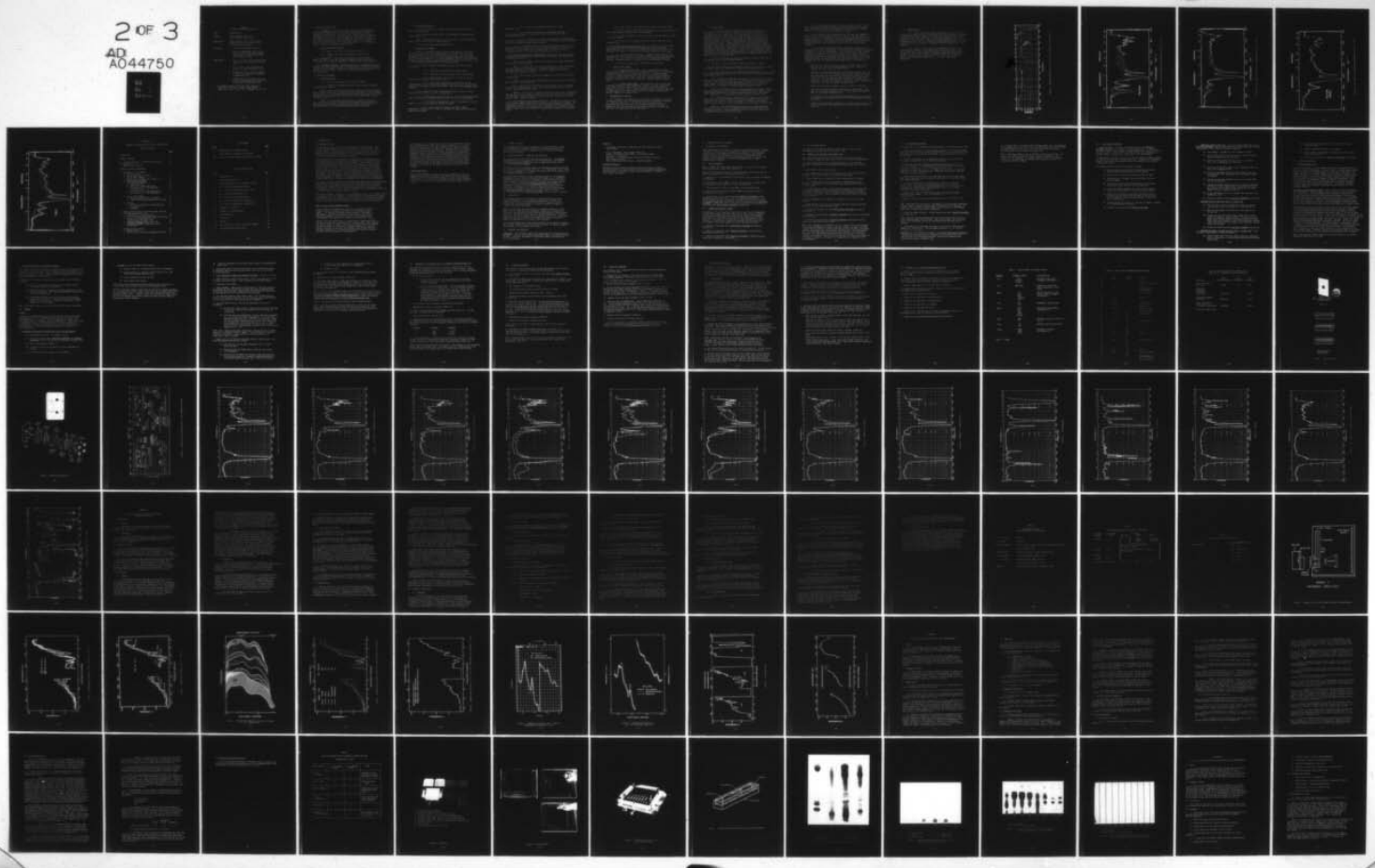

TABLE I
INFRARED FINGERPRINTING OF OILS

Type:	Transmission*
Purpose:	Rapid screening (Mini-cell) Identification (liquid cell)
Applications:	Smaller samples (10 µl) in Mini-cells Larger samples in macro cells (100 µl) For neat oils of all types
Cell Types:	1. Silver bromide (AgBr) Mini-cells. 2. Sealed or demountable cells, 0.05 mm with potassium bromide (KBr) windows; AgBr windows for samples containing small amounts of water.
Sample Types:	1. No. 1, No. 2 fuel oils, lubricants and light crudes can be analyzed in sealed cells. 2. No. 4 and No. 5 fuel oils should be analyzed in sealed demountable cells. 3. Residual oils (No. 6 fuel oil, Bunker C, asphalt) are analyzed as smears in a demountable cell. 4. Lighter oils extracted from solid substrates (adsorbent material, sand, etc.) are analyzed in demountable cells.

* A multiple internal reflectance (MIR) technique is described in "Oil Spill Identification System" Report No. CG-D-41-75 (Acc. No. AD A003803) October 1974.

4. Apparatus and Materials.

4.1 Instrument. The basic instrument used is the Perkin-Elmer 467 Infrared Spectrophotometer. The P-E 467 is equipped with an interlock between the chart paper drive and the diffraction grating, insuring wavenumber reproducibility from scan to scan. Other equivalent commercial instruments could be used. The instrument used should have a minimum wavenumber resolution of 1.6 cm^{-1} at 1000 cm^{-1} and 3.0 cm^{-1} at 3000 cm^{-1} and a minimum wavenumber accuracy of better than ± 4 cm^{-1} from 4000 to 2000 cm^{-1} and better than ± 2 cm^{-1} from 2000 to 250 cm^{-1}. The instrument should also have a photometric accuracy of $\pm 1\%$.

4.2 Cells, Windows and Crystals.

4.2.1 Cells. Sealed, sealed demountable, Mini-cells.

4.2.2 Windows. KBr, AgBr (particularly for Mini-cells).

4.3 Accessories. A reference beam attenuator for the infrared spectrophotometer. Auxiliary equipment includes a window polishing kit, a centrifuge, vortex mixer, hot plate, and a light-box for viewing spectra.

4.4 Expendable Materials. Expendables include the spectroquality solvents necessary for sample treatment and for cleaning cells (cyclohexane, methylene chloride, pentane, hexane), acetone for rinsing glassware, anhydrous magnesium sulfate for drying samples, chart paper, pipets, and calibrated 2.5 ml centrifuge tubes.

5. Analytical Procedures.

5.1 Spectrophotometer.

5.1.1 Warm up the instrument for at least 20 minutes.

5.1.2 Balance the instrument electronics and set gain according to the manufacturer's manual.

NOTE 1. Check the calibration daily by scanning a 0.05 mm polystyrene film. Observe whether the test spectra are within the limits of the instrument specifications. This calibration check should be performed before every oil spill set and the curve retained, with curves from the spill and suspects, as part of the case record.

NOTE 2. The instrument should be kept in a humidity-controlled room (<30% relative humidity to protect the CsI window on the detector) and left with the power plug in continually with the power switch turned off. This permits the built-in humidity-controlling heater to function.

5.2 Transmission Spectra.

5.2.1 Place sample in a liquid cell (see 5.5) and insert the cell into the infrared beam.

5.2.2 Set optical density (O.D.) to an absorbance reading of 0.02A (95%T) at 1975 cm^{-1}.

NOTE 3. The absorbance is set at a fixed value so that the resultant spectra can be compared from a common baseline.

5.2.3 Scan the spectrum from 4000-250 cm^{-1}.

5.3 Preparation of Sample.

NOTE 4. The primary objective in sample preparation is the removal of water to protect the sample cells and get a "clean" spectrum of the oil. If at all possible, the use of solvent should be avoided. It is sometimes necessary to use solvent in order to break refractory emulsions, or to extract the oil from solid substrates. It must be remembered that for valid comparisons of spectra, both oils being compared must have been prepared the same way, i.e., if one is deasphalted with pentane, the other must be also.

5.3.1 Water separation from medium to light oils can be achieved as follows:

5.3.1.1 Centrifuge to separate the oil/water layers.

5.3.1.2 Withdraw the lower water layer with a Pasteur pipet.

5.3.1.3 Repeat steps 5.3.1.1 and 5.3.1.2 until no visible traces of water remain in the centrifuge tube.

5.3.1.4 Before final centrifuging, place a small amount (approximately 0.1 g) of drying agent ($MgSO_4$) into the bottom of the centrifuge tube and mix thoroughly with the Vortex mixer. This will insure complete removal of water.

NOTE 5. Heavier oils may be prepared this way by using an elevated temperature (water bath up to 70°C, if necessary).

5.3.2 To remove water from very heavy oils, use the following procedure which will remove the asphaltene fraction as well as the water (See NOTE 4).

5.3.2.1 Dissolve approximately 1 gram (if available) of the oil sample in 15 ml of chromatoquality pentane or hexane.

5.3.2.2 Allow to sit 30 minutes, then add 1 g $MgSO_4$. Centrifuge at 2500 rpm (R.C.F. 1000) for 20 minutes to remove the precipitated asphaltenes and $MgSO_4$.

5.3.2.3 Decant the supernatant liquid into a 50 ml Erlenmeyer flask.

5.3.2.4 Wash the residue in the centrifuge tube with 2 ml of solvent (pentane or hexane) and combine the washings with water-free supernatant liquid.

5.3.2.5 Evaporate the solvent from the sample by bleeding high purity dry nitrogen over the surface for 1-3 hours (evaporation times are dependent on the oil viscosity-increasing with viscosity).

5.3.3 For oil samples present as thin films on water, extract the film from the surface by placing a layer of chromatoquality hexane (4-5 mm on the surface); stir and withdraw with a Pasteur pipet. Repeat, if necessary, to remove all the oil. Then evaporate the solvent as in 5.3.2.5.

5.4 Cell Loading Procedure (liquid Cells). Liquid cells (sealed or sealed demountable cells) are generally used with potassium bromide (KBr) windows and a 0.05 mm Teflon spacer.

NOTE 6. If the sample is suspected of being wet, use AgBr windows which will resist reaction with water.

NOTE 7. For light oils (low viscosity) such as No. 2 fuel oil, diesels, etc., the sealed cell should be used. It reduces evaporation losses and eliminates pathlength as a variable. DO NOT use a sealed cell if there is ANY chance that the sample is wet!

5.4.1 Fill the cell, tilted at 45 degrees, from the bottom port using a Pasteur pipet.

NOTE 8. Use care to avoid forming bubbles in that portion of the window which will be in the infrared beam.

5.4.2 Stopper the cell with Teflon plugs, taking care to avoid either air bubbles or undue pressure. Insert the bottom plug first using a twisting motion. Gently insert the top plug.

NOTE 9. If an oil is too viscous to flow in a liquid cell, a transmission spectrum still can be obtained. A large drop of the oil is placed on the center of a KBr window. Another KBr window is placed over this with a 0.05 mm Teflon spacer forming a uniform oil smear.

5.5 Cell Loading Procedure (Wilks Mini-Cell). The Mini-cell consists of two silver bromide (AgBr) windows with a 0.025 mm cavity pressed into the AgBr. The advantage of using this cell lies in the small amount of sample required for an analysis and the ease of cleaning the cell. The windows are inexpensive enough to discard when they become scratched or darkened due to exposure to ultraviolet light.

5.5.1 Place a drop of oil sample into the cavity of one AgCl window.

5.5.2 Place the flat side of the second window over the sample and carefully press together to obtain a 0.025 mm pathlength.

5.5.3 Slide the windows around on each other, with one edge up, to work out all air bubbles.

5.5.4 Gently lay the windows in the Teflon holder and screw in the retaining ring.

5.6 Cell and Crystal Cleaning Procedures. For forensic work, cell windows and crystals must be thoroughly cleaned. Complete removal of residual hydrocarbon materials is monitored by examining the infrared absorption in the 3000-2900 cm^{-1} region. A clean cell will show no absorption in this region.

5.6.1 For sealed demountable cells: dismantle cells completely and thoroughly rinse each component. The windows should be rinsed with cyclohexane; the rest of the cell (spacers, end plates, parts and plugs) with methylene chloride. Reassemble and test for absorption in the 3000-2900 cm^{-1} region.

5.6.2 Store cells and window components in a desiccator.

5.7 Care of Cell Windows.

5.7.1 Potassium Bromide (KBr). Keep potassium bromide windows in a desiccator when not in use, since moisture will fog them. If they become scratched, grind them on fine emery paper (3M 600). For deep scratches, first use a coarser grade emery paper (3M 220A), then polish the windows on an optical lap using cerium oxide or Barnsite. (Detailed instructions are given by the manufacturers of the commercially available window polishing kits.)

5.7.2 Silver Bromide (AgBr). AgBr windows are very soft and must be handled carefully to avoid scratching. Keep out of contact with metal. Also, protect them from light as much as possible, since they darken on exposure to ultraviolet light. These limitations require occasional replacment of these windows. The replacement frequency is governed by the spectral baseline changes due to these factors.

6. Interpretation of Spectra.

6.1 Ultimately, oil identification is based on a comparison of the spill spectrum with those of the various potential sources as outlined in Section 6.3. When the results are to be used for forensic purposes, then comparisons must be made on spectra obtained by using the same sample preparation, the same instrument with the same instrumental settings, the same cell, the same windows and the same spacer.

6.2 Overlay Method.

6.2.1 The overlay method consists of a visual comparison of the spectrum of a spill with that of a potential source using a light-box. This comparison is accomplished by first insuring that the spectra have comparable baselines at 1975 cm^{-1}, i.e., that they were arbitrarily set at an absorbance (O.D.) of 0.02 (95%T). Next, the absorbance at 1377 cm^{-1} is examined to obtain qualitiative assurance that the samples were analyzed at the same thickness. The curve is then scanned for overall similarities in shape from 4000-650 cm^{-1}. Between 1300-900 cm^{-1}, the baseline will tend to move downward (to higher optical density), but with little relative change of the peaks in that range. Examination of the 1770-1685 cm^{-1} region will reveal the extent of weathering - particularly in the 1708 cm^{-1} region where carbonyls from oxidative weathering first appear. Finally, the "oil fingerprint" region (900-650 cm^{-1}) is scrutinized for similarities. If slight variations do occur in this region, the peaks are examined for possible weathering influences. The sequential steps are outlined below.

NOTE 10. Before making a detailed comparison, make sure there are no interferences from residual foreign materials:

(a) For water in the spill sample, check in the 3400 cm^{-1} region.

(b) For residual $MgSO_4$ from the drying procedure, check the 610 cm^{-1} region for a small, sharp peak, and look for influences at 1075 and 1175 cm^{-1} .

(c) For residual pentane, if the sample has been deasphalted, look for a small pair of twin peaks at 910 and 920 cm^{-1}. There would be a corresponding increase in the peak at 722 cm^{-1}.

6.2.2 Examine the intensity of the 1377 cm^{-1} peak since it is a good indicator of the thickness of the cell. An absorbance value of between 0.85 and 1.0 at 1377 cm^{-1} gives the optimum fingerprint spectrum. If the two spectra being compared have virtually the same absorbance at 1377 cm^{-1}, then the identification of matching oil spectra becomes considerably easier.

6.2.3 If the spectra are of the same thickness of oil sample, compare the overall shape of the entire curves. If obvious differences appear in the region between 2000-650 cm^{-1}, then the non-matching spectrum of the suspect oil is disregarded. If similarities do exist, proceed to the next step.

6.2.4 Examine the 1685, 1708 and 1770 cm^{-1} regions for indications of weathering. Here, many weathered oils display well-defined carbonyl peaks, particularly at 1708 cm^{-1}. Even if the peaks are present in the carbonyl region in the spilled oil but not in the suspect oil, continue to the next step.

NOTE 11. Unweathered lubricant oils may show a strong sharp band near 1708 cm^{-1} due to a viscosity-modifying additive which diminishes with increased weathering. Unlike the additive peak near 1708 cm^{-1}, the peak due to weathering increases slowly and is broad.

6.2.5 Next examine the peak structures at 1304, 1165 and 1032 cm^{-1}. These peaks generally remain constant with weathering except for a general shift of baseline (between 1300 and 900 cm^{-1}) in the direction of higher absorbance. If they are unlike, disregard the suspect.

6.2.6 The next area scrutinized is the critical "oil fingerprint" region which presents the unique character of an oil. When the spilled oil is the same as the suspect oil, the peak shapes, amplitudes and locations parallel each other. These overlay spectra are designated as a match. For detailed examination of this region, lay one curve over the other in such a way that the curve traces coincide at 875 cm^{-1} prior to a peak-by-peak comparison.

6.2.7 If an oil has been altered by weathering, the analyst must take such effects into consideration. If the effects are moderate, the analyst can account for them as outlined below. If the effects are severe, the best procedure is to weather artificially a sample of the suspect to about the same degree as the spill. In the shorter time frames - under one week - the weathering changes are qualitatively well known. The lighter oils weather faster during this period; the heavier oils progressively more slowly. The following describes the significant weathering effects for different oil classes:

- Fuel oils No. 1 and No. 2 display losses in band structure at 849, 820, 810, 790, 782, 766 and 700 wavenumbers (cm^{-1}). There are apparent increases at 871, 832 and 722 cm^{-1}.

- Lubricant oils have additives which lose structure at 1708, 1355 and 1010 cm^{-1}. The 1235 cm^{-1} additive peak, however, remains stable even with weathering and is an excellent fingerprint for lube oils. The 722 cm^{-1} band is very strong in lubes and diminishes slightly with weathering. It is another excellent clue for the classification of lube oils. (Some No. 2 or diesel oils weather to leave a residue with a large 722 cm^{-1} band and strongly resemble lubes).

- Diesel oils show losses similar to the No. 1 and No. 2 fuel oils.

- No. 4 fuel oils show a decrease in the ratio of 744/722 cm^{-1} peaks. There is an apparent increase at 722 cm^{-1} with apparent decreases at 700, 744, 766, 782, 790 and 810 cm^{-1}.

- No. 5 and No. 6 fuel oils show minimal weathering effects - with slow development of the carbonyl peak at 1708 cm^{-1} and an apparent increase at 722 cm^{-1}.

- Crudes weather differently depending on the nature of the crude. Light crudes will weather like light fuel oils; heavy crudes like heavy fuel oils.

6.3 Sample Spectra.

6.3.1 Figure 1 shows the infrared spectrum of a No. 2 fuel oil to illustrate the general spectral characteristics of an oil analyzed by infrared transmission through KBr windows. This particular illustration is actually a superposition of three independent spectra which graphically show how reproducible replicates are - even with a sealed demountable cell - if proper techniques are used. The "oil fingerprint" region between 900-650 cm^{-1} can be seen to have a large amount of fine detail which is characteristic for a light oil.

6.3.2 Figures 2-5 show spectra from 2000-600 cm^{-1} for four oils weathered over four days. The original spectra were color-coded. They are included here to show the general effects on baselines between 1300 and 900 cm^{-1} and relative changes of individual peaks in the "fingerprint" region. The figures are, respectively: No. 2, No. 4, No. 6 fuel oils, and a Louisiana crude with curves at 0, 1, 2, 3 and 4 days outdoor weathering.

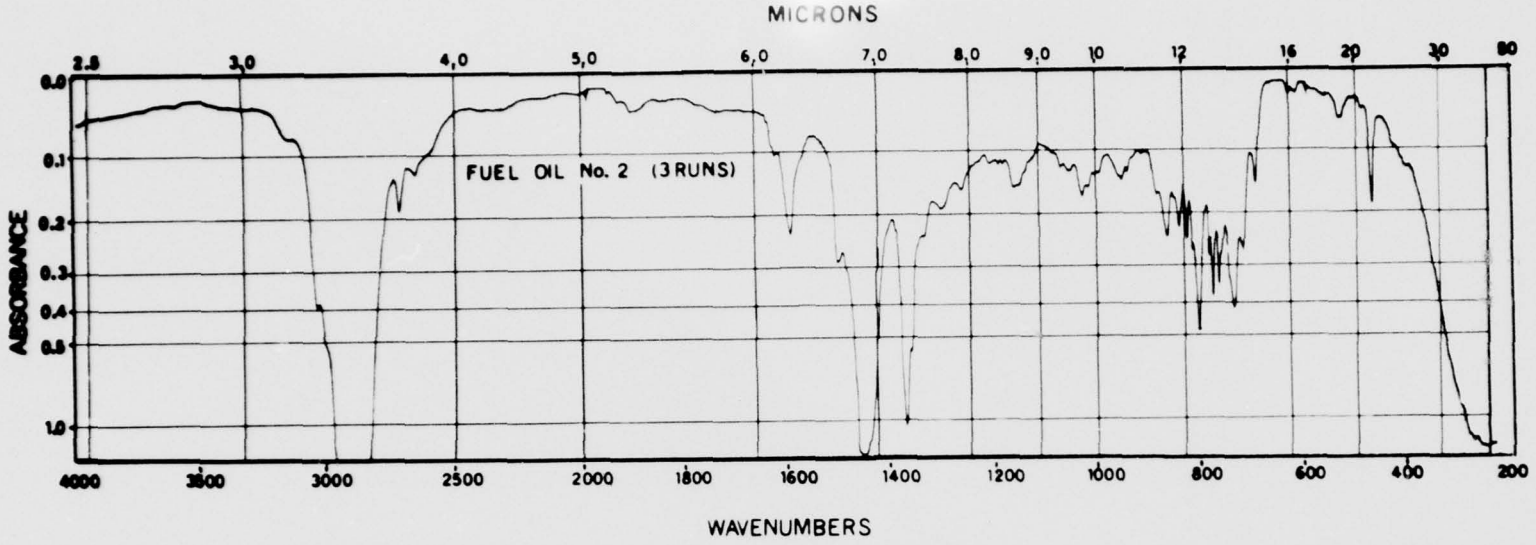

FIGURE 1. COMPLETE SPECTRUM OF A NO. 2 FUEL OIL, ANALYZED IN TRIPLICATE.

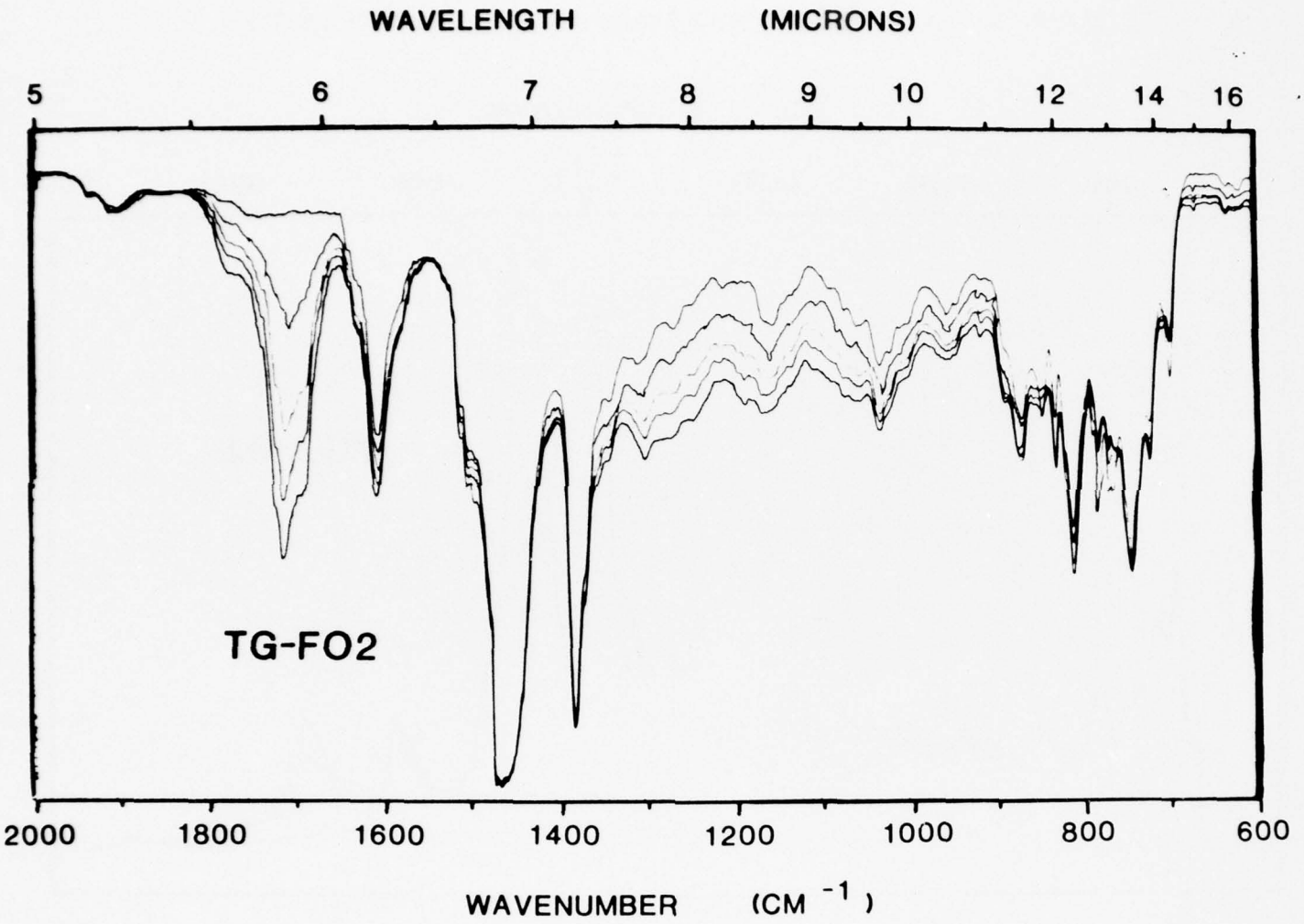

FIGURE 2. NO. 2 FUEL OIL PROGRESSIVE WEATHERING EFFECTS, 0-4 DAYS.

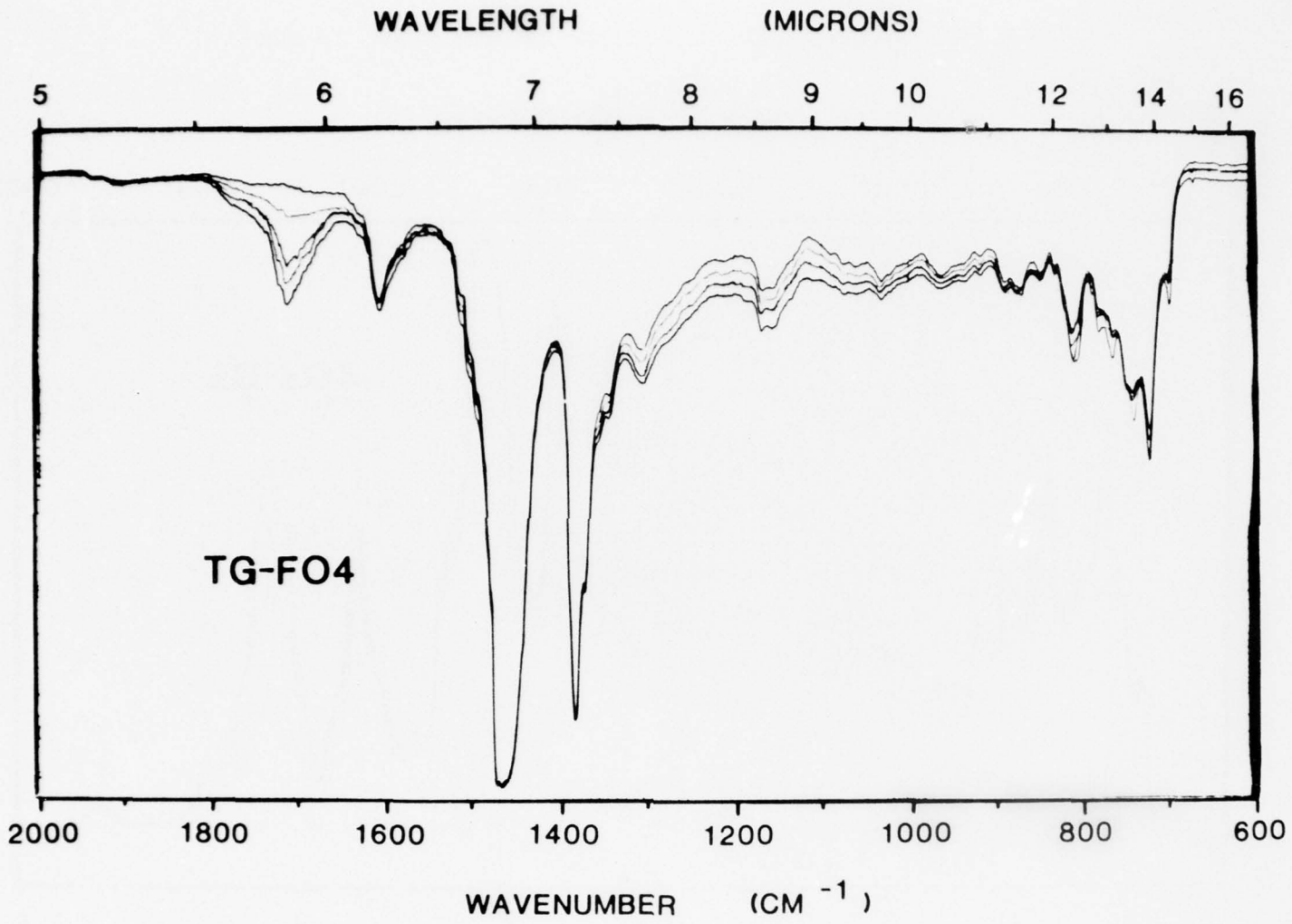

FIGURE 3. NO. 4 FUEL OIL PROGRESSIVE WEATHERING EFFECTS, 0-4 DAYS.

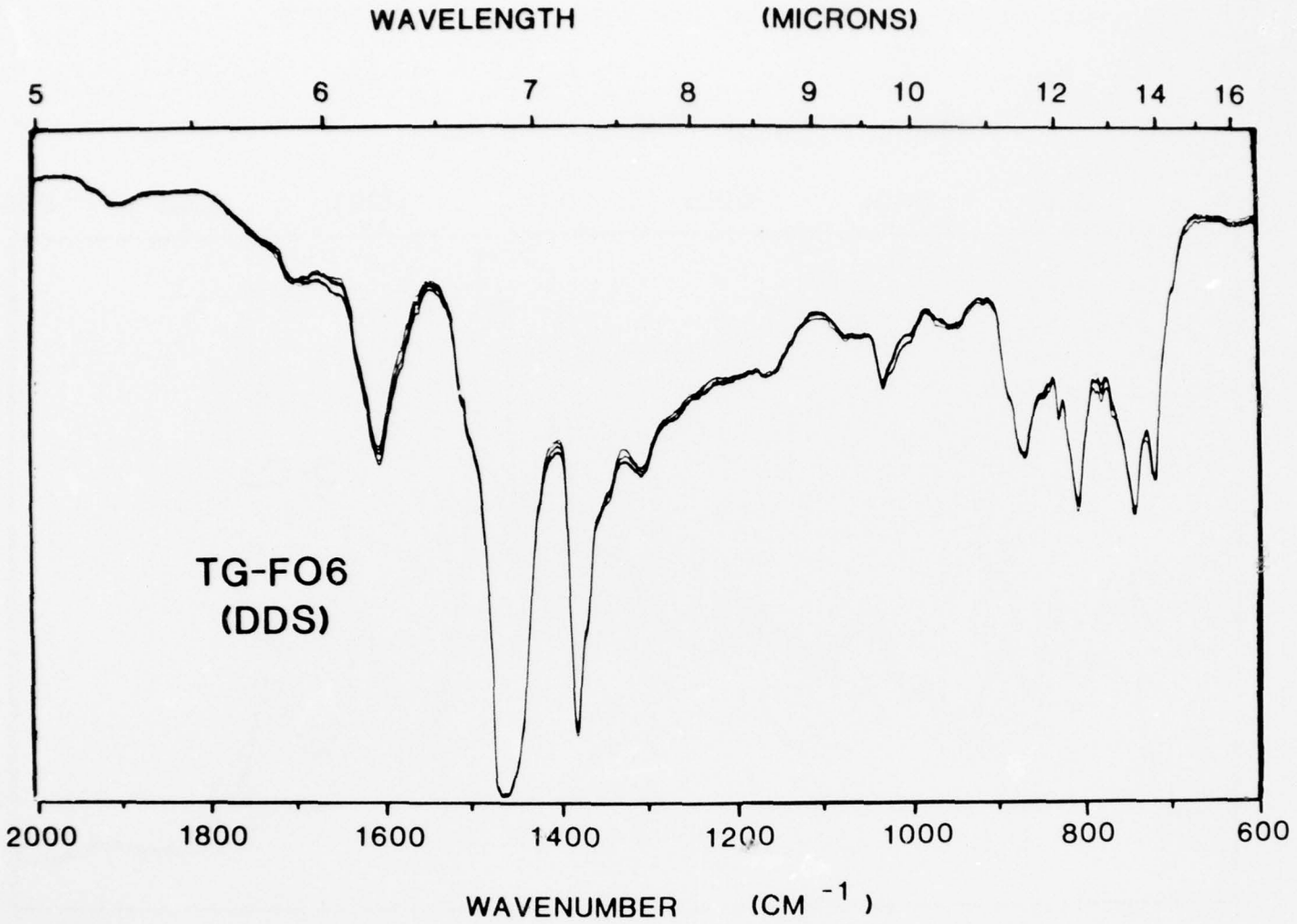

FIGURE 4. NO. 6 FUEL OIL PROGRESSIVE WEATHERING EFFECTS, 0-4 DAYS.

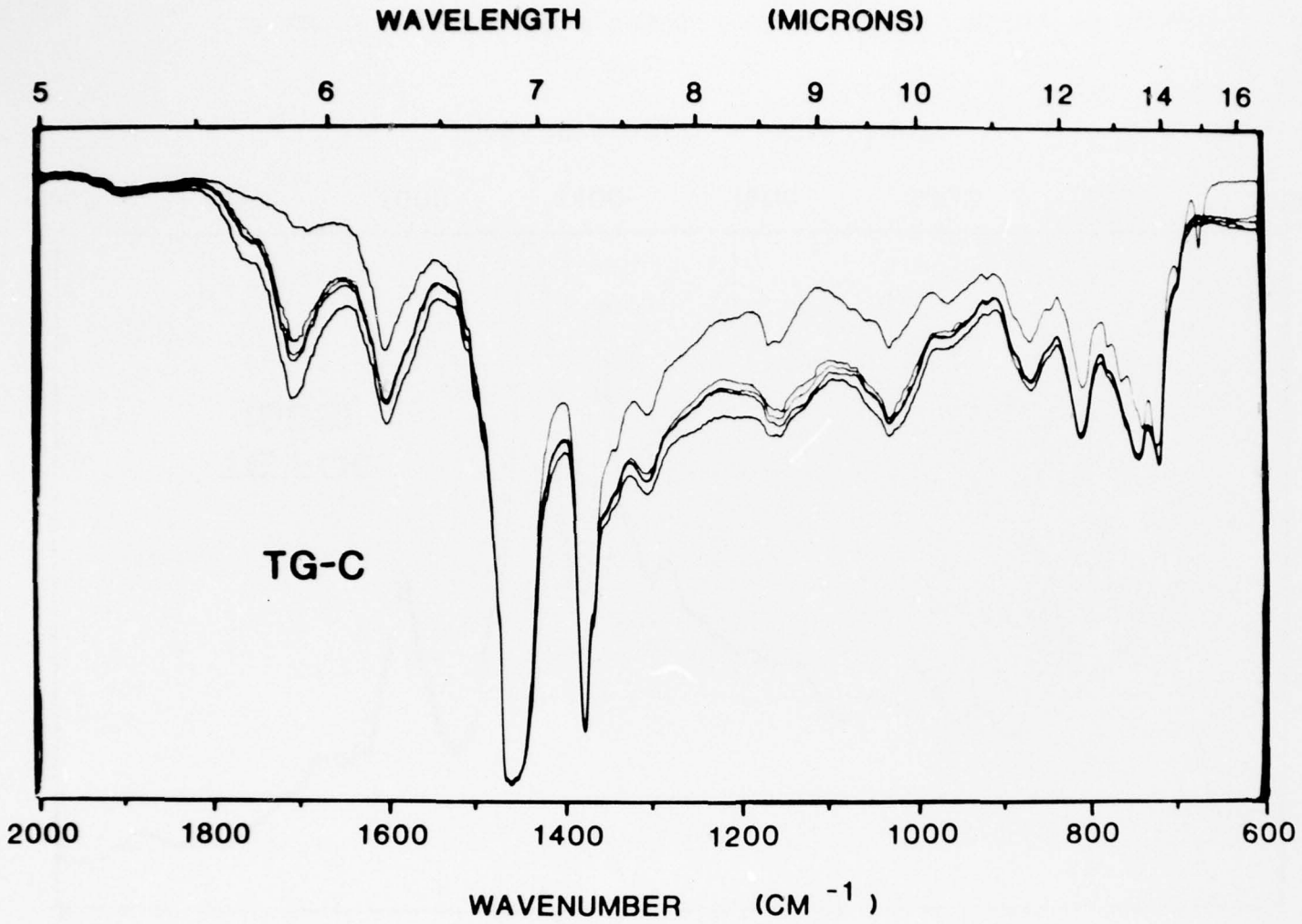

FIGURE 5. CRUDE OIL PROGRESSIVE WEATHERING EFFECTS, 0-4 DAYS.

APPENDIX H

INFRARED FIELD MANUAL FOR OIL SPILL IDENTIFICATION

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I. INTRODUCTION

Infrared Spectroscopy

All molecules are made up of atoms held together by chemical bonds. These act like springs between atoms, leaving the atoms free to vibrate. Each different molecule has its own set of atoms held together by its own set of springs, and the resulting motion of the atoms within the molecule will be different for every kind of molecule. Each bond type within the molecule has its own characteristic vibrational frequency.

Infrared spectroscopy is fundamentally a measurement of the absorptions of infrared radiation at frequencies corresponding to the vibrational frequencies of the molecular species present in an oil sample. This phenomenon can be measured because molecules absorb radiation at frequencies coinciding with the molecular vibrations as long as a net change in electric dipole moment occurs. Thus, if a beam of infrared radiation passes through an oil sample which has molecules vibrating at the same frequency as the beam of radiation, the molecules will absorb some of that radiation with a resultant decrease in beam energy. The absorption of energy is a function of the concentration of the molecules and the thickness of the molecules as well as the nature of the molecules themselves. However, if the vibrational frequency of the sample and infrared radiation do not match, the radiation will pass through undiminished.

The amount of energy transmitted (or absorbed) is measured with a spectrophotometer which passes an infrared beam through the oil sample and scans the infrared portion of the electromagnetic spectrum from 2.5-50 microns in wavelength (4000-200 wave numbers in cm^{-1}) and detects the beam with a thermopile. When a wavelength is reached at which energy is absorbed, the spectrophotometer deflects a pen and records an absorption peak on a chart.

The chart recording is referred to as an infrared spectrum. For oil identification, the spectra of oils are compared at all wave numbers - with particular attention to the 900-650 cm^{-1} region which is referred to as the "oil fingerprint" region. Peaks in this region are characteristic of a given oil and are used to identify a spill source by matching the spectrum of the spill to that of the source.

How an Infrared Spectrophotometer Works

In an infrared spectrophotometer, the source of radiation, usually a ceramic cylinder, is heated electrically to incandescence (about 1200°C). The glowing source emits radiation over a wide range of wavelengths. Only those wavelengths corresponding to the range of 4000-200 wavenumbers (cm^{-1}) are of interest in that region of the electromagnetic spectrum which is called the "mid-infrared" region.

Radiation from the glowing source is divided into a sample beam (which contains the sample to be analyzed) and a reference beam. After the beam has passed through the sample, a diffraction grating is used to disperse the infrared beam into radiation at different frequencies. Then the grating is rotated to scan over the frequency range so that different frequency components of the dispersed radiation are directed through a slit onto the infrared detector (thermocouple).

The intensities of the sample and reference beams remain equal so long as the sample does not absorb any transmitted radiation. At a point where the frequencies of the sample beam match the natural frequency of vibration of the molecule, energy will be absorbed, and the two beams will no longer be at the same intensity. The detector on the infrared instrument sees this difference in energy between the two beams and generates a signal proportional to the energy difference which, in turn, causes an optical attenuator to enter the reference beam and exactly compensate for the energy absorbed from the sample beam by the sample. The final position of the optical attenuator is thus directly related to the energy absorbed by the sample. By linking the recorder pen to the optical attenuator (either electronically or by direct mechanical linkage), the spectrum of the sample is recorded as absorbance versus frequency.

Interpreting Spectra

For oil identification, the spectra of oils are compared at all wavenumbers with particular attention to the 900-650 cm^{-1} region which is referred to as the "oil fingerprint region." Peaks in this region are characteristic of a given oil and are used along with others to identify a spill source by matching the spectrum of the spill to that of the source.

2. SUMMARY OF METHOD

The recommended field infrared spectroscopic procedure provides a means of "fingerprinting" petroleum oil spills. This procedure consists of matching the infrared spectral characteristics of a spilled oil sample with a suspected source.

2A. INFRARED PROCEDURES - SCREENING AND ANALYTICAL

The two techniques used in the field infrared method are: The SCREENING PROCEDURE which uses a Mini-cell (silver bromide windows), and the non-screening ANALYTICAL PROCEDURE which uses a demountable sealed liquid cell (potassium bromide windows).

The initial step is to ascertain whether the preliminary screening technique is necessary for oils in a given spill set. If a large number of oils (8 or more) are to be analyzed, or if water is present in any of the samples, then the SCREENING PROCEDURE is dictated.

The two infrared procedures provide transmission spectra. The SCREENING PROCEDURE which uses the water-insoluble AgBr windows in the Mini-cell allows the analyst to test for the presence of water while also offering a rapid device for screening out those oils that do not resemble the oil spill. The more reproducible ANALYTICAL PROCEDURE which employs the demountable sealed cell and potassium bromide windows (KBr) offers the advantage of confining an oil's volatile components thus preventing evaporation and insuring a uniform oil thickness during the scan. When the SCREENING PROCEDURE verifies that all water (Table 1, Figure 7) has been removed then the ANALYTICAL PROCEDURE can be used with confidence.

2B. APPLICATIONS

The infrared spectrum of any petroleum or non-petroleum oil provides abundant information as to its molecular composition. However, the primary application of the infrared identification method, to satisfy current Coast Guard requirements, is to match a spilled oil sample to a sample from a potential source.

When an oil spill has been on the water for extended periods, or if the spill is a very light petroleum distillate, losses due to evaporation, dissolution and oxidation alter the spectrum sufficiently to make matching difficult. This process of alteration is termed "weathering". In such cases, the oils from a suspect ship or fueling facility can be artificially weathered by placing a portion of the suspect oil sample on water in a beaker and sampling the oil at various time intervals.

The "Infrared Method for Oil Spill Identification" may be applied to samples that are neat (unaltered) oil, films of oil on water, oil coated on sand or other solids, or to oil emulsified in water.

2C. APPARATUS AND MATERIALS

Instruments. The instrument used is the Perkin-Elmer 727B Infrared Spectrophotometer. Refer to the instrument instruction manual for information which includes: operation, maintenance, installation, theory, specifications and troubleshooting.

Apparatus

Centrifuge, Vortex mixer, light box, hot plate, Barnsite, silicon carbide paper

Cells - Demountable sealed liquid, Mini-cell
Windows - Potassium bromide (KBr), silver bromide (AgBr)
Accessories - Attenuator
Fume Hood - To provide adequate ventilation to handle solvents.
Waste solvent container.
Teflon wash bottles for solvents - properly labelled!

Expendable Materials

Expendables include anhydrous magnesium sulfate for drying oil samples, solvents necessary for cleaning cells and polishing windows (reagent grade dichloromethane, cyclohexane, toluene, acetone, methanol), centrifuge tubes, pipet, chart paper.

3. INFRARED ANALYTICAL PROCEDURES

A. Preparation of Oil Samples

Sample preparation is the key to an accurate and reliable analysis. Extreme care must be exercised in the handling of oil samples, and in the use and cleaning of all the equipment involved in the analysis. The following section refers to the procedures to be used in (1) preparing various types of oils for analysis, (2) cell loading procedures, (3) care of cell windows, and (4) cleaning of cells and all glassware used in sample preparation and analysis.

3A1. NEAT OIL SAMPLES

Neat oil samples are those which are unaltered. They are free of foreign matter such as water, sand, dirt, debris, etc.

When neat oil samples arrive at the Coast Guard operational field unit for analysis proceed with the following instructions:

a. Exercise care in the removal of jar and bottle caps to prevent introducing contamination.

b. Carefully draw off a sample of about 2 ml of oil with a Pasteur pipet. This measurement can be made in a 2.5 ml centrifuge tube.

c. Place withdrawn oil into a clean sample vial with Teflon liner.

d. Mark vial with the identical sample number that appears on the oil collection jar or bottle.

e. Scan all neat oils as instructed under ANALYTICAL PROCEDURE, using a demountable sealed liquid cell. This is described in Section 3B5. (If water is suspect in oils that appear to be neat then scan oils first with the SCREENING PROCEDURE, Section 3B4, before proceeding with the ANALYTICAL PROCEDURE. If water is evident in the screening spectrum then proceed as instructed under WATER REMOVAL PROCEDURE, Section 3A5.

3A2. SAMPLES WITH THIN OIL LAYERS

For samples of oil on water where the slick is too thin to be sampled by using a Pasteur pipet, strips of Teflon can be used. Since oil adheres to Teflon more readily than water does, collect the oil by dragging a Teflon strip on the surface of the water in the sample bottle and then allow the oil to drip off the strip onto a clean aluminum pan or into a clean jar. Continue until enough oil, preferably not less than 1 ml, is collected. With water present in the oil, proceed as follows:

a. Proceed as instructed under WATER REMOVAL PROCEDURE described in Section 3A5.

b. Proceed as instructed under SCREENING PROCEDURE, Wilks Mini-cell, described in Section 3B4.

c. Proceed as instructed under ANALYTICAL PROCEDURE, demountable sealed liquid cell, described in Section 3B5.

3A3. OIL AND WATER SAMPLES

For samples of oil that are ample but contain water, follow the three procedural steps listed under Section 3A2a, b and c.

3A4. SAMPLES OF OIL ON SAND, DIRT, DEBRIS, ETC.

Oil can be removed for analysis from oil-soaked material such as sand, dirt, sawdust, vermiculite, grass, glass wool, etc., by the following method:

a. Place several grams of the oil-soaked material, into a centrifuge tube that is labelled with the identification number on the oil collection jar.

b. Cover material with distilled water.

c. Stir contents with a spatula to enable the oil to free itself from the material. Most oils will rise to the surface of the water.

d. Allow to stand for a few minutes to enable remaining oil to surface.

e. Stir contents again if some oil appears to remain adhering to the material. (Continue the stirring and settling procedure until about 2 ml of oil is free.)

f. If heavy dark oils refuse to separate easily from the foreign materials, place the centrifuge tube in warm bath water (temperature kept constant at 35-40°C). With heavy crudes and residual oils, temperatures as high as 60-70°C may be necessary. Allow oil material mixture to stand for 1/2 hour in the warm bath.

g. Stir the warmed mixture repeatedly to enable the oil to rise to the surface.

h. Remove the surface oil in the centrifuge tube with a Pasteur pipet and place this oil in a clean centrifuge tube.

i. Proceed with instructions under WATER REMOVAL PROCEDURE described in Section 3A5.

j. Proceed as instructed under SCREENING PROCEDURE Wilks Mini-cell, described in Section 3B4.

k. Proceed as instructed under ANALYTICAL PROCEDURE, demountable sealed liquid cell, described in Section 3B5.

NOTE: The techniques of floating the oil off of debris offers the advantage of avoiding contamination by organic solvents. However, in some intractable cases it may be necessary to extract with pentane, dry with $MgSO_4$, centrifuge, withdraw the pentane layer and evaporate under nitrogen. The disadvantages are: (1) some asphaltenes may not dissolve and thus alter the spectrum obtained; (2) some residual pentane may alter the spectrum. The residual pentane can be readily observed in the 910 and 920 cm^{-1} region (See Figure 15) to monitor its removal. For details of deasphalting, see Appendix D (Gas Chromatography).

3A5. WATER REMOVAL PROCEDURE

To remove water from oil-water mixtures proceed with the following instructions:

a. Using a Pasteur pipet (5 3/4"), withdraw a sample of the oil-water mixture from the surface of the oil collection jar (about 2 ml, and not less than 1 ml). This amount will fill about 3/4 of the pipet. Avoid drawing water from the bottom layer.

b. Place the oil mixture into a disposable centrifuge tube labelled with the identical number that is on the oil collection jar.

c. Place this tube into a beaker of warm water (temperature kept at 35-40°C) for 10 minutes. For very heavy oils it may be necessary to warm oils in a bath to about 60°C. Avoid such high heat on light and semi-heavy oil types (Nos. 1, 2, 4, 5, light crudes and lubes) as the light components in these oils would be lost. (Exercise caution when using heat with oils to prevent ignition.)

d. Remove water from the base of the centrifuge tube with a Pasteur pipet. When a small amount of water is present in the tube use a disposable syringe with needle (18 gauge).

e. Place the remaining oil in centrifuge for 15 minutes at 2500 rpm (R.C.F. 1000). Place some of the warm bath water in the centrifuge receptacles thus extending the warm environment while protecting the tubes from breaking. Always balance centrifuge with a tube of equal weight opposite to that being centrifuged.

f. Remove water from oil with syringe or pipet and add about 150 mg of magnesium sulfate ($MgSO_4$). This is approximately 0.5 ml in a 2.5 ml centrifuge tube.

g. Mix well on Vortex mixer and centrifuge sample for 15 minutes at 2500 r.p.m.

NOTE: Some heavy oils such as No. 6 and Bunker C's and also highly emulsified oils may require a longer length of time in mixing and in centrifuging. For example, "heavy weathered oils may require up to two hours of centrifuging while light oils may require only 10 minutes."[1].

h. Screen oil sample for water. Follow instructions under SCREENING PROCEDURE in Section 3B4.

NOTE: Exercise care when removing oil from the centrifuge tube in order to avoid disturbing $MgSO_4$ with the pipet. Also, avoid contact with the bottom layer of the centrifuge tube and the side walls where some $MgSO_4$ may be adhering.

i. Check spectrum for entrained water which can be seen at 3450-3333 wavenumbers cm^{-1} and 1640 cm^{-1}. (For an example of water in an oil spectrum see Figure 7.) See Table 1.

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[1]Ahmadjian, M., C. D. Baer, P. F. Lynch, C. W. Brown, "Experimental Conditions for Matching Laboratory and Marine Weathering of Petroleum by Infrared Spectroscopy," 1975 Pittsburgh Conference on Analytical Chemistry and Applied Spectroscopy, Cleveland, Ohio.

j. If water remains in spectrum remove the lower water layer, add 50-100 mg more of $MgSO_4$, mix on the Vortex mixer and recentrifuge for a longer length of time depending upon the type of oil. Follow the same procedure as described above after the addition of $MgSO_4$ (g-i).

NOTE: Avoid excess $MgSO_4$ as it can interfere with an oil spectrum. See Figure 4 for $MgSO_4$ spectrum, and Table 1 for peak assignments. The 610 cm^{-1} peak is diagnostic for residual $MgSO_4$. Since the 727B cuts off at 600 cm^{-1}, the curve must be examined very carefully at 610 cm^{-1} to discern the presence of $MgSO_4$.

3A6. CELL LOADING PROCEDURES

a. WILKS MINI-CELL. This cell, used specifically in the SCREENING PROCEDURE, consists of a threaded, two-piece Delrin body, "O" ring and two silver bromide (AgBr) windows. The AgBr windows each contain a 0.025 mm circular cavity. The rim of the window is flat and the circumference is beveled to ensure a proper seal. (See Figure 1 for illustration.)

The advantage of using this cell lies in the small amount of sample required for analysis and the ease of cleaning the cell. The windows are inexpensive enough to discard when they become scratched or darkened due to exposure to ultraviolet light.

To assemble and fill cell with oil proceed as follows:

(1) Place the body of the Mini-cell (the portion containing internal thread) with the open end facing upward.

(2) Install the "O" ring into the bottom of the cell body.

(3) Place a drop of oil sample into the 0.025 mm. cavity of one AgBr window.

(4) Slide the flat side of the second window over the sample and carefully press together to form a tight seal.

(5) Slide the windows around on each other, with one edge up, to work out all air bubbles or press outer edges firmly.

(6) Gently lay the windows in the Delrin holder and screw in the barrel portion (externally threaded) of the cell until it fits securely. Apply consistent pressure to barrel for each oil application.

(7) Recheck windows for uniform oil film and for bubbles. Reapply oil if bubbles cannot be removed.

(8) Proceed as instructed under SCREENING PROCEDURE.

b. DEMOUNTABLE SEALED LIQUID CELL. This cell, used specifically in the ANALYTICAL PROCEDURE, employs potassium bromide windows (KBr) and a 0.05 mm Teflon spacer. To use this cell proceed as follows:

(1) Cell Assembly. Assemble the cell as shown in Figure 2.

(2) Lay the back plate on a flat surface with the studs up and place the window guide over the studs.

(3) Insert the rubber gasket into the cavity of the window guide, with an undrilled KBr window next.

(4) Place the 0.05 mm Teflon spacer on the window and cover the spacer with a drilled window.

(5) Place the lead gasket over the drilled window so that the filling holes in the lead gasket and in the drilled window are aligned.

(6) Install the top plate assembly and fasten it securely with the quick-acting nuts.

(7) Tighten the quick-acting nuts evenly in criss-cross pattern; turn each nut down a small amount, then proceed to the next and continue in this fashion until all the nuts are tight enough to prevent leakage from the cell.

(8) Do not overtighten the nuts or tighten them unevenly, as the windows may crack.

(9) Proceed as instructed under ANALYTICAL PROCEDURE, Section 3B5.

c. PROCEDURE FOR OILS THAT WILL FLOW IN A LIQUID CELL

(1) Fill the cell, tilted at 45 degrees, from the bottom port using a Pasteur pipet or syringe with a Luer-Lock tip.

(2) When the cell appears filled with oil, place some oil in the top port.

(3) Stopper the cell with Teflon plugs, taking care to avoid either air bubbles or undue pressure. (If bubbles do occur, gently force bubbles through by applying pressure on bottom Teflon Plug or pull bubbles through with a disposable syringe.) Insert the bottom plug first using a twisting motion. Gently insert the top plug.

(4) Proceed as instructed under ANALYTICAL PROCEDURE, Section 3B5.

d. PROCEDURE FOR HEAVY OILS THAT WILL NOT FLOW IN A LIQUID CELL. This procedure is described as a "smear technique."

(1) Place a large drop of the oil, using a spatula or applicator stick, in the center of the KBr window that holds the 0.05 mm Teflon spacer.

(2) Place another KBr window over the oil and press to form a uniform oil smear.

(3) Assemble remaining cell parts. See Figure 2.

(4) Follow previous instructions for stoppering cell with Teflon plugs (Section 3A6c(3)).

(5) Proceed as instructed under ANALYTICAL PROCEDURE, Section 3B5.

3A7. CARE OF CELL WINDOWS

a. POTASSIUM BROMIDE (KBr)

The care and handling of the KBr windows used with the demountable sealed cell is critical to the quality of infrared analysis. Window fogging, caused mainly by etching of the window surfaces by water vapor or moisture in the sample, may result in a sloping baseline or in excessive reduction of the energy transmitted by the cell. Sample residues occluded on window surfaces may absorb energy in a way which will seriously hinder the interpretation of other spectra or reduce the accuracy of quantitative analyses. These difficulties can be overcome by keeping the windows clean and dry, and by polishing them. When a KBr crystal window surface has become scratched, follow the polishing procedure described below. If, however, the window is merely fogged, omit grinding on sandpaper and proceed with the final polishing operation:

Polishing Potassium Bromide Windows - Place a sheet of 3M 600 grit sandpaper on a flat surface. Grind the window surface by moving it back and forth, in a straight line, over the sandpaper until any surface imperfections, such as scratches or insoluble sample residues, disappear. Apply an even pressure while grinding in order to maintain a flat surface. If the windows are badly scratched, use a coarser silicon carbide paper, such as 400 grit, for the first grinding. Follow with a second grinding using 600 grit paper and a motion perpendicular to that of the first grinding. Continue with the 600 grit paper until the scratches from the coarse paper are no longer visible.

After grinding, hold only the ends of the window (in order to prevent fogging from finger moisture) and polish the window surface as follows: Sprinkle a little polishing compound (Barnsite) over a two-inch diameter circle on a lap cloth and moisten the compound with several drops of a 10 percent water-methanol solution saturated with KBr. Polish the window surface by rubbing it over the compound with a circular motion. After about 10 seconds of polishing, wipe it off the window quickly using a dry, clean portion of a polishing cloth. Repeat the above polishing and wiping procedure for about 3 minutes. Inspect the window surface to see whether additional polishing is required. The surface should be clear and free from scratches. If a mottled surface "orange-peel" effect is evident, it is unacceptable and should be removed. If no further polishing is needed, grind and polish the other side of the window. Rinse windows with cyclohexane to remove any residual Barnsite on the window surfaces and in the drilled holes. Re-examine the holes in the drilled window to be certain they are free of Barnsite as this compound could contaminate the oil sample.

NOTE: Keep potassium bromide windows in a desiccator when not in use since atmospheric moisture will fog them.

b. SILVER BROMIDE (AgBr) REPLACEMENT PROCEDURE

AgBr windows are very soft and must be handled carefully to avoid scratching. Also, protect them from light as much as possible, since they darken on exposure to ultraviolet light. These limitations require occasional replacement of the windows. The replacement frequency is governed by the spectral baseline changes due to these factors.

To determine the necessity for AgBr window replacement use the following procedure:

(1) Set Mini-cell sample holder and Mini-cell (blank windows) in the sample beam of the instrument.

(2) Measure the percent transmission at 4000 wavenumbers cm-1 and record in a notebook. New, non-degraded windows register about 43 percent transmission.

(3) After windows have been used 10-15 times, recheck percent transmission at 4000 cm-1. Degraded, darkened, unacceptable windows will register about 33 percent transmission. These windows are to be discarded.

NOTE: Store AgBr windows in the dark and avoid direct contact with metal.

3A8. CLEANING

IR CELLS

All parts of the demountable sealed cell and Mini-cell must be thoroughly cleaned with a solvent. Cyclohexane may be used for the lighter oils, dichloromethane, or toluene, is used for semi-heavy and heavy oils. (Cyclohexane causes less fogging than dichloromethane.) (Always clean ports of demountable sealed cell and drilled holes of the KBr windows with dichloromethane to prevent contamination.) Exercise caution with use of dichloromethane. It is toxic and should be used with a fume hood. Avoid skin contact.

a. PROCEDURE FOR MONITORING CONTAMINATION ON KBr and AgBR WINDOWS

(1) Reassemble clean cells. See Figures 1 and 2.

(2) Follow instructions under ANALYTICAL PROCEDURES and SCREENING PROCEDURES (Sections 3B4 and 5) for instrument adjustment only.

(3) Scan clean windows to 2800 cm^{-1}.

(4) A clean cell will show no absorption in the 3000-2900 cm^{-1} region.

(5) If absorbance peaks do appear, reclean windows.

b. GLASSWARE (2.5 ml centrifuge tubes, beakers)

(1) Remove as much oil as possible with solvent (cyclohexane).

(2) Wash equipment with Sparkleen (Fisher Scientific Co.) and Jansolv-60 (Sunshine Chemical Corp.).

(3) Rinse equipment first with tap water.

(4) Rinse finally with acetone.

NOTE:1 When cells and glassware have been cleaned, store these items in a clean drawer that has been lined with clean disposable towels.

NOTE:2 All the waste organic solvent that is used in the cleaning procedure is to be poured into a Teflon liquid waste bottle that is marked specifically for waste organic solvent. Caution: Never pour inorganic materials into the organic waste bottle. Transfer contents of Teflon liquid organic waste bottle to the organic liquid waste storage can daily after all cleaning operations have been completed.

3B. OPERATING PROCEDURES FOR THE PERKIN-ELMER INFRARED SPECTROPHOTOMETER MODEL 727B

1. Read Perkin-Elmer 727B Instruction Manual for a complete description of instrument parts with photos, maintenance, operation, specifications and troubleshooting.

2. DAILY INSTRUMENTAL WARMUP AND OPERATING PROCEDURE. Proceed as follows:

a. Power Push Button Switch (front panel) - Press to provide power to the instrument (indicator glows). Wait 10 minutes for instrument warmup.

b. Insert pen in pen holder.

c. Chart Alignment - Make certain the chart paper is installed correctly by aligning the 2000 cm^{-1} mark on the paper with the corresponding mark on the chart paper carriage. (Make certain the bottom of the chart paper is flush against the lip on the bottom of the carriage.)

d. Auto-Set Mode Selector Switch (front panel) - Set the Mode Selector Switch to the Resolution position. Do not use Survey (Fast Scan) position for Oil Identification or when calibrating the instrument.

e. Gain Auto-Chek™ Control (slotted knob adjustment at top left of instrument):

(1) With the pen raised, scan off, manually move the paper carriage to 1975 cm^{-1}. Use the 100% control to set the pen at 90% transmission.

(2) Press the Gain Auto-Chek™ knob. The pen should move downscale 10 ± 1% (from 90% to 79-81% transmission). If the pen moves downscale by more than 11% T, the Gain is set too low. Adjust the Gain (clockwise rotation) until the pen displacement from the starting level, 90% in this case, is 10 ± 1%. If the pen moves downscale by less than 9%, the Gain is set too high. Adjust the Gain (counterclockwise rotation) until the pen moves downscale by 10 ± 1%T.

NOTE: With a sample in the beam, the method of setting the Gain is almost identical to the method without a sample. The one exception is that the downscale pen movement is measured from the starting ordinate (%T) level when a sample is present.

f. Balance Control (screwdriver adjustment, hole on recorder base). Set daily and whenever Gain control is reset:

(1) With scan off, set the paper carriage at 1975 ± 20 cm^{-1}. Adjust the Gain.

(2) Partially block the sample beam to bring the pen to about 50% transmission.

(3) Quickly block the sample and reference beams simultaneously. The pen should remain at a constant transmission setting or drift slowly upscale (not more than 2 1/2% in 10 seconds).

(4) If there is a large upscale drift or any downscale drift, adjust the Balance control with a screwdriver.

(5) Recheck the Gain.

g. With the 100% control - Set the pen to 100% transmission with neither beam blocked.

h. Manually move the paper carriage to 4000 cm^{-1}.

i. Scan Switch (front panel) - Press the Scan button to initiate the scan; the Scan button lights when the instrument is scanning. If the scan is to be stopped before reaching the low wavenumber limit, press the Scan button. At the end of the scan, the pen lifts automatically from the chart paper, and the Scan light goes off.

NOTE 1: The P-E 727B instrument should be calibrated daily and whenever an oil spill set is to be analyzed. If a spill set is to be analyzed after initiating the Daily Instrumental Warmup and Operating Procedure, complete instruction 3B2h of that procedure and then continue immediately to instruction 3B3d in Procedure for Calibrating the P-E Infrared Spectrophotometer 727B.

NOTE 2: Turn the scan off before moving the paper carriage manually.

NOTE 3: The zero adjustment should be tested once a week; the pen should rest at zero transmission on the chart when there is no radiation in the sample beam. If the pen does not rest at zero, make the zero adjustment as described in Section 4E of the instrument instruction manual.

3B3. PROCEDURE FOR CALIBRATING THE P-E INFRARED SPECTROPHOTOMETER 727B

This calibration procedure is used as a test to determine that the 727B instrument is performing within the manufacturer's specifications. Before initiating the calibration procedure, first ensure that the Daily Instrumental Warmup and Operating Procedure has been completed.

a. Set the Gain as follows:

(1) With the pen raised and scan off, manually move the paper carriage to 1975 cm^{-1}. Use the 100% control to set the pen at 90% transmission.

(2) Press the Gain Auto-Chek™ knob. The pen should move downscale 10 ± 1% (to 79-81% transmission). If the pen moves downscale by more than 11% T, the Gain is set too low. Adjust the Gain (clockwise rotation) until the pen displacement from the starting level, 90% in this case, is 10 ± 1%. If the pen moves downscale by less than 9% T, the Gain is set too high. Adjust the Gain (counterclockwise rotation) until the pen moves downscale by 10 ± 1% T. (Gain may be adjusted whether the knob is pressed in or not.)

b. Set the pen to 100% transmission with neither beam blocked.

c. Manually move the paper carriage to 4000 cm^{-1}.

d. Place .05 mm polystyrene film in sample beam (see Figure 16). The pen will fall to between 80-85% transmission.

e. Press scan button to initiate scan.

f. Compare the polystyrene spectrum with the one illustrated in Figure 16. The following bands in the spectrum should appear at the frequencies indicated and should not deviate from the designated frequency by more than the tolerance indicated.

Band No.	Frequency	Tolerance
1	2851	± 8 cm^{-1}
2	1601	± 4 cm^{-1}
3	907	± 4 cm^{-1}

g. If the polystyrene test spectrum is not within the tolerances indicated or does not have the same general contour and appearance as Figure 16. then refer to Section 4C of "The Perkin-Elmer Instruction Manual Model 727B."

NOTE: Avoid touching polystyrene film surface. Be consistent in the positioning of the film in the sample beam. For example, mark the cardboard edge of the film "face side up" and always place it in sample beam in that position.

3B4. SCREENING PROCEDURE

This procedure uses the Wilks Mini-cell and AgBr windows with a 0.025 mm cell cavity. To screen oil samples proceed as follows:

a. Fill the Mini-cell with oil as instructed under Cell Loading Procedure in Section 3A6a.

b. Set Mini-cell sample holder and Mini-cell, containing oil sample, in the sample beam of the instrument. (Verify that sample holder is totally down in the sample beam slot.)

c. Set attenuator in the reference beam.

d. Set the Mode Selector Switch to the "Resolution" position.

e. Manually move the paper carriage to 1975 ± 20 cm^{-1}.

f. Adjust the attenuator to set the pen at 0.05 absorbance units (90% transmission).

g. Press the Gain Auto-Chek™ knob. The pen should move downscale 15 ± 1% (from 90% to 74-76% transmission). If the pen moves downscale by more than 16% T, the Gain is set too low. Adjust the Gain (clockwise rotation) until the pen displacement from the starting level, 90% in this case, is 15 ± 1%. If the pen moves downscale by less than 14% T, the Gain is set too high. Adjust the Gain (counterclockwise rotation) until the pen moves downscale by 15 ± 1% T. (Gain may be adjusted whether the knob is pressed in or not.)

h. Manually move the paper carriage to 4000 cm^{-1}.

i. Press the Scan button to initiate the scan.

NOTE: Before moving paper carriage manually verify that Scan button is turned off.

j. See Table 1 for Spurious Bands in Spectrum. See Figure 7 for spectrum of water in oil. If the sample has already been treated with $MgSO_4$ look for the telltale 610 cm^{-1} peak and changes in absorbance at 1075 and 1175 cm^{-1} (See Figure 4).

NOTE: Sometimes water in the oil is not well resolved in the Mini-cell. Be alert to the 3300-3400 cm^{-1} area when analyzing a "dry" sample in the demountable sealed cell.

3B5. ANALYTICAL PROCEDURE

This procedure uses a demountable sealed liquid cell with KBr windows and 0.05 mm Teflon spacer.

a. Assemble the cell (Figure 2) and fill with oil as instructed under Cell Loading Procedures, demountable sealed liquid cell, Section 3A6b.

b. Set the Mode Selector Switch to the "Resolution" position. Place oil sample in sample beam of the instrument.

c. Manually move the paper carriage to 1975 ± 20 cm^{-1}.

d. Set the recorder pen at 0.02 absorbance units (95% T) with the 100% control. Manually move the paper carriage to 650 cm^{-1} to determine if the pen is on scale (slightly above 0.0 absorbance, < 100% T). If the pen is on scale proceed to the next step. (If the pen is not on scale at 650 cm^{-1} set the pen at 0.02 absorbance units at this wavelength and proceed to the next step.)

3e. Manually return the paper carriage to 1975 ± 20 cm^{-1}.

f. Press the Gain Auto-Chek™ knob. The pen should move downscale 10 ± 1%. If the pen moves downscale by more than 11% T, the Gain is set too low. Adjust the Gain (clockwise rotation) until the pen displacement from the starting level is 10 ± 1%. If the pen moves downscale by less than 9% T, the Gain is set too high. Adjust the Gain (counterclockwise rotation) until the pen moves downscale by 10 ± 1% T. (Gain may be adjusted whether the knob is pressed in or not.)

g. Manually move the carriage to 4000 cm^{-1}.

h. Press the Scan button to initiate the scan.

i. After the instrument has completed scanning, inspect the cell to see whether any bubbles have developed that would invalidate the scan. If not, proceed as instructed under Interpretation of Spectra, Section 3C.

3C. INTERPRETATION OF SPECTRA

Ultimately, oil identification is based on a comparison of the spill spectrum with those of the various potential sources. When the results are to be used for forensic purposes, comparisons must be made on spectra obtained by using the same sample preparation, the same instrument, and the same instrument settings. Spectral interpretation is accomplished qualitatively by a visual overlay method. Table 2 lists the useful peaks in petroleum identification.

3C1. OVERLAY METHOD

The Overlay Method consists of a visual comparison of the spectrum of a spill with that of a potential source using a light-box. This comparison is accomplished by insuring first that the spectra have comparable baselines at 1975 $\pm$ cm^{-1} and absorbance at 1375 cm^{-1}; then examining for similarities in overall shape from 4000-650 cm^{-1}; closer study of the 1770-1685 cm^{-1} region; and examination of areas where the baseline is known to shift down (1350 cm^{-1}-900 cm^{-1}) to greater intensities with weathering. The "fingerprint" region (900-650 cm^{-1}) is then examined for similarities. If slight variations do occur in this region, these peaks are examined for possible weathering influences. The sequential steps are outlined below.

a. Examine the intensity of the 1375 cm^{-1} peak since it is a good indicator of oil thickness in the cell. An absorbance value of between 0.85 and 1.0 at 1375 cm^{-1} delivers the optimum fingerprint spectrum. If the two spectra being compared have virtually the same absorbance at 1375 cm^{-1}, then the identification of matching oil spectra becomes considerably easier. Weathering does affect the 1375 cm^{-1} to some degree, but it is still useful as a guide to thickness.

b. If the spectra are of the same thickness of oil sample, compare the overall shape of the entire curves. If the spectra are different in spectral shape (peaks), then the non-matching spectrum of the suspect oil sample is disregarded. If similarities do exist, proceed to the next step.

c. Examine the 1700-1710 region for the generation of a peak which indicates weathering. Here, many weathered oils display a well-defined carbonyl peak (Figure 8). Small amounts of carbonyl peak formation are indicative of weathering, but in some types of oils (e.g. heavy oils - See Figure 9) this carbonyl formation does not result in any important changes in the fingerprint region. Even if a peak is present in the carbonyl region in the spilled oil but not in the suspect oil, continue to the next step.

NOTE: Unweathered lubricant oils may show a strong sharp band at 1710 cm^{-1} (see Figure 11) due to a viscosity-modifying additive which diminishes with increased weathering. Unlike the additive peak at 1710 cm^{-1}, the peak due to weathering increases slowly and is more broad. Compare Figures 8 and 11 which demonstrate these difference.

d. Next examine the peak intensities between 1350-900 cm^{-1}. In this region a shift down to greater peak intensities is observed with weathering.

e. The next area scrutinized is the critical "fingerprint" region (900-650 cm^{-1}) which presents the unique character of an oil. When the spilled oil is the same as the suspect oil, the peak shapes, amplitudes and locations overlay each other. Replicates of the same oil, carefully analyzed, will not vary more than the pen line-width. These overlay spectra are designated as a "definite match" (DM).

f. If an oil has been altered by weathering, the analyst must take such effects into consideration. Weathering alters certain portions of a spectrum; the degrees and locations of the changes are known for various oil types. When these changes are taken into consideration, certain differences from a precise overlay become allowable when matching a weathered to an unweathered oil. The allowable differences are qualitatively indicated in paragraph (g) below. The qualitative peak ratios of all other peaks must remain constant.

g. Two curves: (1) differing in the carbonyl region (1708 cm^{-1}); (2) showing a general lowering of baseline from 1300-900 cm^{-1} with no marked peak ratio changes or changes in peaks in the 900-700 cm^{-1} region which can be attributable to weathering, are designated as a "probable match" (PM).

h. If the changes are excessive and there is any doubt in the analyst's mind whether the oils match or not, the designation is "indeterminate" (I).

i. When the oils are clearly different, with larger differences, peak reversals, absence of some peaks present in other curve, etc., then the designation is "non-match" (ND).

j. Most oil spills are located within 48 hours during which weathering changes can be significant in light oils such as No. 2's, some No. 4's and light crudes. In heavy oils, however, such as some No. 5's, No. 6's, Bunkers and heavy crudes, weathering effects may be negligible. The following describes significant weathering effects on different classes of oil.

--Fuel oils No. 1 and No. 2 display losses at 870, 850, 835, 820, 810, 765, 725, 700 wavenumbers (cm^{-1}).

--Additives in lubricant oils lose structure at 1710, 1355, and 1010 cm^{-1}. The 1235 cm^{-1} additive peak, however, remains stable even with weathering and is an excellent fingerprint for lube oils. The 725 cm^{-1} band is very strong in lubes and diminishes slightly with weathering. It is another excellent clue to the classification of lube oils.

--Diesel oils show losses similar to the No. 1 and No. 2 fuel oils.

--No. 4 fuel oils show ratio intensity changes between the 745 and 725 cm^{-1} peaks.

--Weathering effects on No. 5 and No. 6 oils are less than in lighter oils.

--Crudes weather differently. Light crudes will weather like light fuel oils; heavy crudes like heavy fuel oils. Light crudes display significant losses at 765, 745, 695 and 675 (cm^{-1}).

3C2. SPECTRA OF OIL, SOLVENTS AND MAGNESIUM SULFATE

a. Figure 4 shows a No. 4 fuel oil with varying amounts of residual $MgSO_4$ as might be obtained. Absorption at 610 cm^{-1} is indicative of traces of $MgSO_4$.

b. Figures 5 and 6 show curves of the same No. 2 fuel oil to demonstrate the greater resolution and increased spectral information obtained from a demountable sealed cell when compared to a Mini-cell.

c. Figure 7 shows spectra of a wet and dry No. 4 fuel oil.

d. Figures 8 and 9 show spectra of No. 2 and No. 6 fuel oils respectively, unweathered and weathered four days.

e. Figure 10 is a spectrum of a crude oil.

f. Figure 11 is a spectrum of a lubricating oil.

g. Figure 12 is a spectrum of dichloromethane.

h. Figure 13 is a spectrum of cyclohexane.

i. Figure 14 is a spectrum of pentane.

j. Figure 15 is a spectrum of a No. 4 fuel oil showing the effects of residual pentane. Note the doublet at 910 and 920 cm^{-1}.

k. Figure 16 is a polystyrene spectrum used for calibration.

TABLE I. SPURIOUS BANDS IN INFRARED SPECTRA.

Compound	Frequency (cm^{-1})	Attributable to
H_2O	3710 3450-3333 1640	Entrained liquid water in samples (see Figure 7)
H_2O	2000-1450S	Atmospheric water vapor. Sharp strong bands in this region
$MgSO_4$	1175 1075 1013S 690-700 610S	Residual magnesium sulfate (drying agent) in sample (see Figure 4)
CO_2	2350 667	Atmospheric carbon dioxide
CH_2Cl_2	1425S 1275 1265 900S 700-760	Residual dichloromethane (see Figure 12)
C_5H_{12}	910 920	Residual pentane (see Figures 14 and 15)
CCl_4	795 780	Residual carbon tetrachloride
C_6H_{12}	1258S 905S 863S	Residual cyclohexane (see Figure 13)

Code: S = sharp

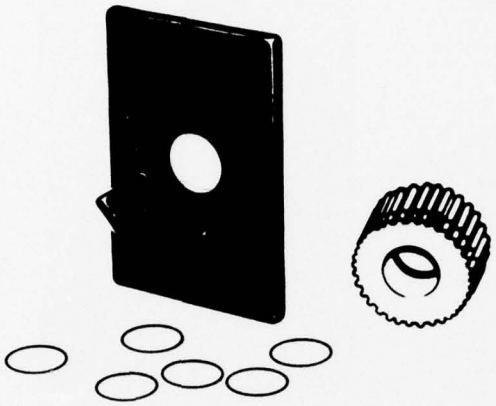
TABLE II. USEFUL PEAKS IN PETROLEUM IDENTIFICATION.

Peak cm^{-1}	Mattson's Ranges	Digitized by Brown	Assignments
3050			Aromatic CH
2925			Aliphatic CH (CH_2 Asym str)
2849			Aliphatic CH
1708	1608-1715		Carbonyl from oxidation of polar products. Lube oil additive.
1700			Santa Barbara seeps
1650			
1600	1594-1609		Aromatic CH
1521			
1460	1446-1465		Aliphatic CH (CH_2 def)
1377	1372-1385		Aliphatic CH ($C-CH_3$ Sym. def.)
1304	1302-1309		Sulfones, alkyl ethers
1235			Lubricating Oil Additive
1165	1155-1188	1160	Secondary alcohols
1145		1145	
1070		1070	
1032	1031-1044	1030	Sulfoxides or ethers
963			
955		955	
918		915	
890		890	
871	864-893	870	Aromatic CH
849		845	
832		835	
810	810-823	810	Aromatic CH (2 adj H wag)
790		790	
782		780	
766		765	
744	741-756	740	Aromatic CH
722	720-734	725 720	Aliphatic CH ($-(CH_2)n>4-$)
700		695	Aromatic (disappears rapidly with weathering)
672			Highly Volatile Fraction

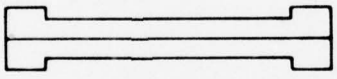
TABLE III. MODE SELECTOR SWITCH SETTINGS, SCAN TIMES, AND SLIT PROGRAMS FOR VARIOUS OBJECTIVES.

Objective	Mode Switch Position	Scan Time*(min)	Slit Program
Survey Spectrum - Fast Scan	SURVEY	2 2/3	Wide
Analytical Procedure - Demountable Sealed Cell	RESOLUTION	8	Narrow
Screening Procedure - Mini-Cell	RESOLUTION	8	Narrow
Single Beam Water Vapor Spectrum for Frequency Calibration*	CALIBRATE	8	Narrow

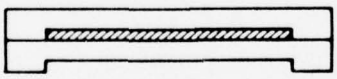
*Block the sample beam.



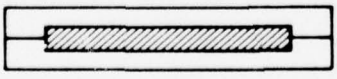
Mini-cell kit



Windows positioned for smears



Windows positioned for 0.025mm



Windows positioned for 0.05mm

Pathlength variations of
Mini-Cell Windows

FIGURE 1. WILKS MINI-CELL.

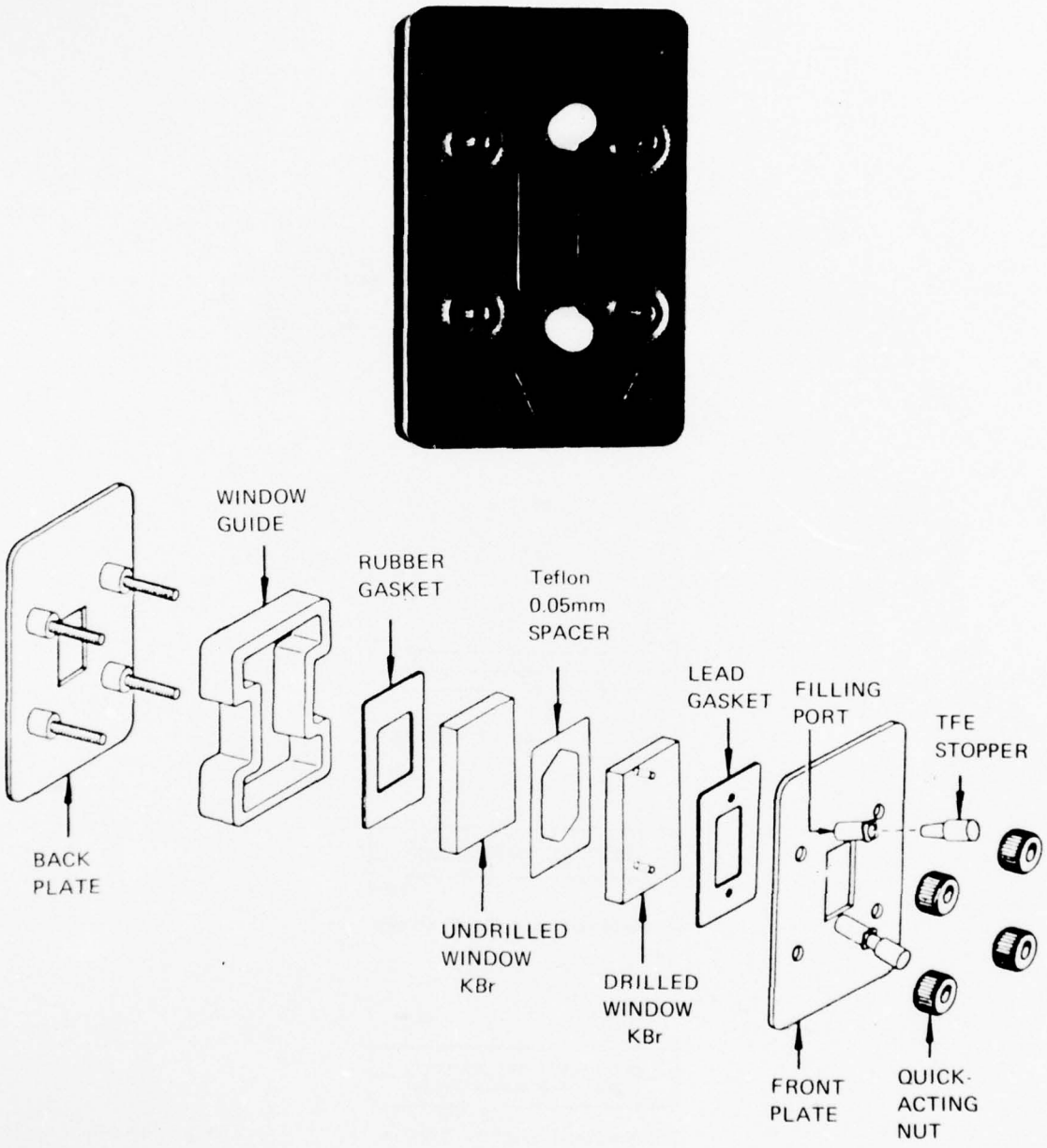


FIGURE 2. DEMOUNTABLE SEALED CELL.

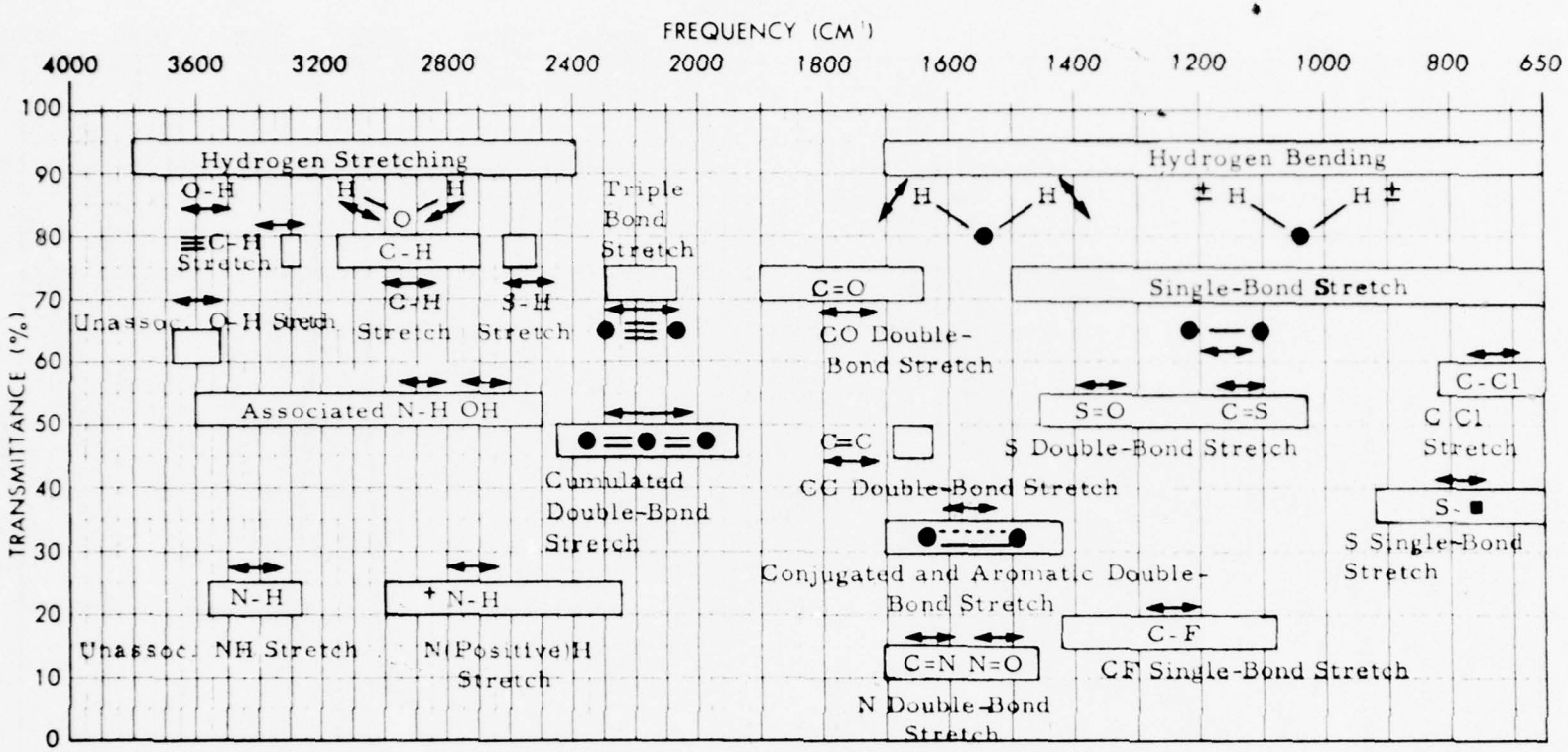


FIGURE 3. ABSORPTION IN DIFFERENT REGIONS OF THE INFRARED SPECTRUM.

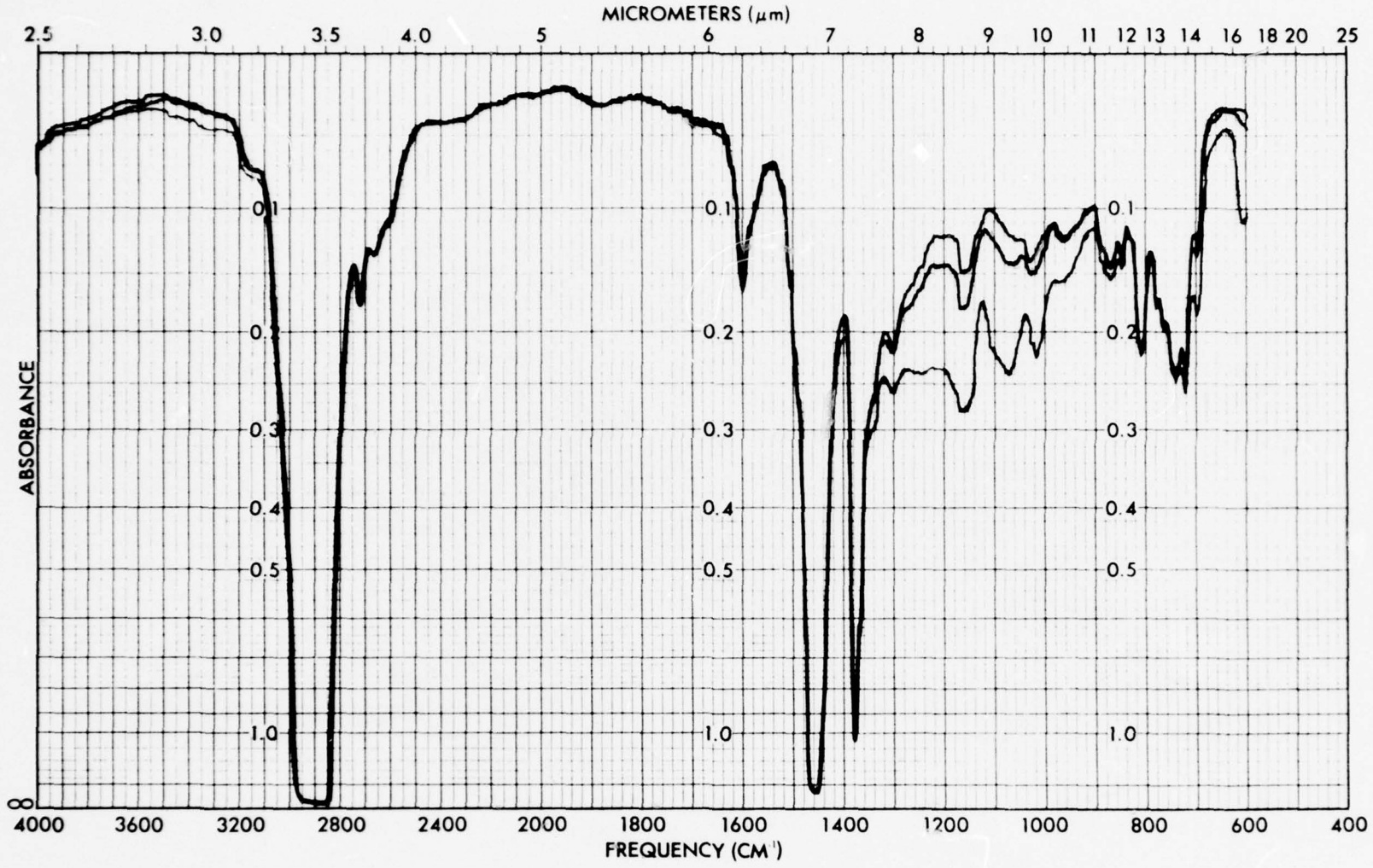


FIGURE 4. NO. 4 FUEL OIL WITH RESIDUAL MAGNESIUM SULFATE.

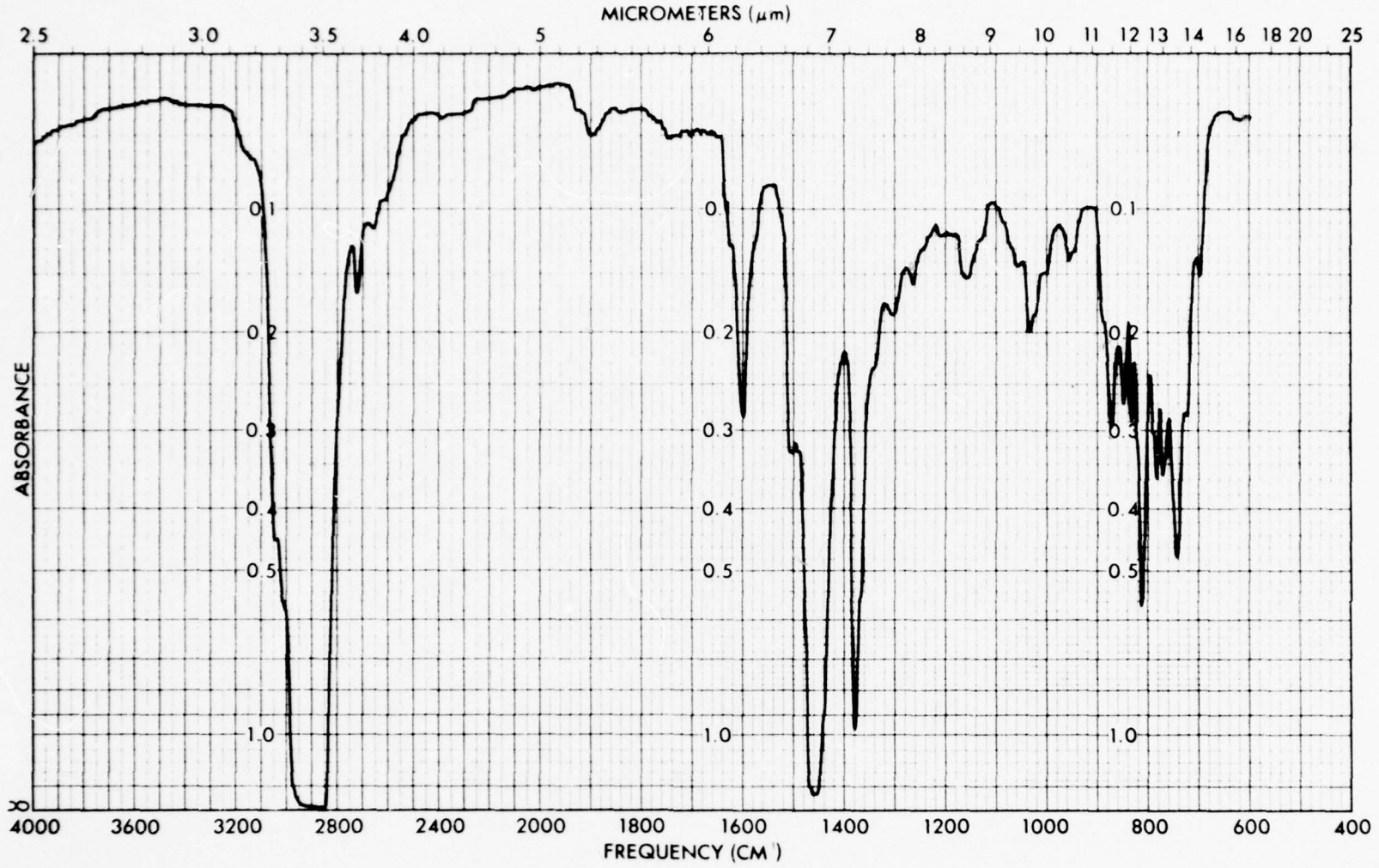


FIGURE 5. NO. 2 FUEL OIL (DEMOUNTABLE SEALED CELL).

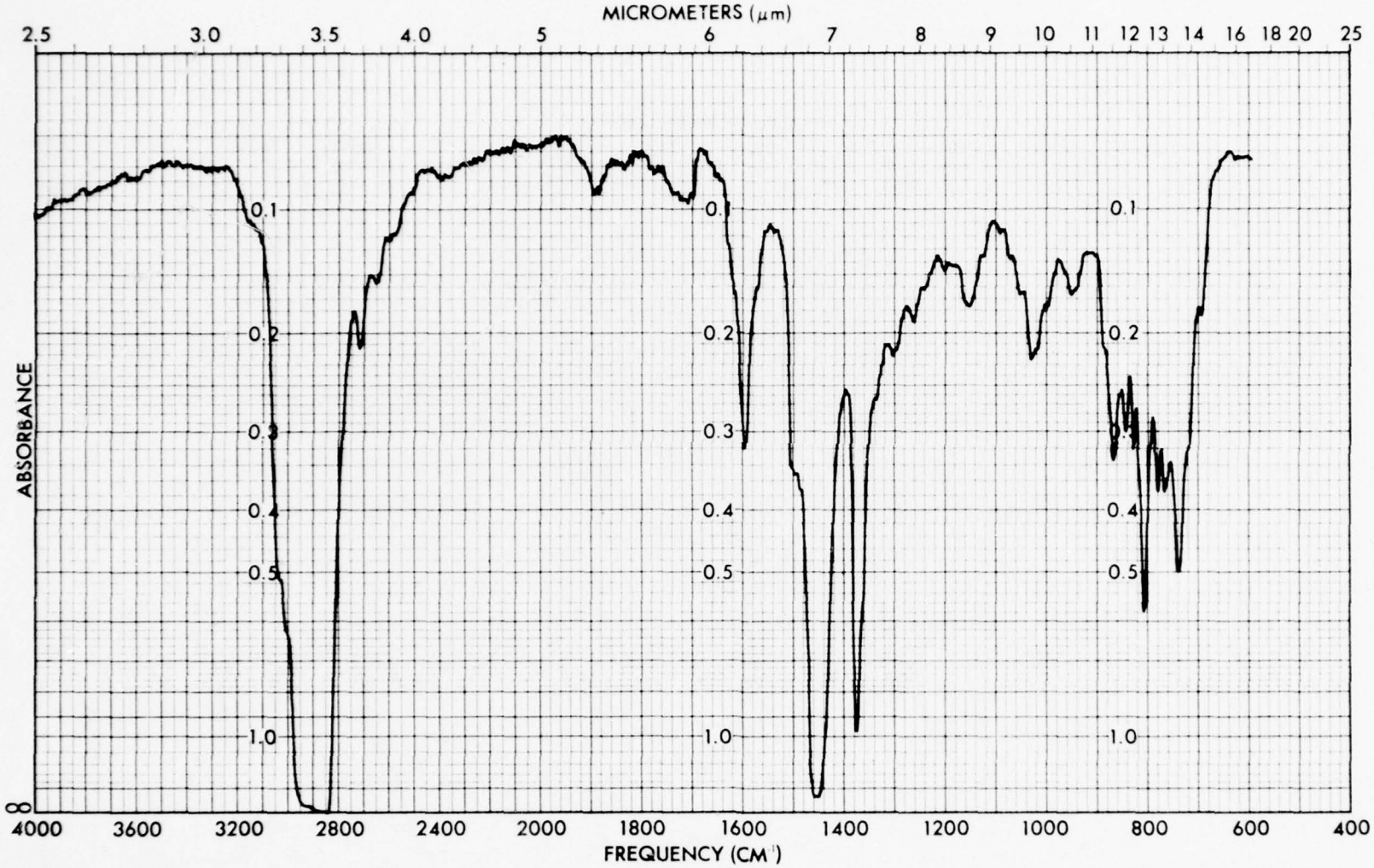


FIGURE 6. NO. 2 FUEL OIL (WILKS MINI-CELL).

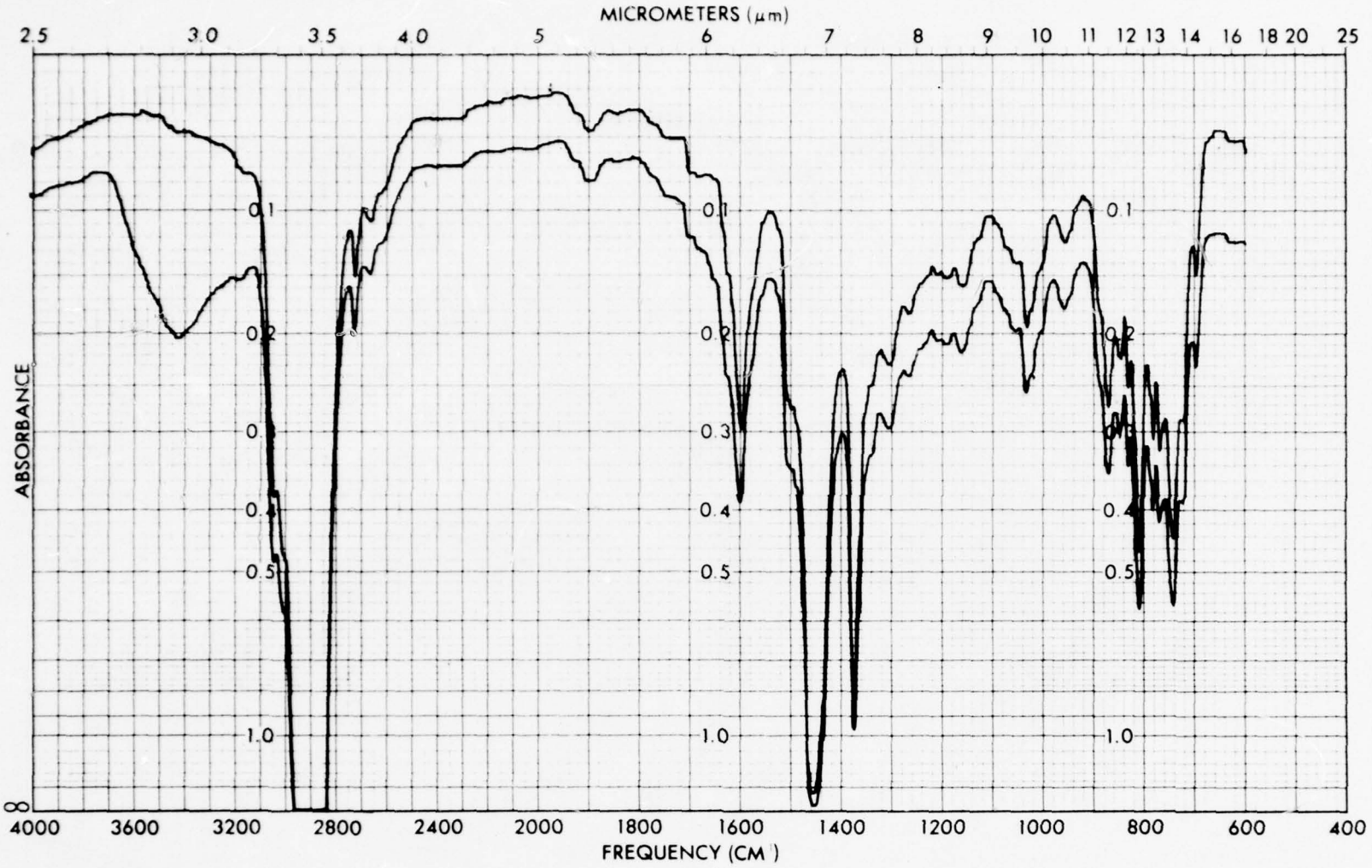


FIGURE 7. NO. 4 FUEL OIL WITH RESIDUAL WATER.

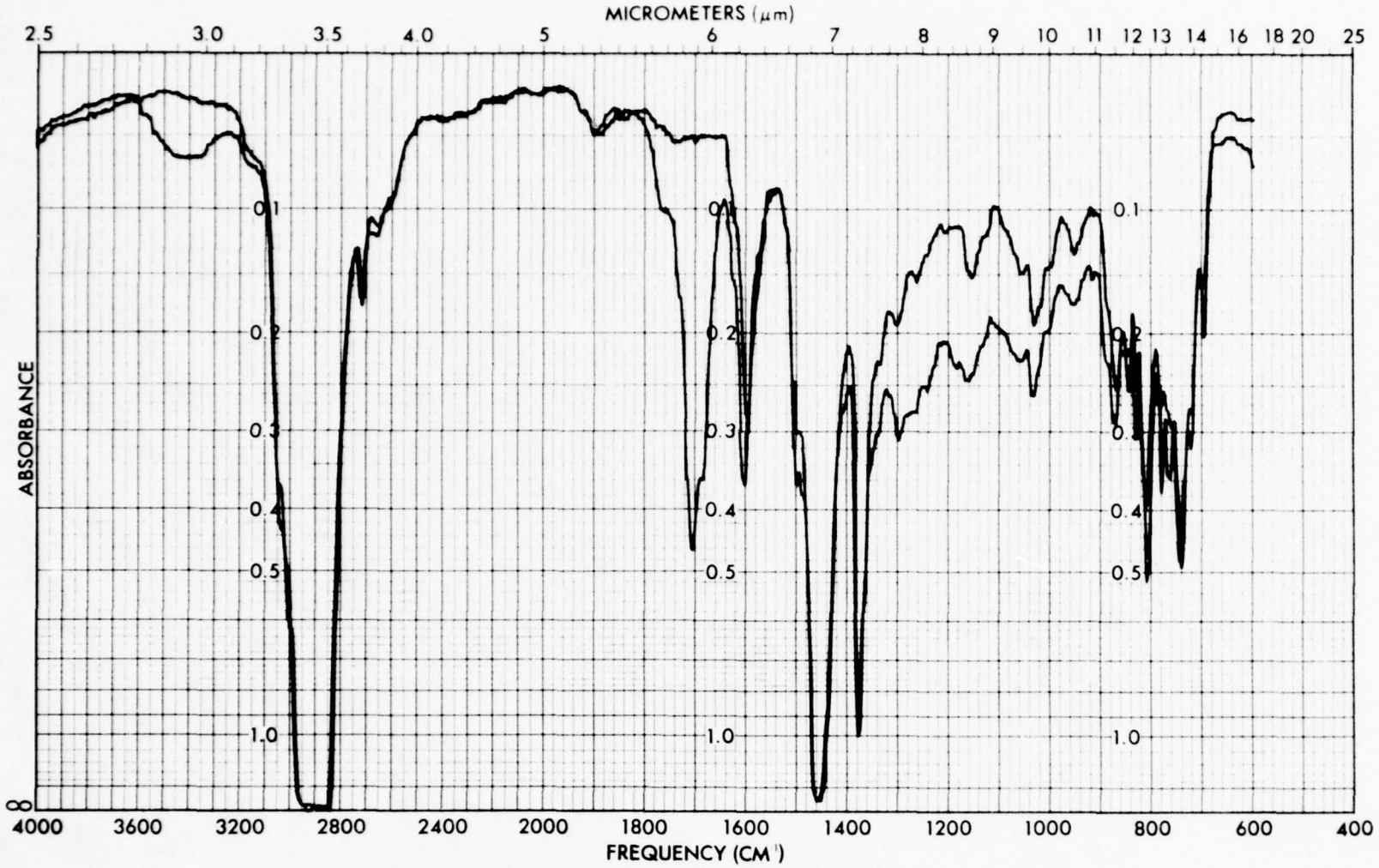


FIGURE 8. NO. 2 FUEL OIL UNWEATHERED AND WEATHERED.

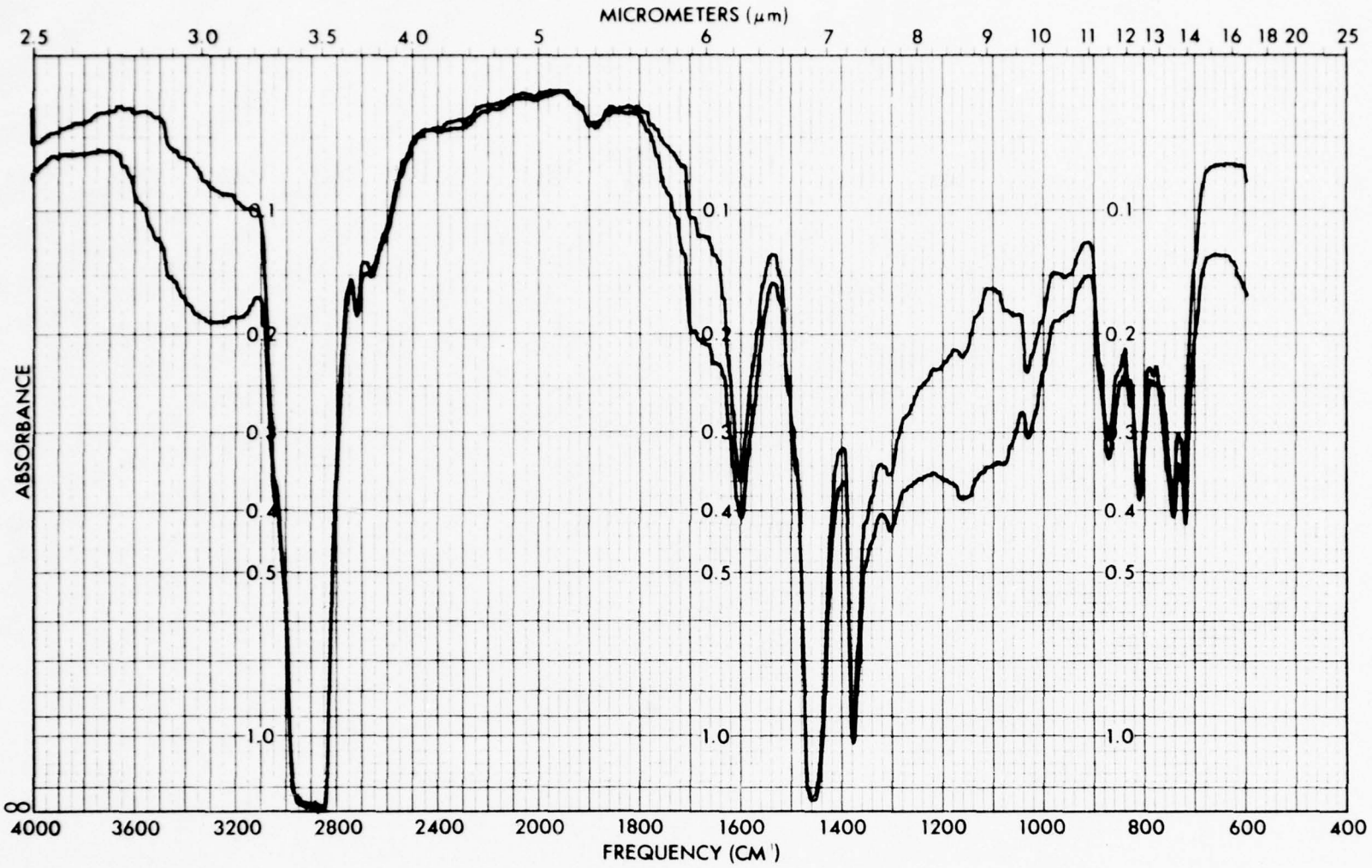


FIGURE 9. NO. 6 FUEL OIL UNWEATHERED AND WEATHERED.

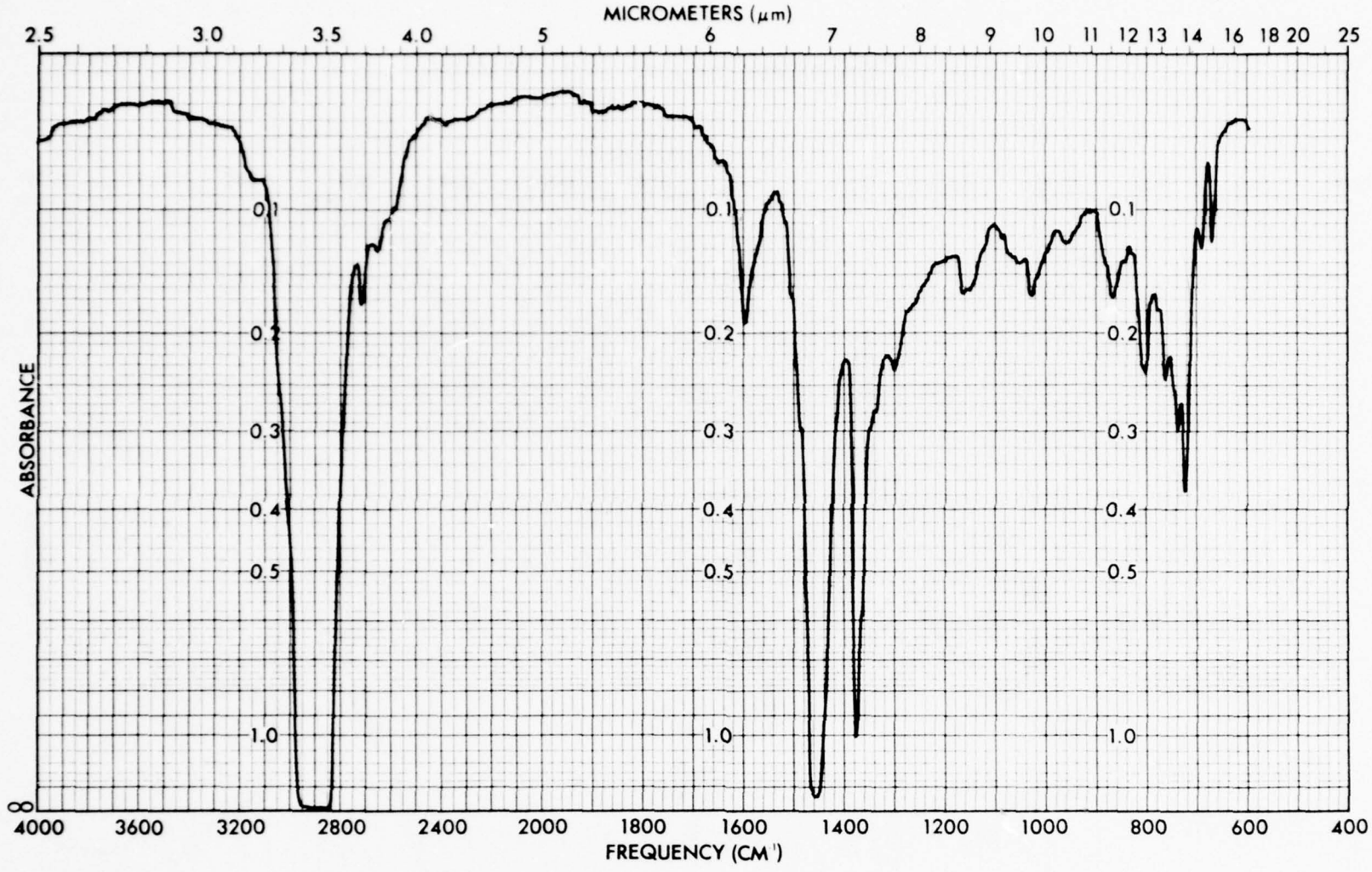


FIGURE 10. EXAMPLE OF A CRUDE OIL.

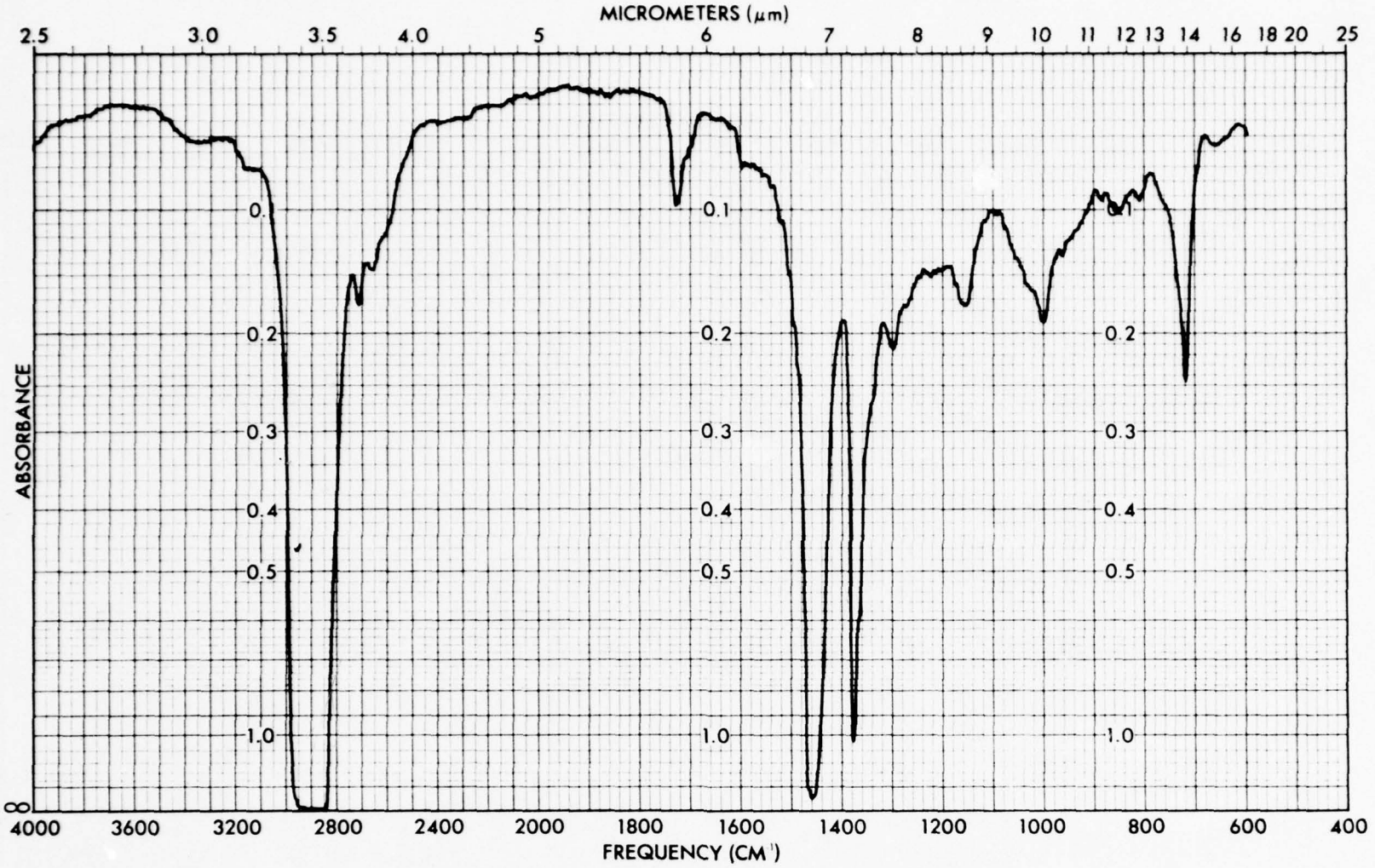


FIGURE 11. EXAMPLE OF A LUBRICATING OIL.

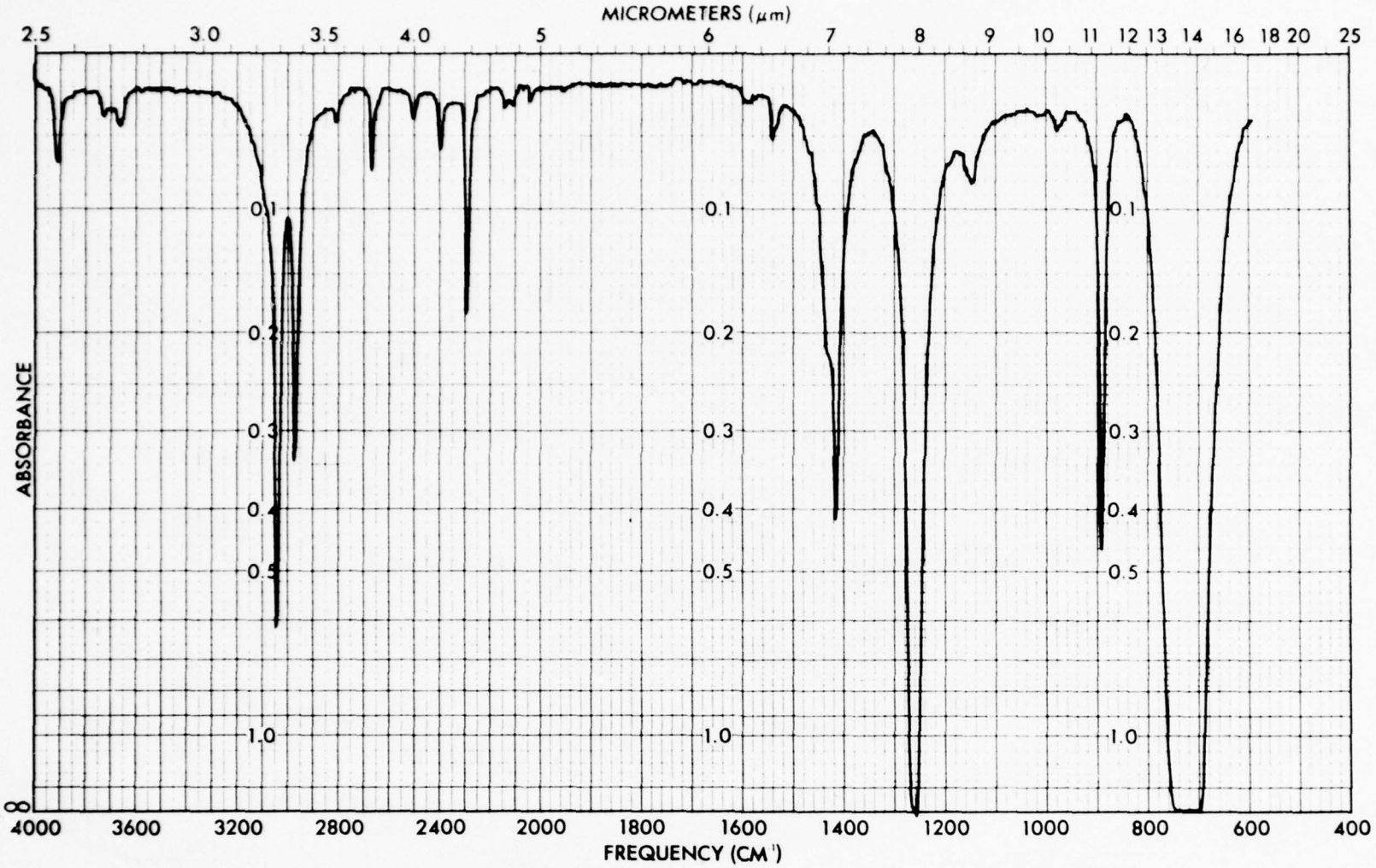


FIGURE 12. DICHLOROMETHANE.

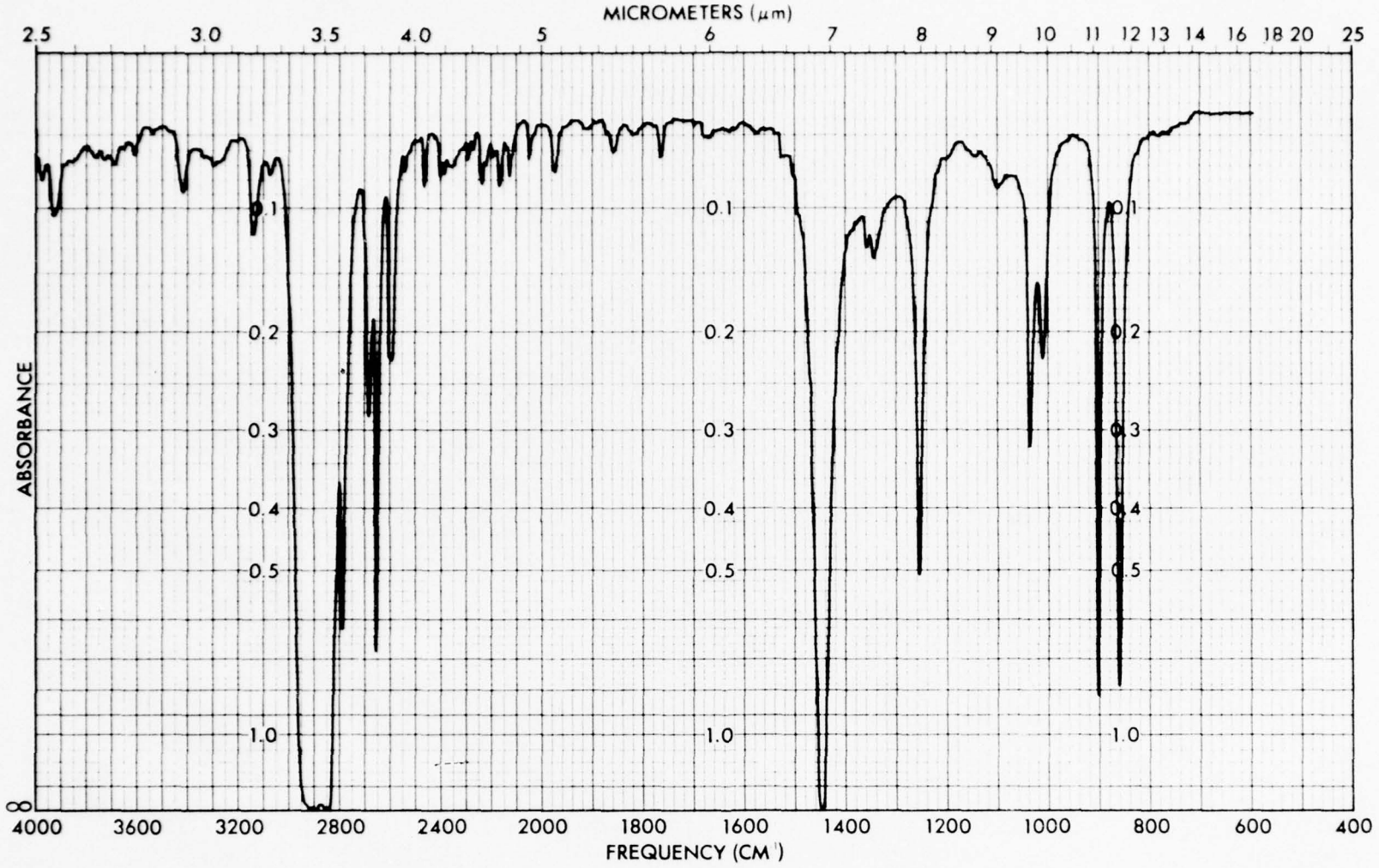


FIGURE 13. CYCLOHEXANE.

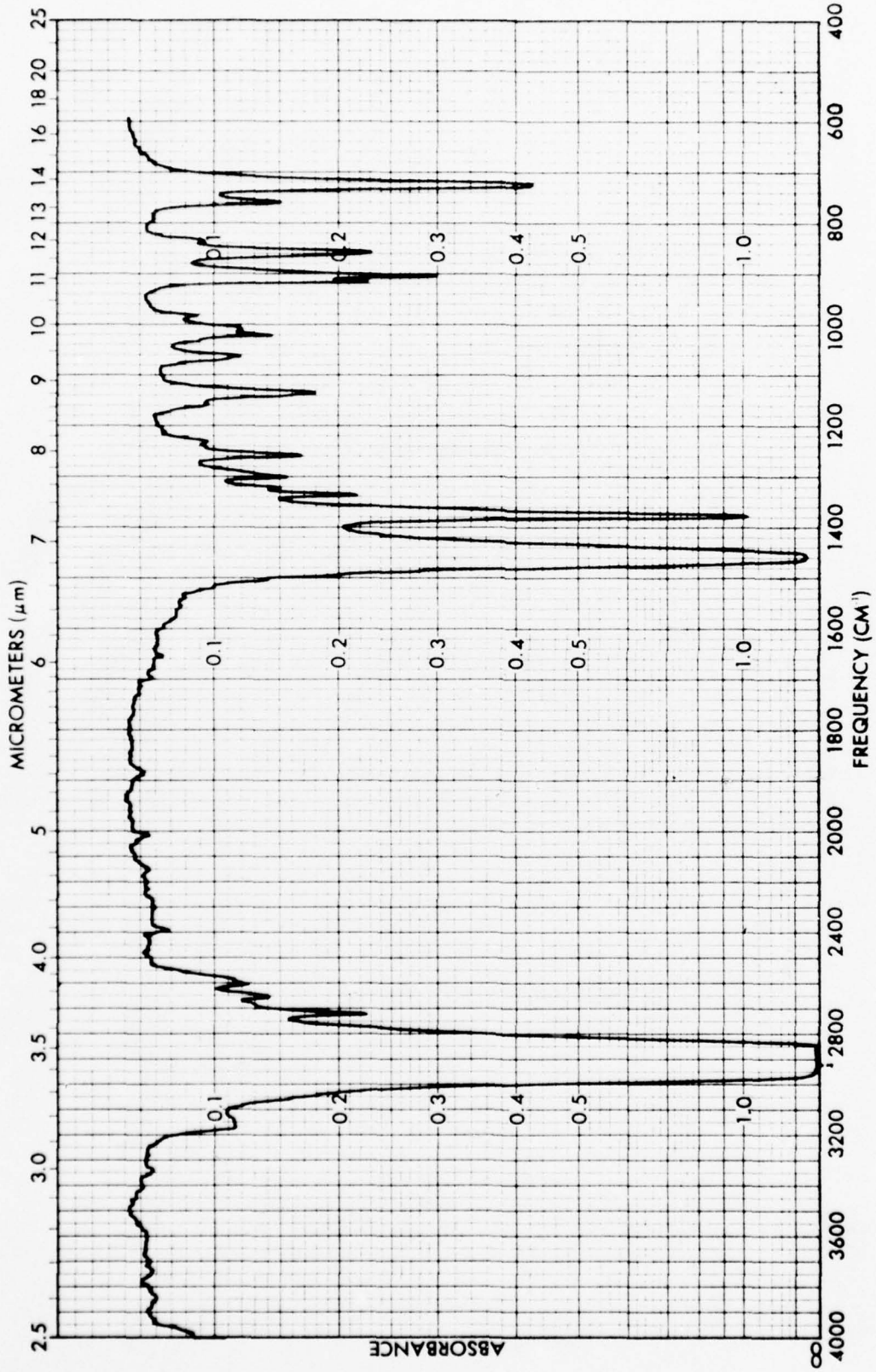


FIGURE 14. PENTANE.

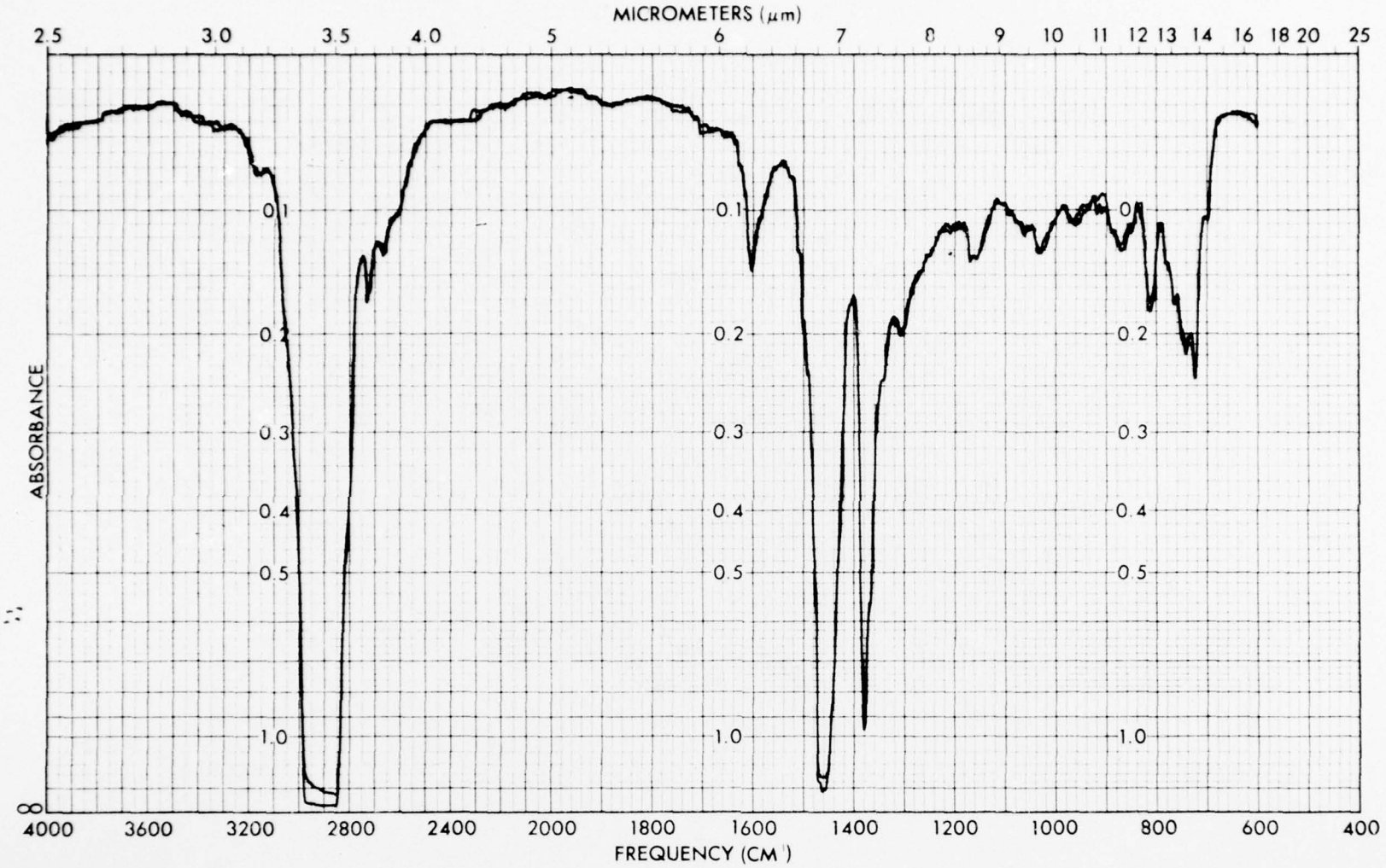


FIGURE 15. NO. 4 FUEL OIL - EFFECTS OF RESIDUAL PENTANE.

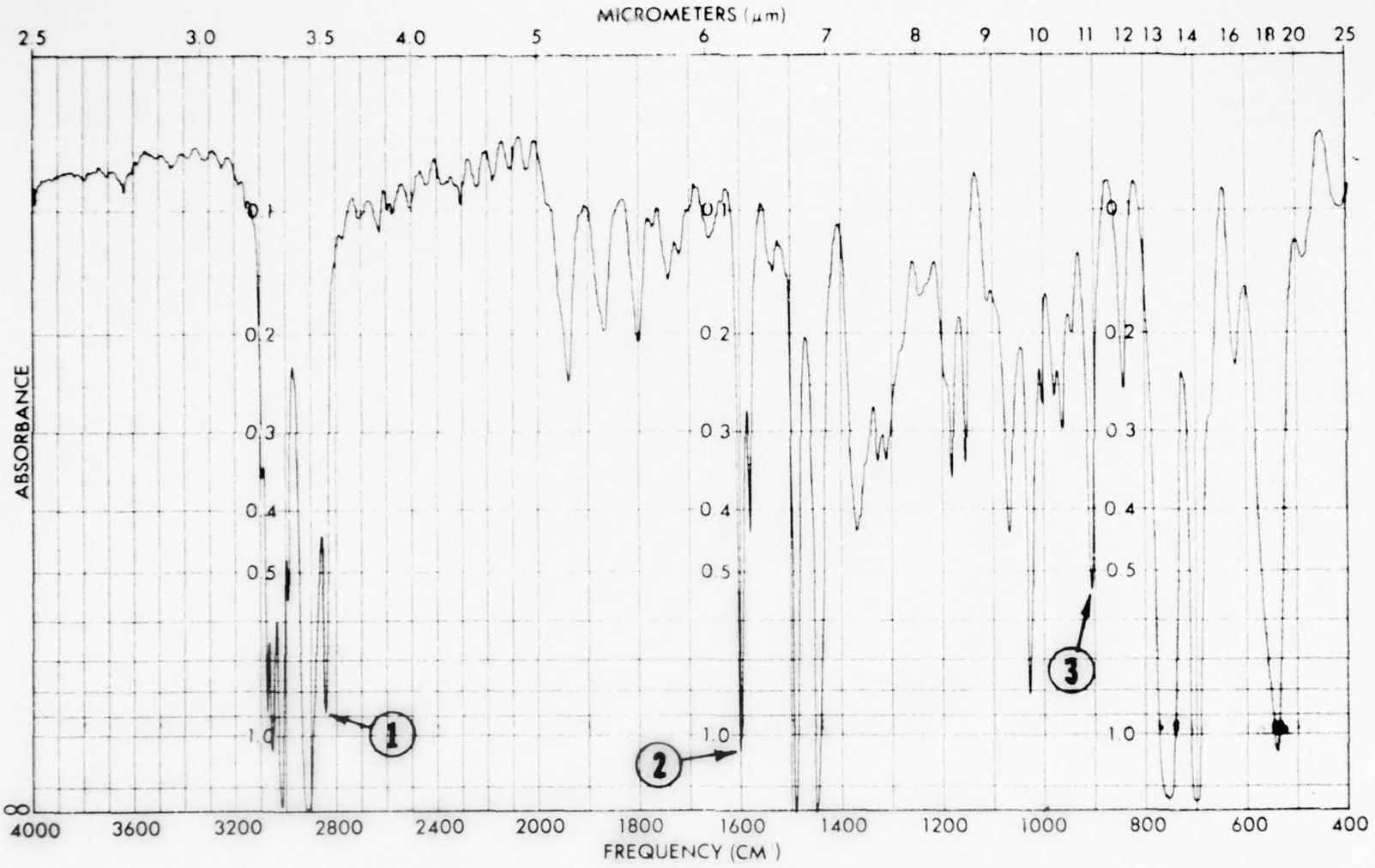


FIGURE 16. POLYSTYRENE TEST FILM (0.5 MM).

APPENDIX I

FIELD CLASSIFICATION OF OILS BY SINGLE-BEAM INFRARED SPECTROSCOPY

1.0 INTRODUCTION

1.1 Scope

The purpose of this project was to develop a simple method for use in the field to classify oils using an inexpensive single-beam infrared spectrophotometer.

1.2 Application

The proposed application was to classify oil spills rapidly so that the proper potential sources could be sampled. It was to classify oils as to whether they were crudes, lubes or fuel oils (No. 2, No. 4, No. 5, No. 6 or Bunker C).

2.0 SUMMARY OF METHOD

2.1 The neat, weathered or unweathered, petroleum crude or refined oil sample is first placed in either a sealed demountable cell with silver bromide (AgBr) windows and a 0.1 mm Teflon spacer or in a Wilks Mini-cell also with AgBr windows and a 0.1 mm pathlength. If the sample is known to contain water, additional sample preparation steps are required.

2.2 The cell is placed in the single-beam infrared analyzer (Miran I, Model 5649) and the infrared spectrum on the transmittance scale is scanned from 2.5 to 14.5 μ. Then, the spectrum is scanned from 8 to 11 μ on the (0-1 O.D.) or 1x linear absorbance scale and from 11 to 14.5 μ on the (1-2 O.D.) or 10x linear absorbance scale. Afterwards, spectra are analyzed by examining major spectral features or by using the overlay method (see Section 5) to classify the oil as to type.

3.0 PRINCIPLES

3.1 Method

The infrared absorption spectrum of a petroleum oil is the sum of the absorptions of all the infrared active vibrations of all the component molecules in this highly complex mixture. All vibrations are infrared-active if they result in a change in electric dipole moment (displacement of electric charge) within the molecule. For hydrocarbons, this means the asymmetric vibrations which include bending, stretching and twisting motions of the molecule as a whole or of individual bonds. When the frequencies in the light from the infrared source coincide with the vibrational frequencies of the molecules, energy is absorbed - resulting in an absorption maximum, or peak, in the spectrum.

Certain peaks may be found to have mainly aromatic or mainly aliphatic character, whereas other peaks will be found to represent the stretching of a particular type of bond such as carbonyl (C=O), or hydroxyl (OH). Therefore, the infrared spectrum gives us a good handle on the overall composition of the oil including components such as aliphatic hydrocarbons - not readily seen by other methods, e.g., fluorescence spectroscopy. Since oil is such a complex mixture, individual components of the oil are not generally determined. The infrared spectrum permits classification of the oil because different classes of oils have different characteristic infrared absorption patterns.

Infrared spectroscopy is not a good method for studying trace contamination of the oil, especially if the contaminant absorbs energy in a region of general hydrocarbon absorption. Certain solvent contaminant peaks such as pentane, used in deasphalting or extraction procedures, may be present and may distort the underlying infrared envelope without being noticeable except to an experienced operator. Therefore, it is advisable to avoid adding solvents to the oil unless absolutely necessary. This is also the reason for evaporating off all organic solvents before filling the cell. Also, many inorganic compounds such as magnesium sulfate, used in sample preparation, absorb in the infrared and may cause contamination peaks of which the operator must be aware. Finally, atmospheric absorption peaks may be noticed and changes in the spectral baseline may be caused by scratching and darkening of the cell windows, especially silver bromide (AgBr). The latter effects may be checked by running an empty cell baseline.

3.2 Instrument.

Figure 1 shows a schematic diagram of the instrument. The source is a resistively-heated nichrome wire. A circular variable interference filter is used in combination with a slit to select a single narrow wavelength band from the source radiation. The selected wavelength bandpass is varied over the range 2.5-14.5 microns (in three steps) by rotating the filter.

A pyroelectric lithium tantalate detector is exposed to chopped radiation which has passed through the sample cell and the resulting signal is sensed as an alternating voltage across a large-value, parallel resistor. This detector is used because of its ruggedness and rapid response. It is not degraded by normal exposure to the atmosphere but must be protected from strong drafts when in use. The basic signal-to-noise ratio of the instrument is determined by the "Johnson noise" in the parallel resistor due to the random thermal motion of electric charges and by the intensity of radiation falling on the detector.

The instrument settings of the Miran I model 5649 for oil classification are shown in Table I.

4.0 DIFFERENCES BETWEEN SINGLE AND DOUBLE-BEAM INFRARED SPECTRAL METHOD

In modern infrared spectrophotometry, double beam instruments are used almost exclusively. Consequently, not everyone is familiar with the distinctions between single and double-beam spectrophotometers. The major distinctive features of the single-beam instrument are outlined below.

For the single-beam method:

4.1 The instrument resolution is lower. This is a disadvantage in that some information is lost, but the important features of the infrared spectra are still observable.

4.2 The spectral baseline will be sloping rather than flat as for the double-beam instrument. Also, interferences due to atmospheric absorbances of CO (4.25 μ) and H_2O (2.7 μ) may occur.

4.3 The gain is set with reference to a polystyrene standard. (For the double-beam spectrophotometer, the gain is adjusted at a fixed wave length with the filled cell in place.) When using the single-beam instrument, it is important always to use the same Teflon spacer (for the sealed demountable cell) and/or the same Mini-cell windows, so that the relative absorbances can be compared directly, since small differences in cell thickness are not compensated by the calibration procedure. This method has the advantage that relative absorbance differences among oils will be maintained. Therefore, relative API gravities ("weights") of oils can be compared by observing the absorbances at certain wavelengths (e.g., 7.5 μ).

4.4 The Miran instrument has a linear absorbance capability, in addition to the linear transmittance which is available in most double-beam instruments. The Miran has two absorbance ranges (0-1 and 1-2 Optical Density).

4.5 The Miran abscissa is linear in microns rather than wavenumbers as are most double beam instruments. The Miran abscissa is divided into three segments because of the filter wheel construction (see Figure 1 and Section 6.1).

5.0 INTERPRETATION

A spectral library of 47 oils was chosen as being representative of all the main fuel oil types as well as of crude and lubricating oils, based on a larger library of 300 infrared oil spectra run on a double-beam infrared spectrophotometer. Transmittance templates were produced for each type of oil over the spectral range from 2.5 to 14.5 μ. Such transmittance templates for No. 2 and No. 6 fuel oils are shown in Figure 2.

The oils may be classified as to type by examining the main spectral features (and comparing with Table II). Useful wavelengths (in microns) include 5.85, 6.2, 6.6, 7.5, 9.7, 10.4, 11.4 and using an absorbance scale of 12.35, 12.85, 13.05 and 13.85. Figure 3 indicates some of these wavelengths for a template of No. 4 fuel oils.

The utility of the 6.2 μ peak is that it can distinguish lubes (lubricating oils) from non-lubes since it is weak and generally not observable in lubes. To distinguish lubes and crudes from fuel oils the ratio of the 12.35 μ peak to the 13.85 μ peak is used. It is >0.5 for fuel oils and <0.5 for crudes and lubes. (In determining the ratios for these peaks, baselines were drawn from absorption minimum to absorption minimum by the standard ASTM method (E168-67). Some lubes show an additive peak at 5.85 μ.

Inspection of the 7.5 μ absorbance minimum will give the relative absorbance of the oil which is roughly proportioned to the density (or API gravity) of the oil. This is illustrated for fuel oils in Figure 4. Each oil template represents the spread of data for all oils of that type in the library which was analyzed. Table III summarizes the information given in Figure 4, listing the range of transmittance at 7.5 μ for each fuel oil template. Figure 5 shows the variation of transmittance as a function of API gravity for individual crude oils.

The classification scheme indicated here was developed for neat unweathered oils. The following comparisions for a medium weight (No. 4 fuel) oil unweathered and weathered 3 days on the transmittance scale (Figure 6) and the absorbance scales (Figure 7) show that classification should still be possible after a reasonable period of weathering. Note in Figure 6 the appearance of a carbonyl peak at 5.85 μ due to oxidation and the change in absorbance at 7.5 μ which corresponds to a change in the density of the oil with weathering. Figure 8 shows an absorbance scale on a No. 1 fuel oil weathered for nearly 24 hours in a real world spill case. More extensive weathering studies were beyond the scope of the project.

For classification, it was found sufficient to make all comparisons by examining main spectral features or using spectral overlays. Peaks could be ratioed quantitatively by drawing slanted baselines from absorbance minimum to absorbance minimum. For single-beam spectral which have a sloping baseline to start with, the quantitative results are not too precise.

Since the Miran has good photometric accuracy ($\pm$ 0.5%) and since good overlays (better than 1% T) could be obtained using the precautions described in this procedure, the principal sources of error are in weathering and/or baseline determinations (if peak ratios are to be used).

6.0 APPARATUS AND MATERIALS

6.1 Instrument

The basic instrument is the Wilks Miran I Infrared Analyzer. The Miran I is a single-beam variable circular filter instrument with a nichrome wire source and a solid-state detector. The instrumental settings are listed in Table I. The linear absorbance scales are required to observe sufficient detail for identification in the fingerprint region from 8-14.5 μ. The instrument is used with any compatible strip-chart recorder with time response <1 second full scale and 1 volt input.

Any other equivalent commercial infrared spectrophotometer (single- or double-beam) with similar photometric accuracy and resolution at least as high could be used with minor modifications to this procedure.

6.2 Cells and Windows

Sealed demountable cell with 0.1 mm Teflon spacer and AgBr windows or Wilks Mini-cell with flat cover AgBr window and 0.1 mm cell depth AgBr window or RIIC disposable cell with AgCl windows and 0.1 mm cell thickness.

6.3 Auxiliary Equipment

Portable hood (for field work), hotplate for sample preparation, light box.

6.4 Expendable Materials

Expendables include: spectroquality solvents necessary for sample treatment (pentane) and for cleaning cells (toluene and hexane which are recommended for field use because of their relatively low toxicity), anhydrous magnesium sulfate for drying samples, standard 0.05 mm polystyrene film, chart paper, extra recorder pens, Pasteur pipets, micropipets, centrifuge tubes, Kimwipe or equivalent tissues and lens paper (non-silicone treated).

7.0 SPECTROSCOPIC PROCEDURES

7.1 Infrared Analyzer and Recorder

Refer to the instrument manual for information on operation, maintenance, specifications and troubleshooting.

7.1.1 Turn on instrument and recorder and warm up for 15 minutes.

7.1.2 Zero the strip-chart recorder.

7.1.3 Insert a standard 0.05 mm polystyrene film in the sample position.

7.1.4 Set Scale Expansion -1x (low gain setting)

Function switch - 100 % T

Time Constant - 0.25 sec. (Setting 1)

Slit Width - 0.5 mm.

Filter Wheel - 3.34 microns

7.1.5 Adjust gain to read 0.90 transmission at 3.34 microns.

7.1.6 Close slit and remove film.

NOTE 1: Close slit when the instrument is not in use to prevent detector overload.

NOTE 2: Be careful not to alter the gain adjustment after it has been set against the polystyrene standard.

NOTE 3: Usually, the external detector/preamplifier unit is locked into a position corresponding to the maximum signal; however, if the unit has been packed, moved or severely jarred, it may be necessary to check the alignment of the external detector unit and reposition for maximum signal.

7.1.7 At least once a week run a spectrum of the polystyrene film to check the wavelength accuracy of the instrument. **(See Figure 9)**

7.1.8 At least once a day run an empty cell blank to check the degree of atmospheric absorption due to water vapor (2.7 μ) or carbon dioxide (4.25 μ). At the same time, check for possible changes in spectral baseline due to scratching or darkening of the cell windows. (See Section 7.6).

7.2 Sample Analysis

7.2.1 Place the sample holder and cell in the sample position.

7.2.2 Open slit to 0.50 mm.

7.2.3 Set the filter wheel at 2.5 microns. Check that function switch is set at 100% T scale.

7.2.4 Engage scan motor on Miran and chart drive on recorder at the same time. Scan past 14.5 microns. Mark beginning and end of scan on the chart paper and indicate the wavelength values to which the marks correspond for each segment of the scan. Each segment is linear in microns. Close slit and disengage the scan and chart.

7.2.5 Change function switch to 1, linear absorbance scale (0-1 O.D.). Set the filter wheel at 8.0 microns. Open slit to 0.50 mm.

7.2.6 Engage scan and chart drive. At approximately 11 microns the pen will go off scale. At this point, change scale expansion to 10x (high gain setting), this corresponds to (1-2 O.D.) linear absorbance scale. Scan from 11 to beyond 14.5 microns. Close slit, disengage scan and chart; return scale expansion to 1 x (low gain) and change range switch to 100% T scale.

7.3 Preparation of Sample

7.3.1 Pipet 2 ml of oil sample into a centrifuge tube.

7.3.2 Remove any visible water with a micropipet.

7.3.3 Place the centrifuge tube in 60°C water bath for 10 minutes. (For intermediate viscosity oils, use 35°C; for light oils, eliminate this step.)

7.3.4 Add 0.5 gram of anhydrous $MgSO_4$ (magnesium sulfate). Mix.

7.3.5 Immerse centrifuge tube into holder in centrifuge after filling holder partly with water. Centrifuge at 2500 rpm (relative centrifugal force = 1000) for 15 minutes. For many viscous oils, it may be necessary to repeat this step after removing water with pipet or to centrifuge for a longer time interval.

7.3.6 Take 1 ml of oil from the surface of the centrifuge tube with a clean pipet and place in a clean vial.

7.3.7 When running infrared spectra, make sure that there are no H_2O (2.7 μ) or sulfate (SO_4^{-2}) (8.5-9.3 μ) peaks.

7.4 Filling the Cell

7.4.1 Sealed Demountable Cell

7.4.1.1 Assemble clean cell with 0.1 mm Teflon spacer (always use same spacer for spectra which will be compared since spacer thicknesses may vary by ± 10%). Tighten nuts uniformly with a criss-cross sequence.

7.4.1.2 Fill the cell, tilted at 45 degrees, from the bottom port using a Pasteur pipet or syringe with a Luer-Lock tip. NOTE: Use care to avoid forming bubbles in that portion of the window which is in infrared beam.

7.4.1.3 Stopper the cell with Teflon plugs. Insert the bottom plugs first using a twisting motion. Gently insert the top plug. NOTE: If the oil is too viscous to flow in a liquid cell, smear a drop of oil in the center of the AgBr window before assembling the cell.

7.4.2 Wilks Mini-Cell

7.4.2.1 Dismantle the cell and place base and gasket on a clean Kimwipe.

7.4.2.2 Pipet one drop of oil sample into the 0.1 mm cavity of the AgBr window.

7.4.2.3 Slowly lower flat cover window onto the sample.

7.4.2.4 Inspect the window for bubbles, if present, clean the windows and repeat 7.4.2.2 using a larger drop of oil.

7.4.2.5 Insert the windows into the base and place outer threaded barrel over windows. Screw the cell together, always tightening cell in a uniform manner.

7.4.2.6 Inspect window again for bubbles.

7.4.2.7 The Wilks Mini-cell is useful especially for field use; however, it may not be sufficiently reproducible for the overlay method for similar oils using the single-beam spectrophotometer. In this case, either a sealed demountable cell or the RIIC Disposable Cell should be used.

7.4.3 RIIC Disposable Cell (Beckman) (AgCl Windows). (0.1 ± 0.002 mm. pathlength.) This cell is useful for field work and has a highly reproducible path length which is important for this application; however, it can only be used on oils with relatively low viscosity because of difficulty in filling. It can be used several times by rinsing with solvent and drying by blowing air through the cell.

7.4.3.1 Fill cell using a syringe with a Luer-Lock tip (low-viscosity oils only). Plug or crimp cell shut.

7.4.3.2 Check that there are no bubbles in optical path.

7.5 Cell and Window Cleaning Procedures

For forensic work, cells and cell windows must be thoroughly cleaned. Complete removal of residual hydrocarbons is monitored by looking for a hydrocarbon peak in the 3.5 micron region. See Figure 10 for a comparison of clean and dirty cells.

7.5.1 Turn on the fan in the portable fume hood. Make sure that the exhaust hose carried fumes outside the building.

7.5.2 Use spectroquality hexane for cleaning cells used for low and medium viscosity oils. Use spectroquality toluene for cleaning cells used for very high viscosity oils such as natural seeps. NOTE: Since these solvents are very flammable, observe safety precautions. Do not smoke or use open flames in the vicinity. Avoid getting solvents on skin. Use gloves or forceps to avoid contact. Avoid breathing solvent vapor by working within hood.

7.5.3 Dismantle cell completely. Thoroughly rinse each component with spectroquality solvent. Hold windows only by the edges. Catch waste solvent in a beaker. Dispose of waste solvent immediately in safety can. Let windows air-dry on clean Kimwipes. Reassemble cell and test for absorption in 3.5 μ region.

7.6 Care of Cell Windows

Silver Bromide (AgBr) and silver chloride (AgCl) windows are soft and must be handled carefully to prevent scratching. It is important to protect them from light as much as possible, since they darken on exposure to ultraviolet light. Check spectra of empty cell frequently to see if the spectral baseline had changed enough (> 5% Transmission at 7.5 μ) to warrant replacement of windows. Silver bromide (and chloride) reacts with certain base metals (such as aluminum, zinc, brass, copper and iron) especially at higher temperatures and in the presence of moisture. Although no reaction has been observed with stainless steel and/or lead, as a precaution, do not leave the cell assembled for prolonged periods of time. Dismantle cell, dry windows and wrap them in lens paper before storing.

TABLE I

INSTRUMENT SETTINGS,
MIRAN SPECTRA OIL CLASSIFICATION

TIME CONSTANT	0.25 sec
SLIT WIDTH	0.50 mm
SCALE EXPANSION	10x = 1-2 O.D. (Above 11 microns in absorbance mode)
	1x = % T or 0-1 O.D.
VARIABLE GAIN	90% @ 3.34 microns (0.05 mm polystyrene)
CELL (STEP A)	Sealed Demountable, AgBr, 0.1 mm
(STEP B)	RIIC Disposable, AgBr, 0.1 mm
	or Wilks Minicell AgBr, 0.1 mm
ANALYSIS	By both transmission and absorbance modes

TABLE II

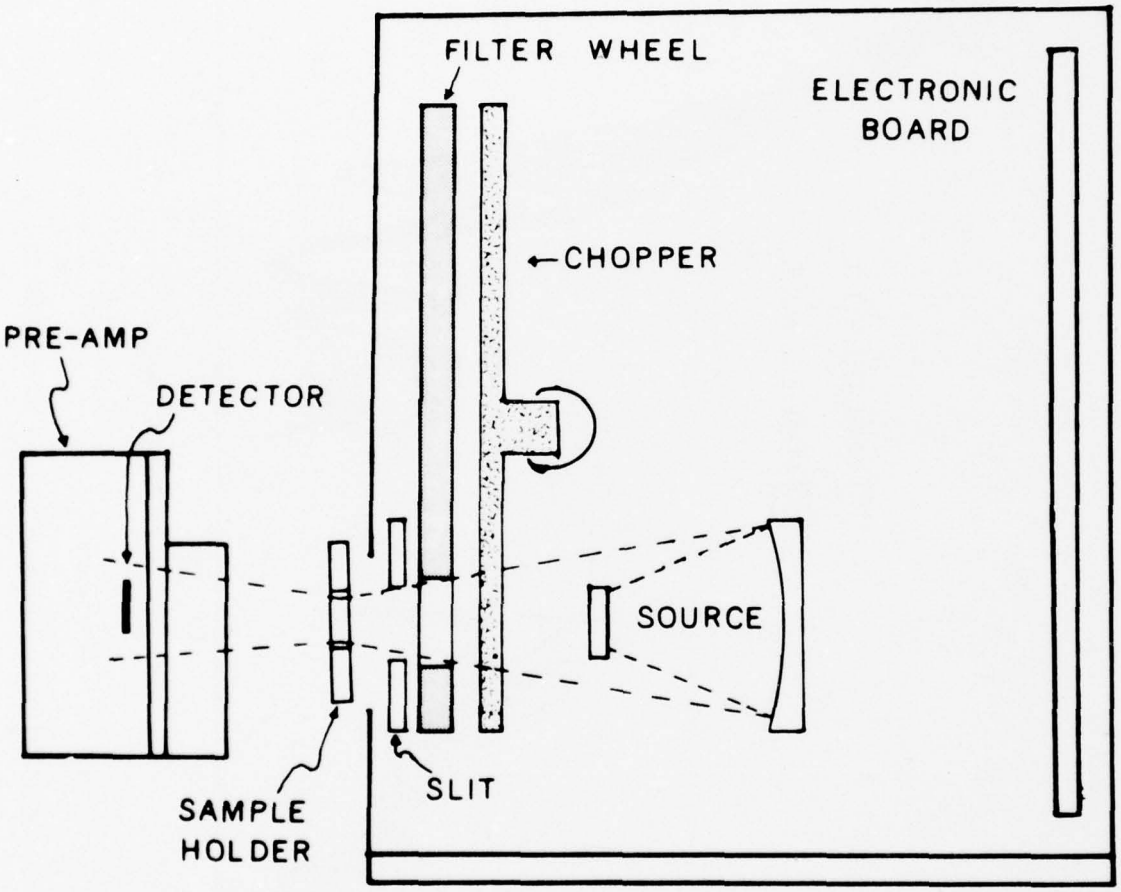
IMPORTANT WAVELENGTHS USED FOR DISCRIMINATION

SPECTRAL FEATURE	WAVELENGTH (μ)	OIL TYPE		
		FUEL OIL	CRUDE	LUBE
ABS MAX	6.2		Weak to medium	Very weak to absent
ABS MIN	7.5	Function of the Weight of the Oil (API Gravity) Weak for No. 1 Fuel Oil to Strong for No. 6 Fuel Oil		
ABS MAX	9.7			
ABS MAX	11.4			
PEAK RATIO	12.35/13.85	>0.5	<0.5	<0.5

TABLE III

OIL CLASSIFICATION READINGS AT 7.5 μ

FUEL OIL TYPE	7.5 μ INTENSITY RANGE (% T)
1	20.2 - 21.1
2	18.4 - 19.9 (16.4)
4	13.7 - 16.3
5	10.1 - 13.1
6	5.6 - 8.7



MIRAN I
INFRARED ANALYZER

FIGURE 1. DIAGRAM OF SINGLE BEAM (MIRAN) INFRARED SPECTROPHOTOMETER.

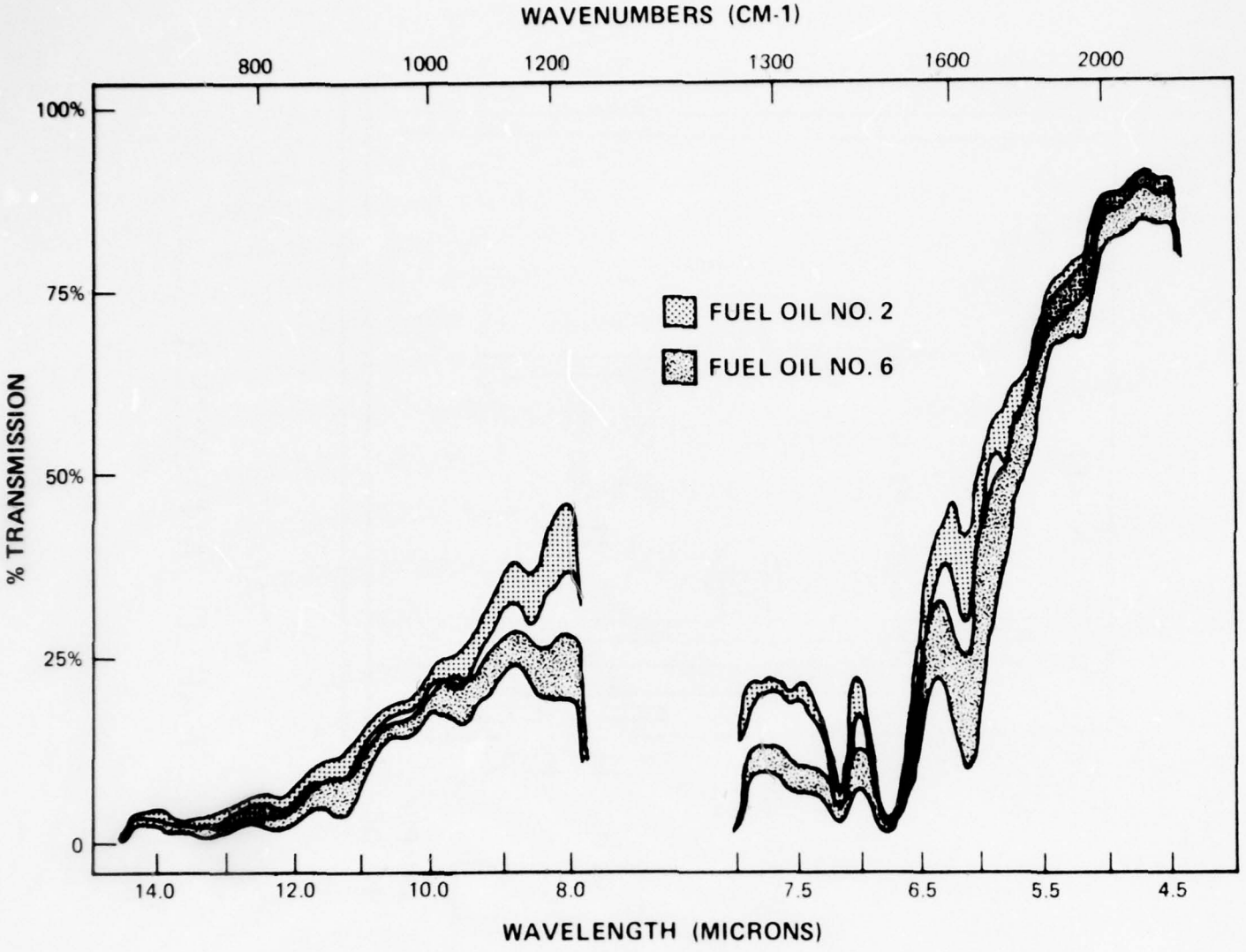


FIGURE 2. TRANSMITTANCE TEMPLATES FOR NO. 2 AND NO. 6 FUEL OILS.

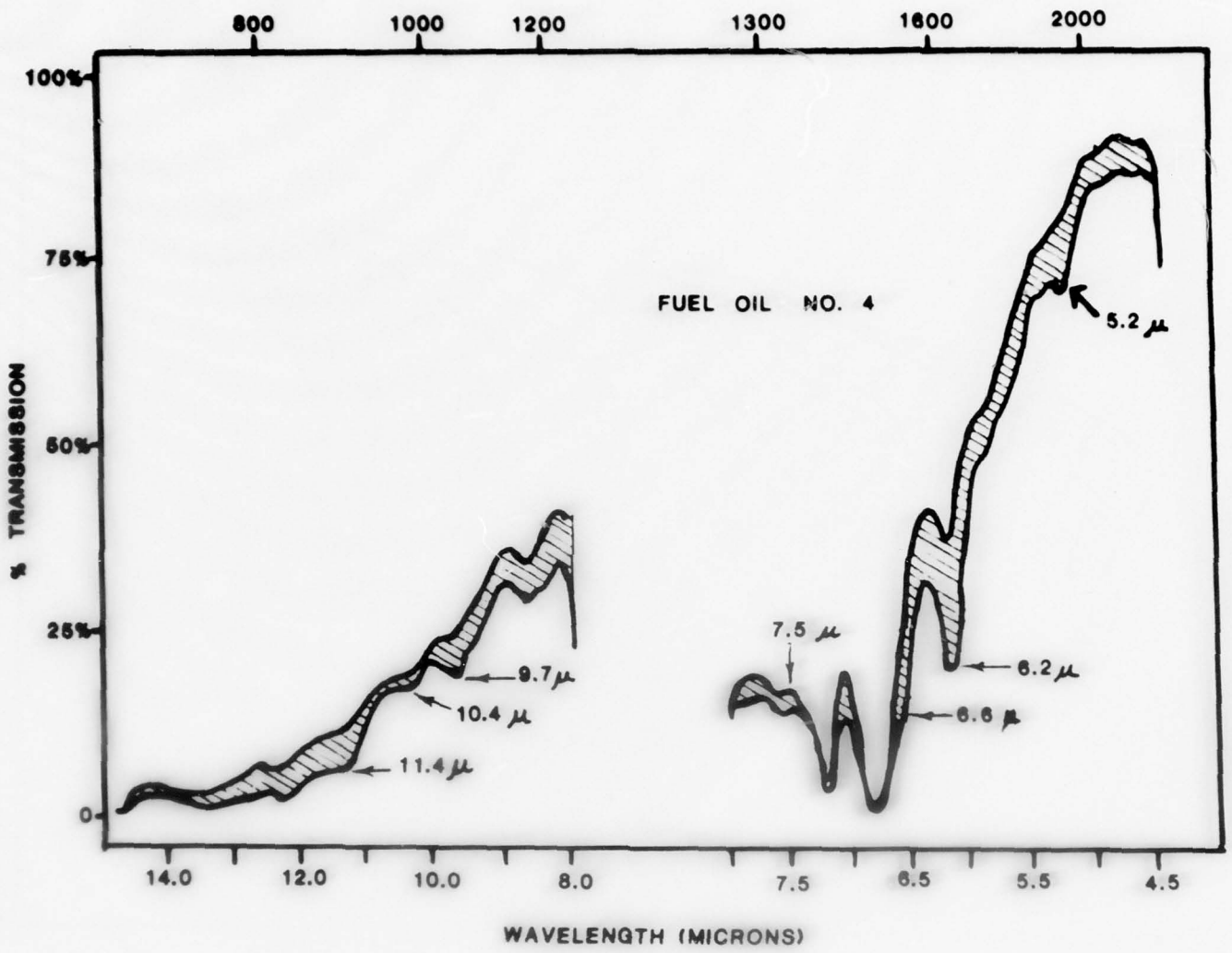


FIGURE 3. TRANSMITTANCE TEMPLATE FOR NO. 4 FUEL OIL.

FIGURE 4. TRANSMITTANCE TEMPLATES FOR FUEL OIL CLASSES IN 7.0-8.0 MICRON REGION.

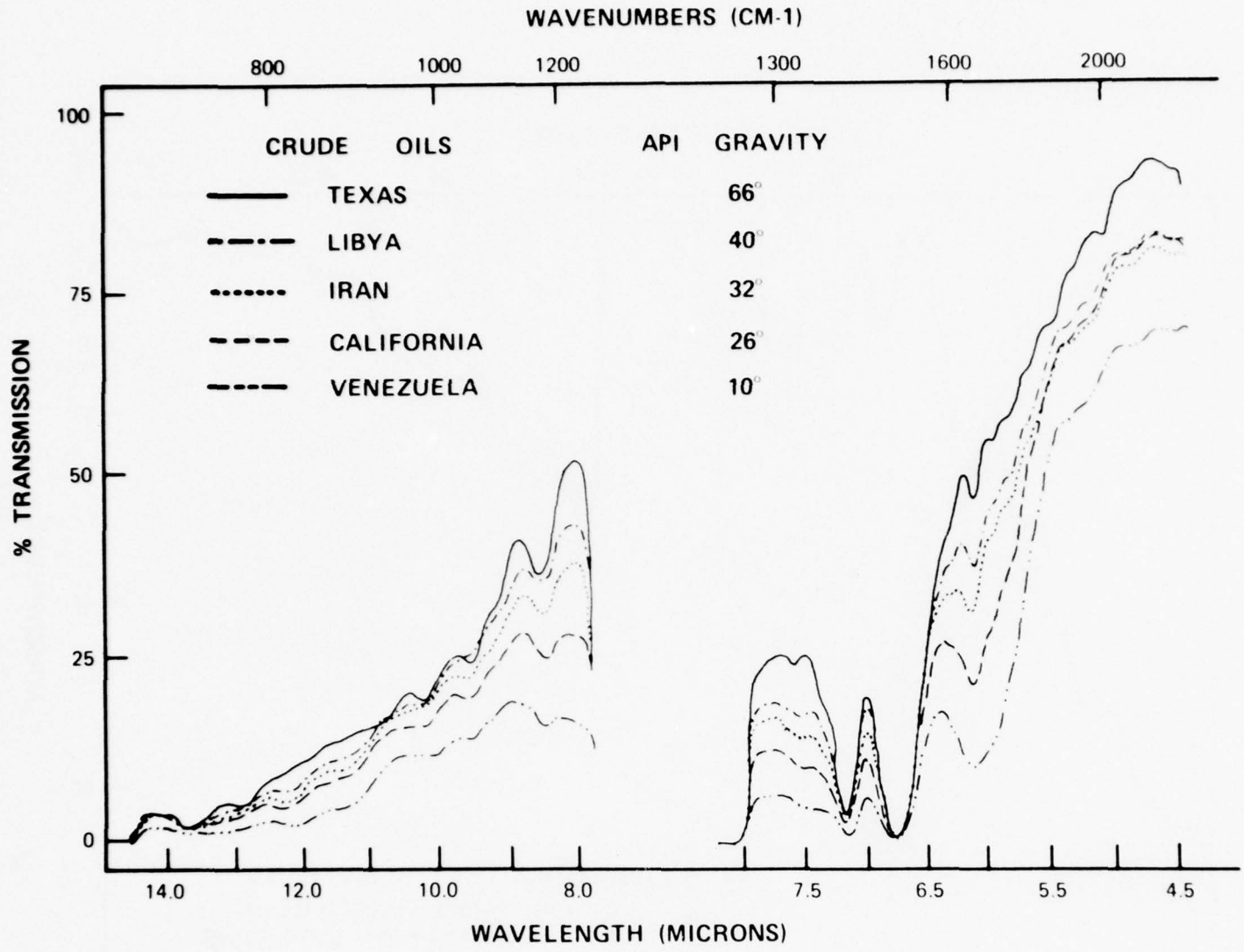


FIGURE 5. TRANSMITTANCE SPECTRA OF CRUDE OILS WITH DIFFERENT API GRAVITIES

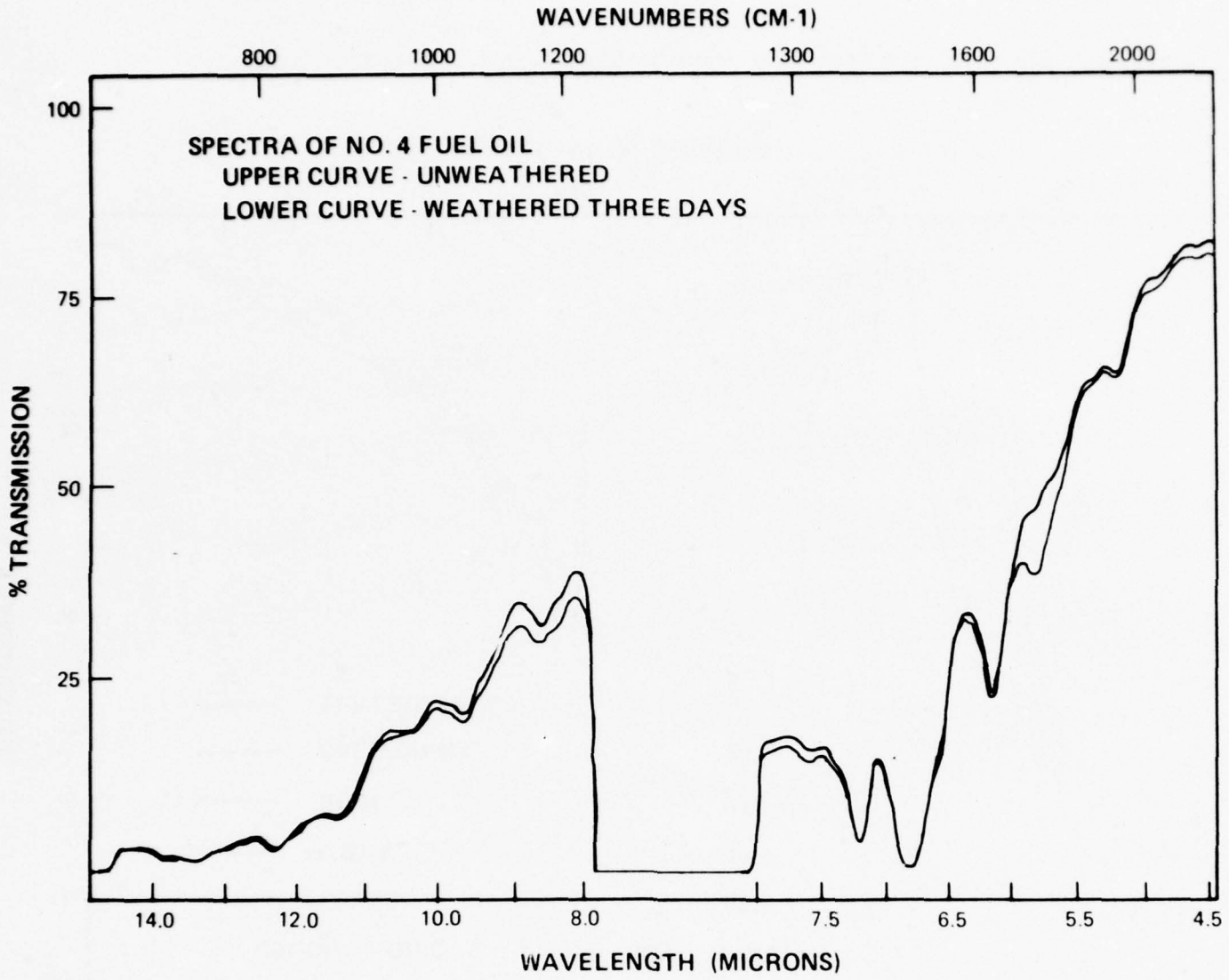


FIGURE 6. TRANSMITTANCE SPECTRA OF A NO. 4 FUEL OIL, UNWEATHERED AND WEATHERED (3 DAYS).

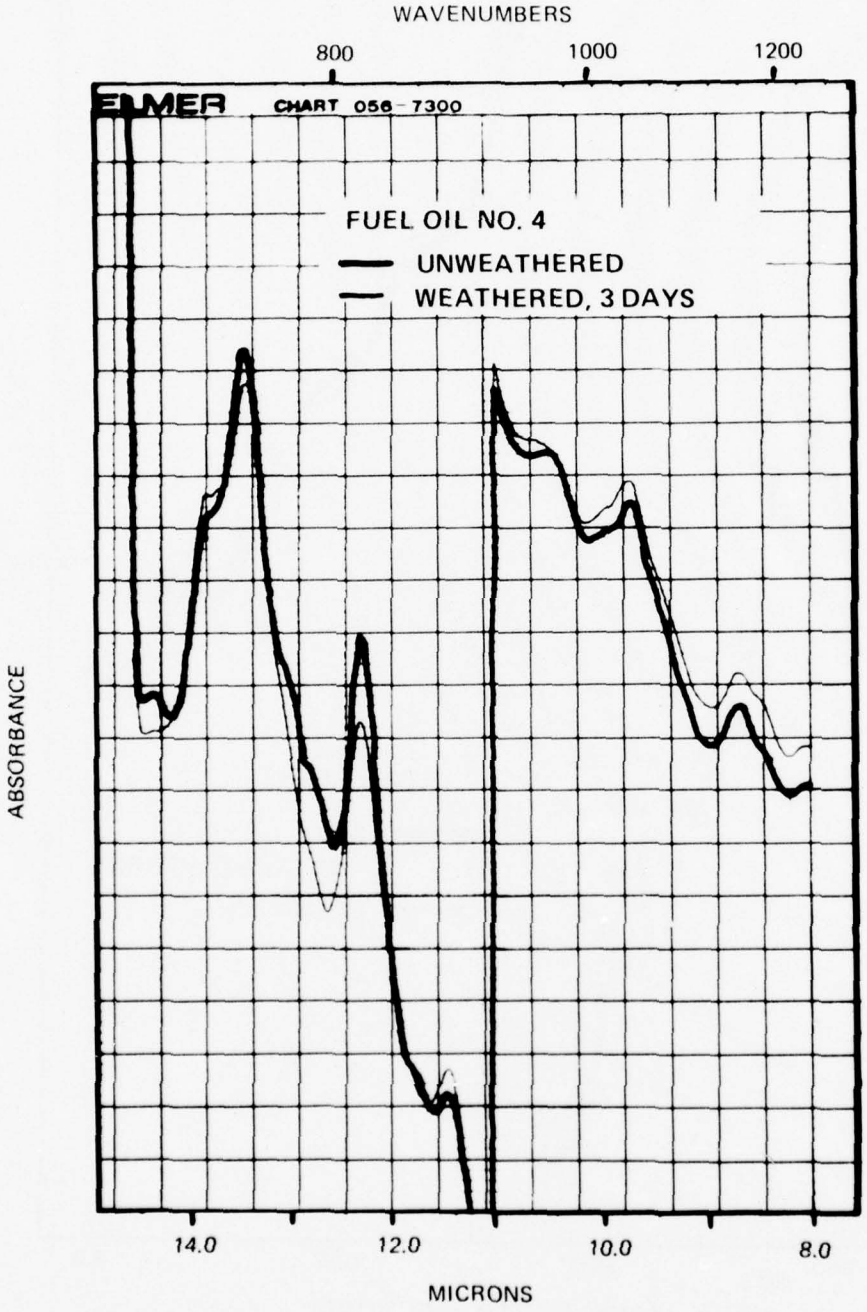


FIGURE 7. ABSORBANCE SPECTRA OF A NO. 4 FUEL OIL, UNWEATHERED AND WEATHERED (3 DAYS).

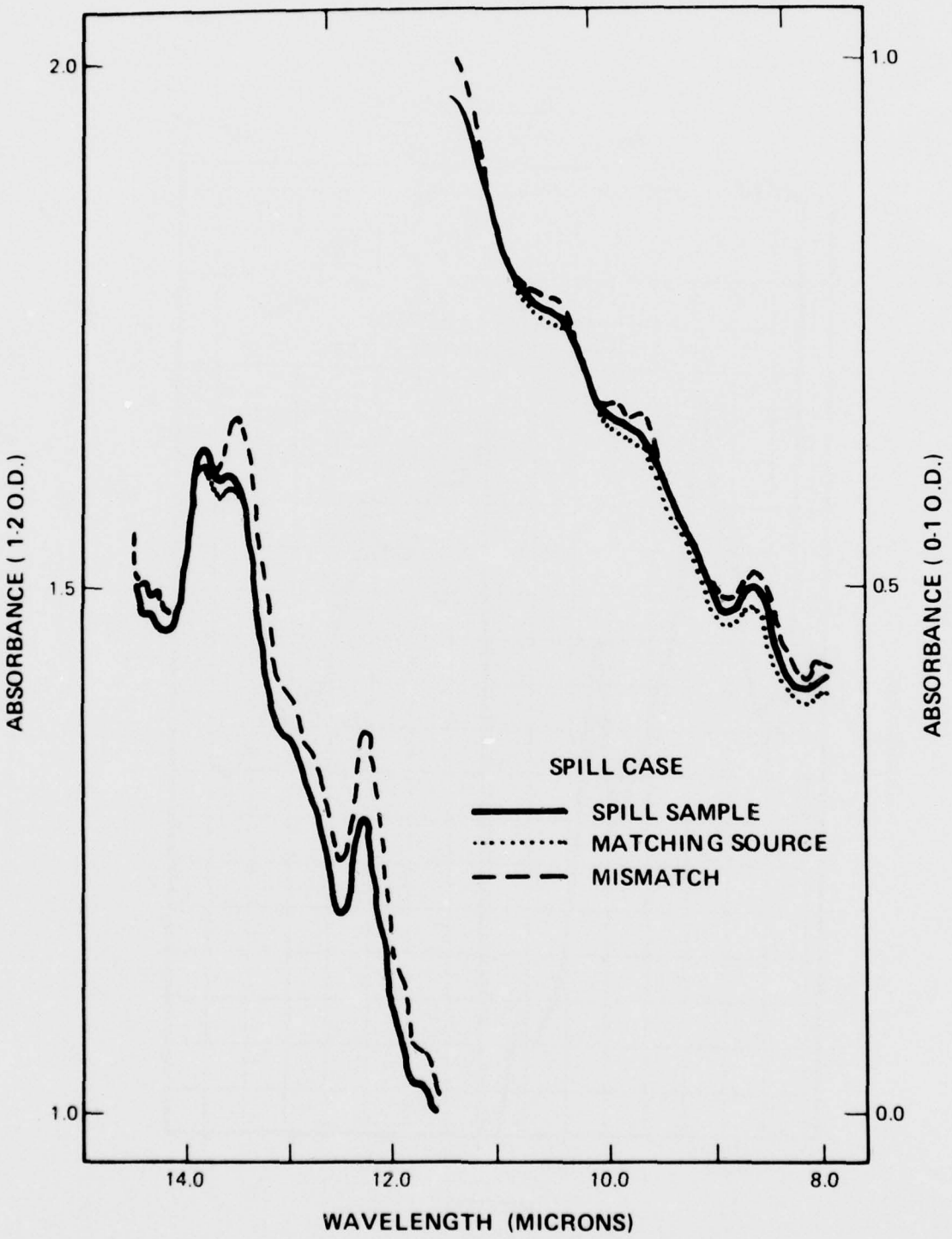


FIGURE 8. ABSORBANCE SPECTRA OF NO. 1 FUEL OILS, REAL-WORLD COMPARISON.

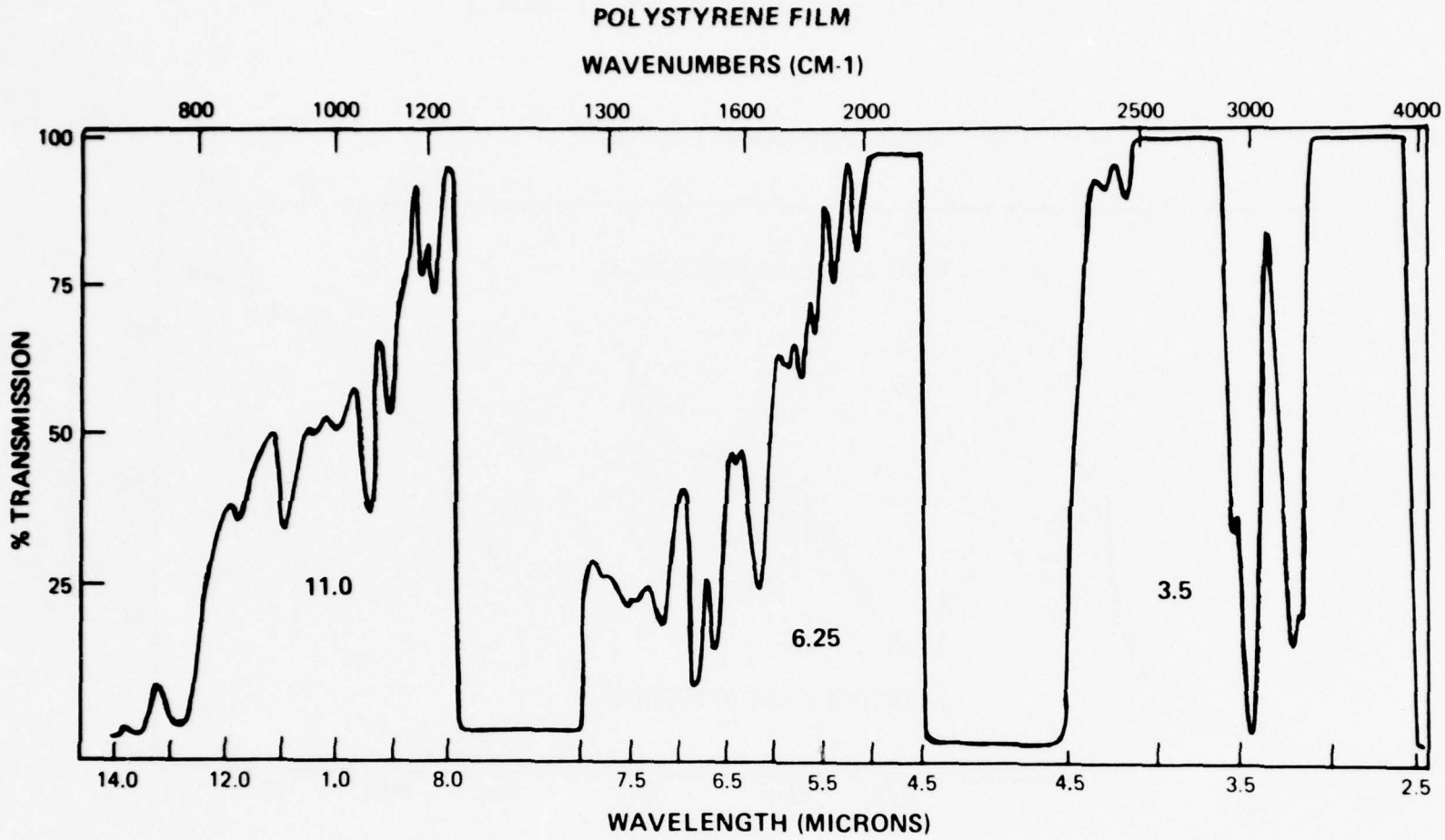


FIGURE 9. POLYSTYRENE FILM.

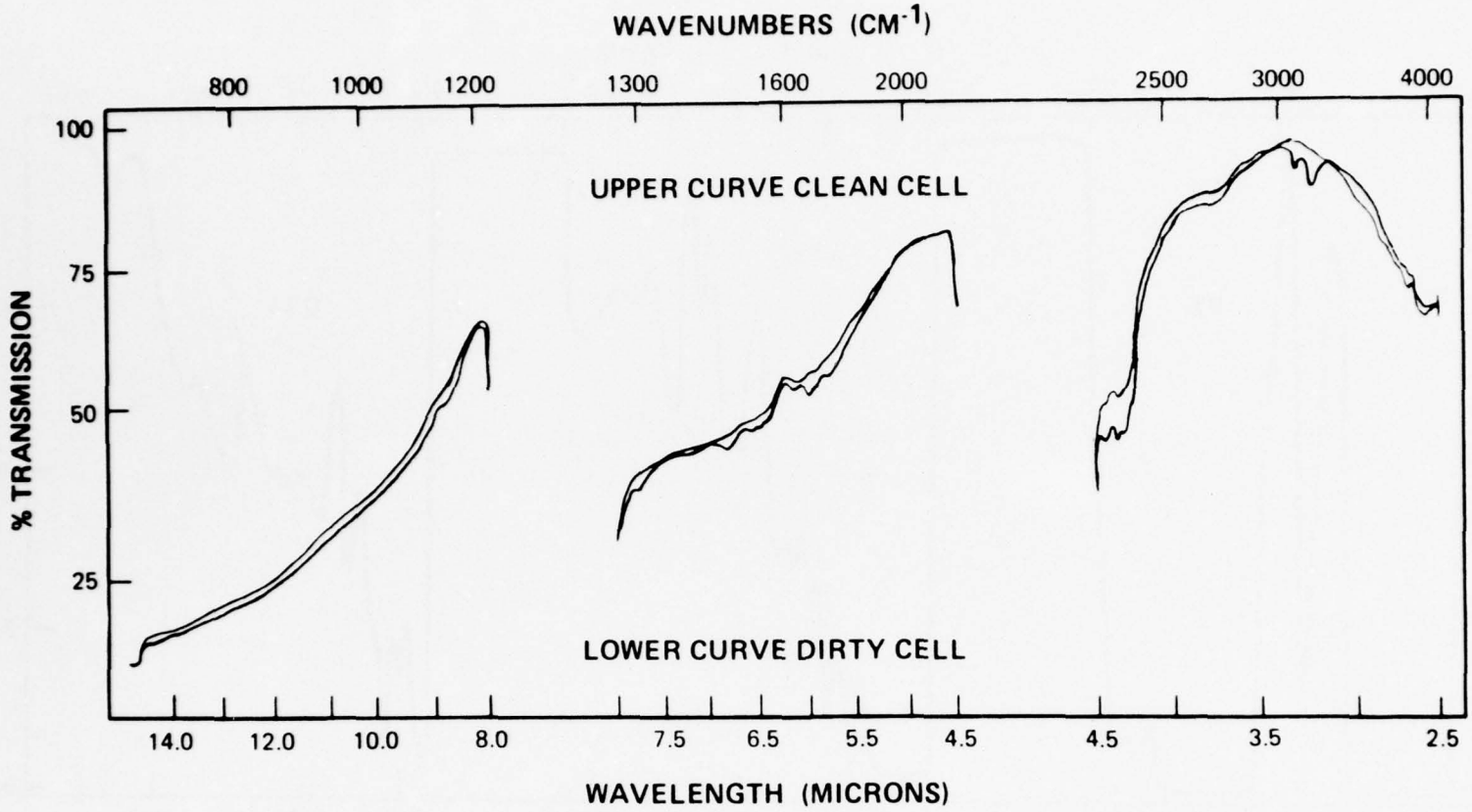


FIGURE 10. EFFECT OF RESIDUAL HYDROCARBON IN A CELL.

APPENDIX J

OIL SPILL IDENTIFICATION BY THIN-LAYER CHROMATOGRAPHY

1.0 Scope

1.1 The recommended TLC (thin-layer chromatographic) procedure providing a means of identifying a waterborne petroleum oil with a suspect source by visual comparison of thin-layer chromatograms under ultraviolet light.

1.2 This procedure can be applied to neat oil samples and to waterborne oil samples which have been weathered (environmentally or artificially) for periods of time. However, after about four days, matching becomes difficult, particularly for lighter oils. If samples are on solid substrates, the degree of confidence in the results falls markedly.

2.0 Summary of Method

2.1 Known aliquots of methanol-extracts from the oil samples are spotted on thin-layer, glass-supported, chromatographic plates coated with silica gel and developed with a methyl alcohol/hexane solution. The total development time is 45 minutes. Visual interpretation of the dried chromatograms is made under ultraviolet light.

3.0 Applications

3.1 The TLC procedure provides a means for the rapid identification of waterborne petroleum oil samples.

3.2 This method is applicable to all neat unweathered oil samples.

3.3 This method is also applicable to weathered (environmentally or artificially) oil samples which have undergone weathering effects for periods of less than about four (4) days.

3.4 The spilled oil is identified by the visual comparison of its thin-layer chromatogram with the thin-layer chromatograms of source samples (selected because of their possible relationship to the spill). A match between two thin-layer chromatograms indicates that they are from a common source. A match is defined as two separations on the same plate which are indistinguishable to the eye as to fluorescence color and location of the separated spots.

3.5 Extensive testing of this technique has demonstrated that it is very sensitive to impurities. Extraction of oils from solid substrates frequently extracts sufficient extraneous material as to make matching impossible. This method is therefore NOT recommended for oil samples furnished on solid substrates. However, certain instances require the analysis of samples on solid substrates. Table I indicates reasons for negative correlations which may be obtained.

4.0 Apparatus

4.1 Thin-layer chromatographic plates, Absorbosil-5-SG, or equivalent, silica gel pre-coated 20 x 20 cm glass plates, are to be used throughout this procedure. These plates are used directly as they come from the manufacturers without any pre-treatment, i.e., heat activation.

4.2 Developing Chamber (Figure 1, Camag Vario - K-S Chamber) and the following accessories:

a. Chamber body, complete
b. Solvent troughs, glass (2)
c. Clamping springs for solvent wick (2)
d. Solvent trough, glass, with 5 subdivisions
e. Conditioning tray, glass, with 5 subdivisions
f. Conditioning tray, glass, with 10 subdivisions
g. Conditioning tray, glass, with square subdivisions
h. Sandwich slide
i. Flat slide
j. Solvent wicks, packages of 50

4.3 Spotting Guide - consisting of plateholder, Camag template, scraper and scorer pen (Figure 2).

4.4 Ultraviolet light box with short (254 nm) and long (366 nm) wavelengths provided (e.g., Ultraviolet Products, Inc., Model C-5).

4.5 Vortex Mixer (S/P Deluxe or equivalent).

5.0 Reagents and Materials

5.1 Glacial Acetic Acid - Reagent grade.

5.2 Methyl Alcohol - Spectroquality Matheson Coleman/Bell (MX-475), or its equivalent.

5.3 Calcium Sulfate - Reagent grade, anhydrous, indicating Drierite (pellet form), or its equivalent.

5.4 Hexane - Spectroquality Matheson Coleman/Bell (HX-290) or its equivalent.

6.0 Preparation of Sample

6.1 Place a one-gram sample (approximately 1 ml of the neat oil) into a 15 ml centrifuge tube using a Pasteur pipet.

NOTE 1: In those few instances when it is imperative to analyze an oil-coated debris sample (i.e., none other available), an oil sample can be obtained in the following manner: fill a 15 ml centrifuge tube to 1/3 of its capacity with the oil-soaked material. Add an equal

volume of distilled water; then centrifuge this mixture for five minutes at ~1000 RCF. The oil will float at the top of the water layer where it is accessible. This procedure is included since it is sometimes necessary to analyze samples from solid substrates. This, however, as pointed out above is not recommended.

NOTE 2: If only a thin coating of oil is present on the water, it is not possible to obtain sufficient sample with a Pasteur pipet. In this case, the use of Teflon strips is recommended. Dip the lower half of a hand-held strip into the sample jar, passing through the oil slick. Quickly remove the strip and allow the oil adhering to it to drip into a test tube. Repeat this operation until approximately one ml of oil has been removed.

6.2 Add one gram of anhydrous calcium sulfate to the oil sample contained in the 15 ml centrifuge tube. (The anhydrous calcium sulfate removes any water entrapped within the oil sample.) Shake the test tube on the Vortex mixer for 1 minute.

NOTE 3: If the oil sample is of a very viscous, heavy consistency, carry out this mixing in a hot water bath. This will allow the calcium sulfate granules to circulate through the oil thoroughly when mixing in order to insure complete desiccation of the oil sample.

6.3 An equal volume of acidified methy alcohol (0.4% glacial acetic acid/methyl alcohol, by volume) is added to the mixture in 6.2. The combined mixture is shaken on the Vortex mixer for one minute until the oil is well emulsified and then centrifuged at 1000 RCF for five minutes.

6.4 The methanol phase is then removed with a Pasteur pipet, and retained in a 1 dram capped glass vial.

7.0 Care and Cleaning of Apparatus

7.1 Inevitably, some silica gel from the TLC plates will become dislodged and accumulate on the surface of the developing unit. This can be removed by rising with distilled water. An organic solvent such as methyl alcohol should be used on a soft tissue or cloth to remove residual traces of oil prior to reuse.

7.2 The paper wick must always be renewed after each development to prevent cross-contamination from one plate to the next.

8.0 General Procedure

8.1 Spotting the samples

8.1.1 Place a thin-layer plate into the spotting guide holder (Figure 2).

8.1.2 Using the scraper, remove a 1 cm wide strip of powder from three edges; from both sides of the glass plate, and top.

8.1.3 With the Camag template locked into the pair of holes on the sides of the plate holder nearest the bottom, gently mark the plate (just enough to see) with the scorer pen at every point where a recess in the saw-toothed spotter occurs.

8.1.4 Turn the spotting guide ninety degrees and lock it into place in the outermost holes of the top and bottom rows of the holder on the left side. Score the plate vertically using the scorer pen.

8.1.5 Repeat Step 8.1.4 on the right side of the plate.

8.1.6 Remove the plate from the plate holder and gently tap on the bench top to remove the loosened adsorbent into a waste basket. Avoid breathing dust.

8.1.7 Spot five microliters (4 µl) of the methanol extracts from 6.4 along the lower edge of the TLC plate. The extracts are removed and spotted on the plate using disposable 5 µl capillary blood pipets, delivering each extract to the plate where it was marked in 8.1.3.

NOTE 4: After spotting the plate as in 8.1.7, check the spot intensities in the ultraviolet light box. If the fluorescence intensity of any spotted samples is decreased (dull in appearance, hard to see), place another 5 µl of that sample extract on the original spot. Re-check under ultraviolet light. All sample spots should be roughly equal in fluorescence intensity. Most, if not all, samples displaying decreased fluorescence will be spill samples. (The loss of fluorescence intensity is primarily attributed to oxidative weathering processes.)

8.2 Developing the plate (Figure 3)

8.2.1 The clamping wings (1) are opened by lever (not shown). The solvent trough is lowered by pulling knob (3). The chamber is exactly leveled by means of adjustable feet and referring to spirit level (4).

8.2.2 Lid (5) of the solvent trough is opened. A solvent wick with the clamping spring is placed into the dry solvent trough. The wick is creased to a [shape before inserting (Figure 4).

8.2.3 The conditioning tray (Figure 1e) is inserted into the developing chamber in such a way that the troughs are parallel to the solvent trough.

8.2.4 Enough 10% acetic acid/methanol solution is added to the solvent trough to saturate the paper wick completely.

8.2.5 The TLC plate is placed, layer facing downward, on the chamber so that the edge of the plate parallel to and nearest the sample spots is touching the base of the chamber. Note that the solvent trough lid must be open and the sandwich slide in place, all the way down toward the solvent trough.

8.2.6 The clamping wings (1) are closed and tightened by lever, and the 10% acetic acid/methanol-soaked wick is brought into contact with the absorbent layer by pushing knob (3) inward. (Note the wick should make contact with the adsorbent near the sample spots.) Allow the solvent to travel on the adsorbent until the sample spots are covered (about 5 mm). Lower wick.

8.2.7 Release the clamping wings and slide the plate away from the trough until the solvent trough is fully exposed. N.B. DO NOT LIFT PLATE FROM CHAMBER.

8.2.8 Remove the solvent trough and wick assembly containing 10% acetic acid/methanol and replace with a clean dry solvent trough and wick assembly.

8.2.9 Replace the solvent trough cover and slide the TLC plate forward all the way to the trough cover.

8.2.10 Open the trough lid. The developing solvent (4% methanol/hexane) is poured into the solvent trough. The solvent trough lid is then closed and knob (3) is pushed, starting development of the chromatogram. The solvent should migrate uniformly.

NOTE 5: If the solvent front does not migrate uniformly, this may be due to any one of the following: (a) the chamber may not be level; (b) the clamping wing seals may not be airtight. This may, in turn, be due to improper cleaning of the sandwich slide, or scoring of TLC plate edges. If the solvent front is slanted, but straight, the plate can be used; if it is concave, or convex, repeat entire separation with new plate carefully observing all precautions.

8.2.11 After development has proceeded for 45 minutes, lower the solvent trough, open clamping wings, and lift off the TLC plate. After the plate has air-dried for about one minute, examine under UV light.

8.2.12 If cursory examination of the plate under UV light indicates very poor resolution (likely due to presence of crude oil), it is necessary to pre-condition the plate with solvent vapor (Step 8.2.13) and repeat steps 8.1.1 through 8.2.11.

8.2.13 Solvent pre-conditioning is effected by proceeding from 8.1.1 through 8.2.5. In 8.2.6 omit the paper wick. Instead, fill the solvent trough half full of 10% acetic acid/methanol solution and allow the vapor to condition the plate for 5 minutes. Proceed then to 8.2.7.

9.0 Visual Interpretation

9.1 Visual interpretation is carried out by examining the TLC plate under short (254 nm) and long (366 nm) wavelength ultraviolet light, as well as under both simultaneously. Occasionally an apparent match under one wavelength will be a non-match under the other. Therefore, it may be necessary to switch back and forth several times in order to make a final judgment as to the correct match - using the criteria discussed below.

9.2 Observe the colors of the separated bands under ultraviolet light. The colored sequence of bands must be identical for two oils to be considered a match.

NOTE 6: Exceptions to this rule are light distillates, i.e., refinery cuts from gasoline and kerosene through (and including) No. 2 fuel oils. These are light-colored oils which are highly susceptible to weathering processes. As a result, fluorescence shifts to shorter wavelengths for certain bands result in color change. The only susceptible bands are lime-green which change to light blue. As little as four hours' exposure to bright sunlight (in the case of a No. 2 fuel oil) can effect this color shift. This color shift also applies to blended fuel oils (dark-colored oils which contain green bands); however, these oils are not as quickly affected as the lighter oils since they contain an asphaltene fraction which acts as a light shield to slow the color shift reaction. Any unweathered suspects not possessing green bands may immediately be eliminated as potential matches for a spill sample which does possess green band(s); however, if the spill sample displays no green bands, then potential sources which contain green bands cannot be dismissed as non-matching sources.

9.3 Observe the relative distances of the bands from the origin. These distances must be visually identical, spot for spot, for a suspect/spill match (all bands must correspond). Figure 5 shows some representative chromatograms of different crudes and refinery products using the American Petroleum Institute Oil Standards (from left to right: No. 2 fuel oil, Kuwait crude, Bunker "C", and Louisiana crude). In Figure 6, a spill sample (2) is compared to five (1,3,4,5,6) oils taken from different locations from the same tanker. The spills and samples 3, 4, and 5 are identical. Figure 7 shows the results of a second spill case in which samples 2 and 3 from the spill are indistinguishable from suspect 4.

NOTE 7: All corresponding bands must fluoresce the same color (except as in NOTE 5) for two oils to be identical. In order that a match may still be made for oils possessing a green-to-blue color change, the green (or blue) band on the spill chromatogram must correspond exactly to a green band on the suspect. (Although the band can change in color, it will not shift its location on the thin-layer chromatogram.)

9.4 Observe the intensities of the separate bands. For a spill/suspect match, relative intensity differences from band to band in the spill chromatogram must remain constant in the suspect chromatogram, although the overall intensities of the spill chromatogram may be diminished due to weathering.

NOTE 8: Incomplete drying of a sample prior to extraction will result in a non-uniform diminution of fluorescence intensity over the entire TLC plate. If the bands are poorly resolved (streaked) and weak in intensity, redry the sample and repeat the analysis.

9.5 After taking into account all factors listed in 9.2 through 9.4 (color, relative distance and intensity), under all three UV exposures (short wavelength, long wavelength, and combined), designate a match or non-match for each separation on the same plate as the spill sample.

9.6 After UV light examination, iodine staining may be used, if necessary, to provide additional information or support the judgment made from UV examination. It is used only with lubricating oils or mixtures, such as bilge oils, which contain non-fluorescing additives. Visualization of these non-fluorescing additives may be the key to the source of the spill. Figure 8 shows the iodine-stained plate of a spill case. The spill (1) is identical to suspect (2). All suspects (2-8) represent bilge oils from different tugboats.

9.7 Chromatograms can be recorded by photographing the developed plate. A Polaroid Model 195 Land camera is used with the following lens and filters placed in the order cited (all connected by Series Seven 45 mm adapters):

+3 close-up lens
#3 yellow filter
CC40Y filter
Hood

The same light box can be used for both visual inspection/interpretation and UV photography. This is accomplished by mounting a bracket with an extension arm to swing the camera into place after visual examination of the plates. After the camera is in place, the shutter speed is set on "B"; the f-stop at 8 and a cable release is installed. Polacolor 2, Type 108 film is used. One photograph per plate per wavelength is sufficient; shot as follows:

	WAVELENGTHS		
	254 nm	366 nm	254+366 nm
Exposure Time (minutes)	4.5	2.5	1.5

All photographs are developed for 1.5 minutes.

It is important that the bottom of the developed plate (the edge with the original spots) always be nearest the ultraviolet light source being used. This means that the plate must be rotated 180° when taking a long wavelength after a short wavelength plate or the reverse. When using both lights simultaneously, the bottom of the plate is nearest the short wavelength light.

10.0 Field Versus Laboratory Method

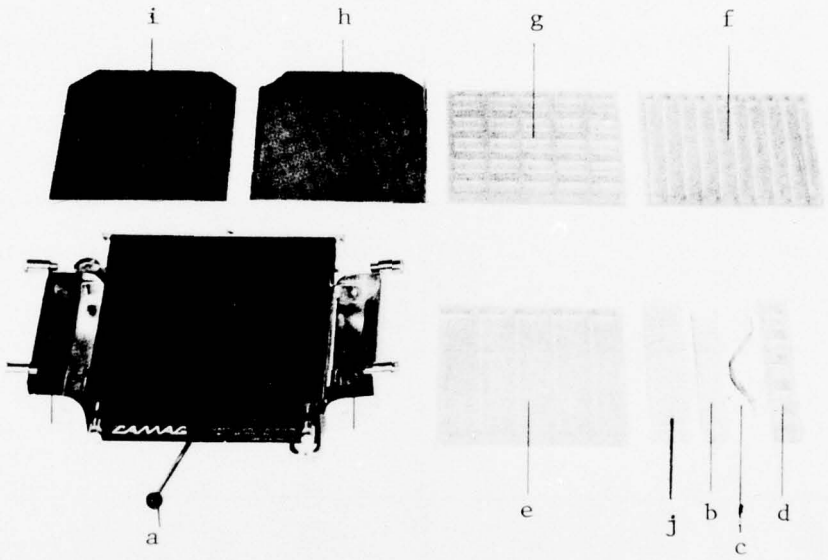
10.1 In the research laboratory environment, the TLC system can be used for more sophisticated analyses, such as: grading gasolines, additive detection in lubes, classifications of oils, etc.

TABLE I

EFFECT OF SUBSTRATE ON TLC MATCHING OF SAMPLE FROM TEN

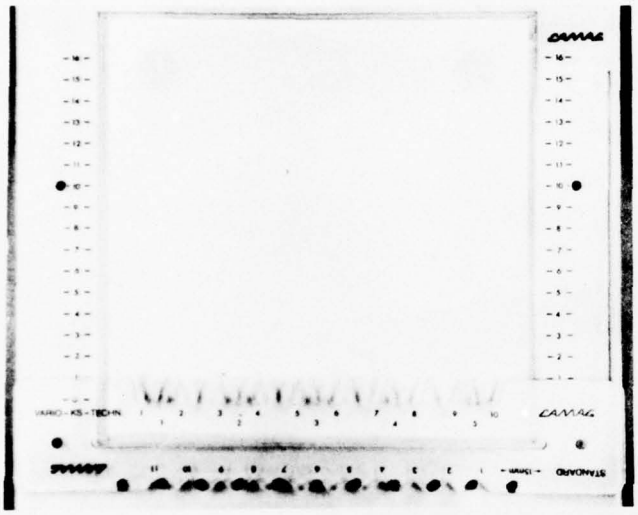
SIMULATED SPILL CASES

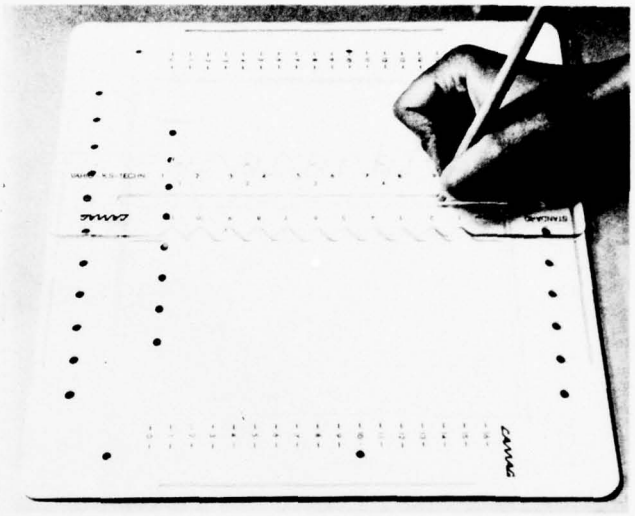
SPILL SAMPLES	NO. OF POSSIBLE MATCHES	NO. OF MATCHES MISSED	CAUSES
Oil on saltwater	9	1	Inability to distinguish two crudes from same oil field
Oil on sand	9	4	Oil-contaminated sand
Oil on sawdust	4	4	Compounds extracted from sawdust substrate
Oil on vegetation	4	1	Polar compounds and lipids extracted from vegetation
Oil on dirt	1	0	
Mixture of 2 oils (suspect)	3	3	Lost identity (i.e., Oil A + Oil B = Oil C, not A + B)



a. Chamber body, complete
b. Solvent troughs, glass (2)
c. Clamping springs for solvent wick (2)
d. Solvent trough, glass, with 5 subdivisions
e. Conditioning tray, glass, with 5 subdivisions
f. Conditioning tray, glass, with 10 subdivisions
g. Conditioning tray, glass, with square subdivisions
h. Sandwich slide
i. Flat slide
j. Solvent wicks, packages of 50

Figure 1. Apparatus.





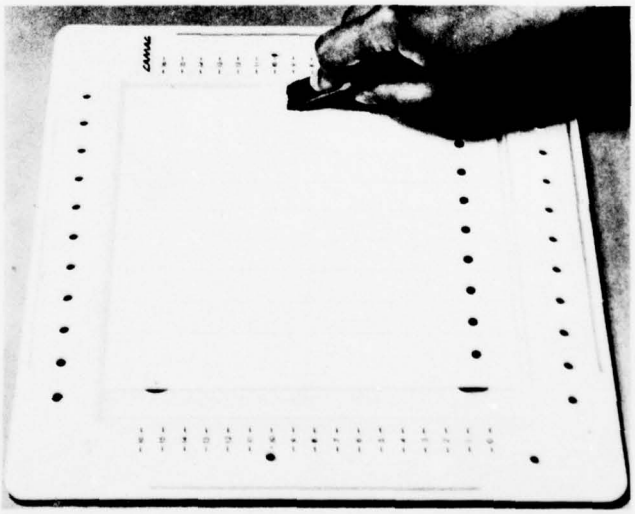


Figure 2. Spotting guide.

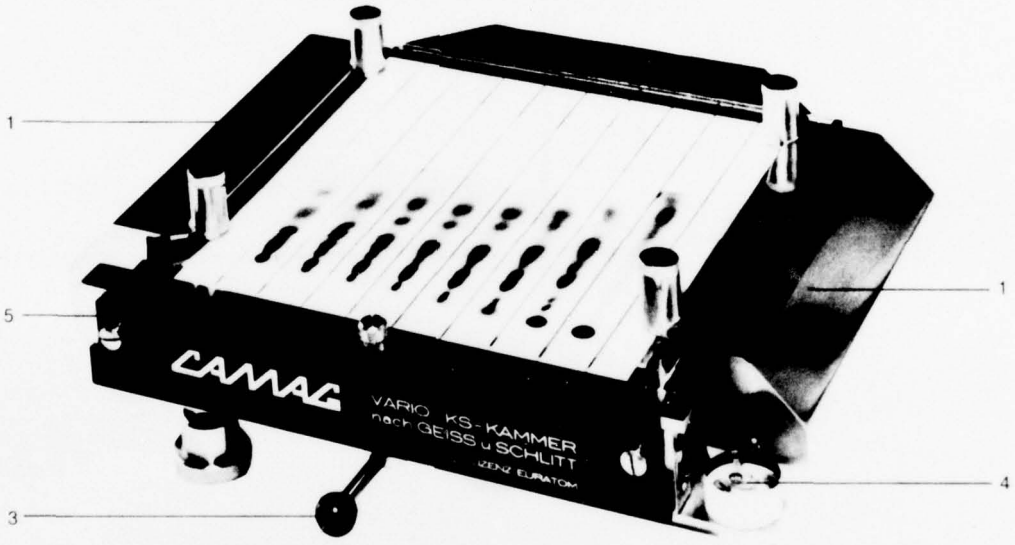


Figure 3. Developing TLC plates in the Camag Vario-KS-Chamber.

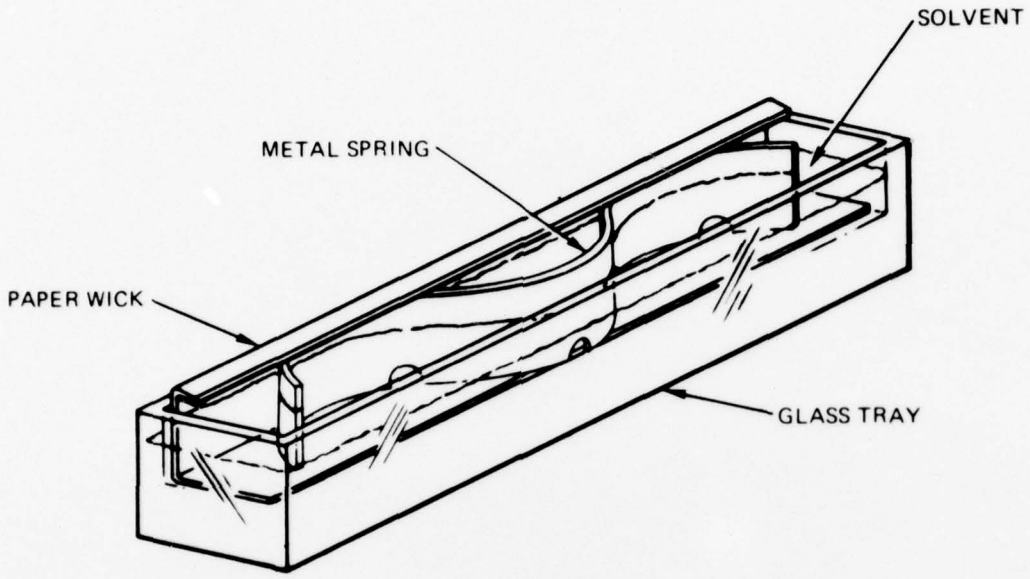
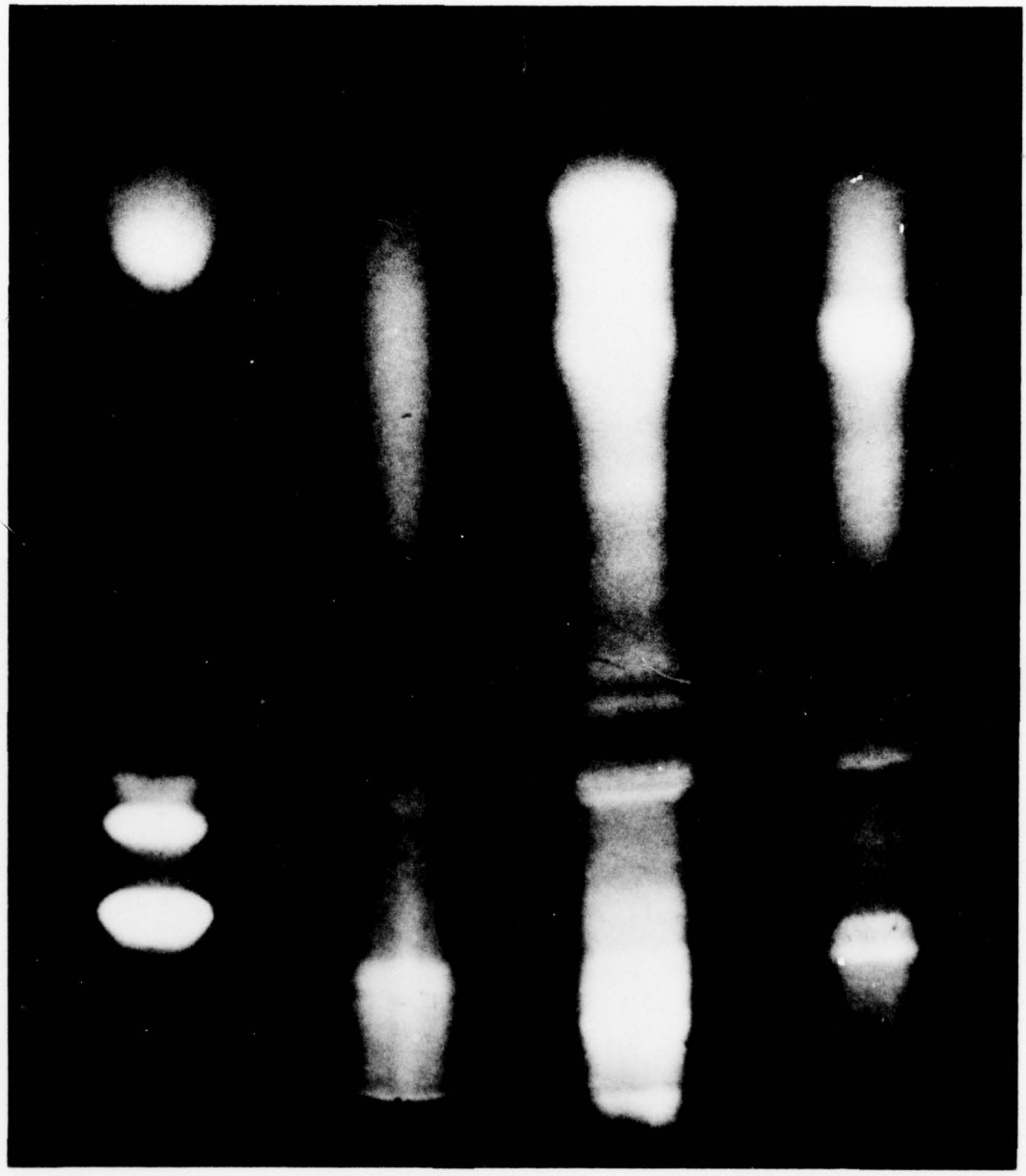
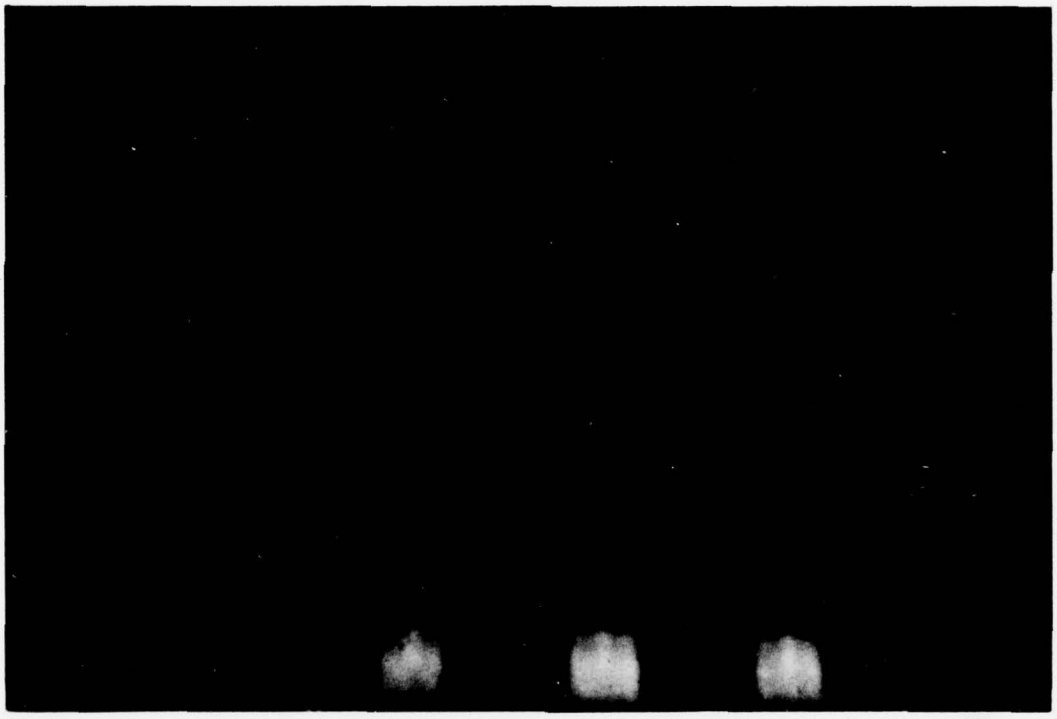


FIGURE 4. SOLVENT TROUGH WITH WICK HELD IN PLACE BY METAL SPRING.



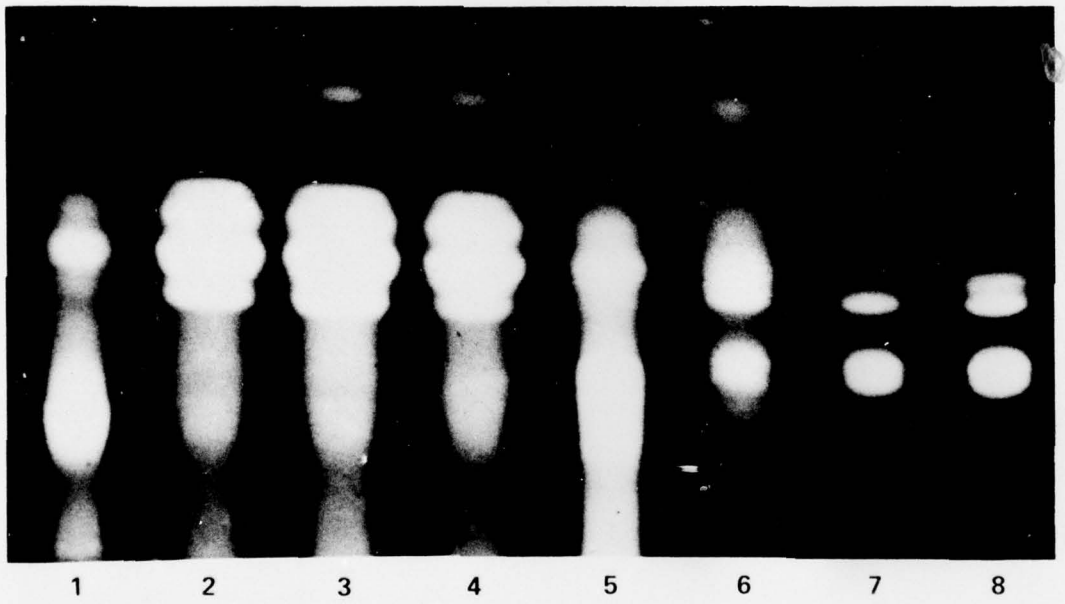
1. No. 2 Fuel Oil
2. Kuwait Crude
3. Bunker "C"
4. Louisiana Crude

Figure 5. Thin-layer chromatograms of API standard oils.



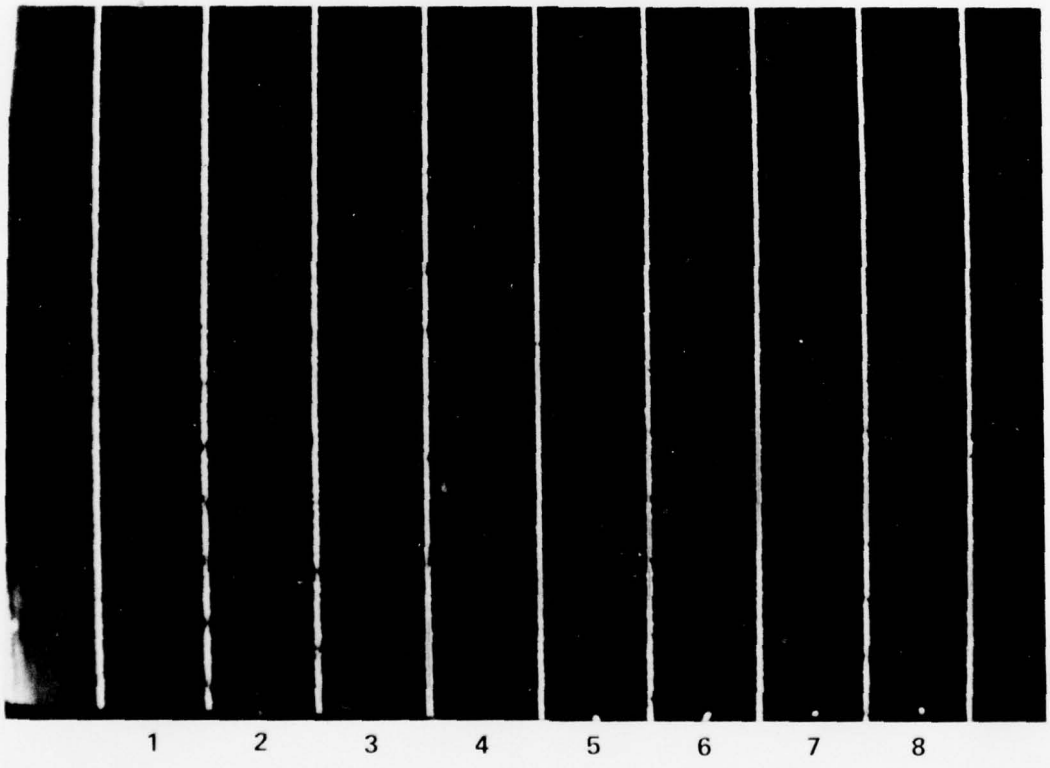
1. Solvent bucket
2. Spill
3. Aft pump room
4. Slop oil tank
5. Cargo tank
6. Bunker tank

Figure 6. Spill sample chromatogram matched to three samples taken from a single vessel.



2, 3 Spill Samples
1, 4, 5, 6, 7, 8 Suspect Samples

Figure 7. Spill case in which one of the six suspects (4) is indistinguishable from the spill samples.



1. Spill sample
2-8 Bilge samples from seven tugboats

Figure 8. Spill case in which sample 2 was matched to the spill by visualizing the TLC plate with iodine.

APPENDIX K

OIL SPILL IDENTIFICATION BY HIGH PRESSURE LIQUID CHROMATOGRAPHY

1.0 SCOPE

1.1 The following High Pressure Liquid Chromatographic procedure was developed as an adjunct to those methods in the "Oil Spill Identification System" (CG-D-41-75). This technique is useful in those oil spill cases when other analytical methods do not concur with each other. High pressure liquid chromatography provides not only a "fingerprint" but a rough estimate of the time an oil has been on the water.

2.0 SUMMARY OF METHOD

2.1 The liquid chromatographic method as applied to oil spill identification utilizes a methanolic extract of the oil injected onto a reverse phase liquid chromatographic column and developed with a water/methanol gradient (from 50% to 100% methanol). The components of the oil extract are separated on the chromatographic columns and detected in the effluent as they pass through dual photometric cells monitored at 254 nm and 210 nm. The resulting chromatograms are then used to screen suspect sources and then to match a suspect to the spill.

3.0 APPLICATION

This method is applicable to all types of oils and is useful with spill samples which have not been weathered more than about four days.

4.0 APPARATUS

1. Perkin-Elmer Model 1220 liquid chromatograph equipped with two 3000 psi piston pumps with an air bath column oven and gradient elution capability.

2. Waters Associates U-6-K injection system.

3. Perkin-Elmer Model 250 Ultraviolet Adsorption Detector

4. Coleman Model 55 UV-Vis Digital Spectrophotometer

5. 2 Water Associates μBondapack C_{18} ODS columns

6. 2 Perkin-Elmer Model 56 strip chart recorders (or a dual recorder)

7. 5 μl side-port Auto Sampler Syringe, Precision Sampling Corp.

8. Corning Model PC-35 hot plate

9. Milliport Vacuum - pressure pump #XX60000000

10. International Equipment Centrifuge Model HN-S

11. Lab-Line Pyro-Magnestir, Model 1266

12. Millipore All-Glass Filter Apparatus, 47 mm #XX15 047 00

13. Scientific Products Deluxe Vortex Mixer

5.0 MATERIALS AND REAGENTS

1. Glacial Acetic Acid - Reagent grade

2. Methyl Alcohol - Spectroquality Matheson Coleman/Bell (MX-475)

3. Calcium Sulfate - Reagent grade anhydrous

4. Distilled water, UV cut off 190 nm or less

5. Filters, Millipore 47 mm, 0.22 μ

6.0 PREPARATION OF SAMPLE

6.1 Place a one gram sample (approximately 1 ml of the neat oil) into a 15 ml centrifuge tube using a Pasteur pipet.

NOTE 1: In those few instances when it is imperative to analyze oil-coated debris sample (i.e. none other available), an oil sample can be analyzed in the following manner: fill a 15 ml centrifuge tube to 1/3 of its capacity with the oil-soaked material. Add an equal volume of distilled water; then centrifuge this mixture for five minutes at ∿ 1000 RCF. The oil will float to the top of the water layer where it is accessible. (This procedure is included since it is sometimes necessary to use samples on solid substrates. This, however, as pointed out above, is not recommended.)

NOTE 2: If a sample of oil is present as a thin slick on the water it is not possible to obtain sufficient sample with a Pasteur pipet. In this case, the use of Teflon strips is recommended. Dip the lower half of a hand-held strip into the sample jar, passing through the oil slick. Quickly remove the strip and allow the oil adhering to it to drip into a test tube. Repeat this operation until approximately 1 ml of oil has been removed.

6.2 Add one gram of anhydrous calcium sulfate to the oil sample contained in the 15 ml centrifuge tube. (The anhydrous calcium sulfate removes any water entrapped within the oil sample.) Shake the test tube on the Vortex mixer for 1 minute.

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NOTE 3: If the oil sample is of a very viscous, heavy consistency, carry out extraction in a hot water bath. This will allow the calcium sulfate granules to circulate through the oil thoroughly when mixing in order to insure complete desiccation of the oil sample.

6.3 An equal volume of acidified methyl alcohol (0.4% glacial acetic acid/methyl alcohol, by volume) is added to the mixture in 6.2. The combined mixture is shaken on the Vortex Mixer for one minute until the oil is well emulsified and then centrifuged at 1000 RCF for five minutes.

6.4 The methanol phase is then removed with a Pasteur pipet, and retained in a 1 dram capped glass vial.

6.5 Place the sample vial from 6.4 into a 60°C water bath on a Lab-Line Pyro-magnestir hot plate. Suspend a Pasteur pipet with the tip about 0.5 cm above the methanol phase.

6.6 Connect the Pasteur pipet to a vacuum source and apply suction. Evaporate until oil droplets appear in the glass vial.

6.7 Replace cap and centrifuge at 1000 RCF for 10 minutes. The sample should now be crystal clear with oil droplets at the bottom of the vial. If not, repeat this step. Insure that the sample vial is properly labelled.

7.0 SOLVENT PREPARATION

This procedure is used separately for both water and methanol.

7.1 Vacuum filter 1 L solvent using a Millipore all-glass filtration apparatus with a 0.22 μ filter in place.

7.2 Place the filtered solvent in a 2 L volumetric flask. Install a ground glass vacuum adaptor with ground glass stopper on the flask.

7.3 Place this flask on the Lab-Line Pyro-Magnestir hot plate at 60°C (ambient temperature for methanol) and apply a vacuum (15 in. Hg for H_2O, less for methanol to avoid boiling). Adjust the magnetic stirrer until swirling bubbles appear in the solvent. Allow this degassing to proceed until bubble formation ceases (about 30 minutes for water).

7.4 Remove the flask from the degassing apparatus and immediately fill the solvent pump reservoirs on the chromatograph. Avoid unnecessary agitation of the solvent while filling.

7.5 Store the unused portion of the solvent in a 2 L flask with an air-tight ground glass stopper in place.

8.0 GENERAL PROCEDURE

8.1 Columns

Two columns (0.64 x 30 cm) connected in series and packed with Waters Associates μBondapak™ C_{18}. They are connected with reducing end fittings containing a 5 mm stainless steel frit. Column effluent is transferred to the detectors via 0.015" ID stainless-steel tubing. NOTE: Any commercially available micro C_{18} reverse phase packing may be used if it provides comparable efficiency (i.e., approximately 3,000 theoretical plates per foot).

8.2 Detectors

A Perkin-Elmer Model 250 Ultraviolet Absorption Detector monitoring effluent at 254 nm is used as the primary (first) detector (nearest the end of the column); the sample cell only is connected to the column, with the reference cell cleaned and sealed off containing room air as a blank. The shortest possible length of 0.015" I.D. stainless steel tubing is used to connect the above detector to the Coleman spectrophotometer, set at 210 nm. Both detectors are operated in the absorption mode. The photometric cell should have a minimum flushing efficiency of 2.5 volumes (as described in the manufacturer's instruction manual).

8.3 Column Installation

When installing new columns in the instrument it is important to pre-rinse each column separately to remove any particulate matter on the column. The particulate matter can settle out on the detector's flow cell windows. This will make it difficult, if not impossible, to zero the detectors.

8.3.1 Install the inlet end of the first column to the solvent inlet fitting in the oven.

8.3.2 Set the solvent delivery at 50% methanol in water and start the pumps at a flow rate of 1.5 ml/minute. Allow the solvent to flow through the column into a waste container for 15 minutes. Check to make sure that the solvent effluent is crystal clear before proceeding to the next step.

8.3.3 Connect the second column in series with the first using a column coupling adaptor. Make sure that the inlet of the second column is connected to the outlet of the first. Repeat step 8.3.2.

8.3.4 Connect the outlet side of the second column to the solvent outlet in the oven. Turn on detectors and chart recorders and wait until the detectors stabilize. The chart recording will indicate many sharp peaks. These are caused by air bubbles passing through the detectors. The detectors are stable when the chart recorders show a straight line. The stabilization time will vary with the amount of air trapped in the system.

8.4 Calibration of Detectors and Recorders

8.4.1 Perkin-Elmer Model 250 - Set attenuator to 10, O.D. to "S" position, with the detector knob in Calibrate position. Using chart recorder zero adjust, set pen deflection at 0. Set O.D. to "%T" (the meter reading should be 50). Adjust pen deflection using "calibrate adjust" on detector to 30% full scale on strip chart. Set detector to "operate,"and reset O.D. to 0.02. Recorder pen should be zero; if not, use coarse and fine zero adjusts, on detector.

8.4.2 Coleman LC 55

Set detector in absorbance mode. Set spectrophotometer to 0.00 digital display using coarse and fine adjusts. Set pen deflection to zero using chart recorder zero adjust knob.

8.5 Column Conditioning

The following procedure is used not only for brand new columns, but is also necessary for the first run of the day.

Turn oven to "on" and set the temperature to 60°C. Set chromatograph into linear gradient mode. Set "hold/run" switch to "hold" position when 50 is shown on the digital display. Set time to 10 x 10 minutes. Set "hold/run" switch to "run." Set flow rate at 1.5 ml/min (the pressure should be 2,000 psi ± 100 psi). Allow gradient to run to completion (99 on digital display, finish light on), and allow chromatograph to continue pumping for exactly 20 minutes more (with 100% methanol).

8.5.1 The following procedure is used for repetitive sample injections on the same day after first conditioning the columns as outlined above in 8.5.

Set time to 1 x 10 minutes. Place chromatograph in "retrace" mode, and push "start" button. Allow digital display to run backwards from 99 to 50, then reset "run-hold" switch to "hold," retrace-gradient switch to "gradient," and time to 10 x 10 minutes. Allow column equilibration for exactly 20 minutes before introducing a sample.

8.6 Sample Introduction

Fill syringe with 4 µl of a sample prepared in 6.7 (do not draw up air or oil droplets). Set U-6K "load/injection" lever to "load." Set plug retaining lever to "open" position. Remove sample plug and place in hole in "load/injector" lever. Insert syringe as far as possible into the sample loading port. Inject sample, remove syringe, and replace plug. Set "plug retaining" lever to "closed" position. Set "load/injector" lever to "inject" and set "hold-run" switch (on chromatograph) to "run."

NOTE 4: Amplifier attenuation must not be changed on either detector at any time between 25 and 40 minutes, since these regions of the chromatograms are for data reduction in matching oils. The maximum pen deflection, however, must be less than 90% within this region.

9.0 DATA REDUCTION

9.1 The 254 nm absorption chromatogram is used primarily as a screening technique to eliminate unlikely suspects. For all oils a doublet peak elutes at t=34 minutes. Figure 1 demonstrates the liquid chromatograms of 2 #6 fuel oils from the same feedstock; the peak-height reversal of the 34 minute doublet is apparent.

9.2 Peak heights of the 34-minute doublet are measured in mm with a ruler from baseline constructed as shown in Figure 1.

9.3 The heights are ratioed by dividing the first peak height by the second.

9.4 Any suspect sample whose calculated 254 nm ratio is not within 6% of the ratio for the spill can be eliminated as a potential spill source.

9.5 All potential sources not eliminated by the above ratio technique must be considered further by means of the 210 nm chromatograms. Only those peaks eluting between 25 and 40 minutes, which possess both positive and negative slopes, with a minimum peak height of 5 mm are to be considered. Figure 2 indicates these peaks and the constructed baselines.

NOTE 5: The data points defined above must be common to both spill and suspect.

9.6 Peak heights are measured as in 9.2.

9.7 The peak heights are used to generate a series of intra-chromatographic peak height ratios for each chromatogram not eliminated in 9.4. This series is an ordered sequence of ratios generated by ratioing the first peak to the second, second to third, third to fourth, etc. (i.e., $\frac{n}{n+1}$ $\frac{n+1}{n+2}$ $\frac{n+2}{n+3}$ ...). Table 1 lists the serial ratios generated from the chromatograms in Figure 2.

NOTE 6: Since qualitative differences between spill and suspect chromatograms in the 25 to 40 minute region of the 210 nm chromatogram occur frequently, the spill sample must be made the standard for comparison. Only corresponding peaks common to both spill and suspect are used for comparing oils. Extraneous peaks existing in the 25-40 minute elution range of the suspects are ignored in calculating the series of ratios for that suspect if no such peak(s) exist in the spill chromatogram, since both weathering

and possible contamination could account for these discrepancies. Consequently, zero values are not included in the series of ratios for any suspect oil.

9.8 The serial ratios are compared statistically for matching purposes. This method compares any two chromatograms and assigns a numerical degree of "match" between any spill/suspect pair. This procedure involves the following:

(1) Computation of the standard error of the standard deviation of the serial ratios (collectively considered for each oil, i.e., Table 1).

(2) Computation of the correlation coefficient between the individual ratios of the two oils.

(3) Comparison of the standard errors of the standard deviations for any two ratio series.

9.8.1 For any chromatogram, X, the standard error of the standard deviation (S) of the ratio series for X is defined by the equation.

$$S_{\sigma X} = \frac{S}{\sqrt{2n}}$$

where the standard deviation (S) is defined by

$$S = \sqrt{\Sigma(x^2) - \frac{(\Sigma x)^2}{n-1}}$$

where x represents the individual ratios for the series of chromatogram X, and n is the number of ratios (x's) in the series.

9.8.2 Repeat 9.8.1 for chromatogram Y

9.8.3 For any two chromatograms, X and Y, the correlation coefficient (r) is defined by the equation

$$r_{XY} = \frac{n\Sigma xy - \Sigma x \Sigma y}{\sqrt{[n\Sigma x^2 - (\Sigma x)^2][n\Sigma y^2 - (\Sigma y)^2]}}$$

where n = the number of ratios in the chromatogram

9.8.4 The correlation coefficient (r_{XY}) and the standard errors of the standard deviations for chromatograms X and Y are now used to test the hypothesis that the serial ratios of X and Y are not significantly different. This comparison (S_D) is defined by the following equation:

$$S_D = \sqrt{S_{\theta X}^2 + S_{\theta Y}^2 - 2r_{XY}\, S_{\theta X}\, S_{\theta Y}}$$

9.8.5 Repeat steps 9.4 thru 9.8.4 for all suspects.

9.9 Interpretation of the Numerical Value of S_D

9.9.1 The value of S_D is a statistical measure of the differences between two oils. The larger the S_D, the greater the differences. Duplicates of three different oils gave S_D values of 0.1±0.01. Weathering of a spilled sample generates a larger S_D value when it is compared to the unweathered source.

9.9.2 To quantitate the effects of weathering on S_D values, between a spill and source, seven simulated spill tests were conducted with samples weathered up to 96 hours. Thirty-three S_D values were calculated. Ten values of matching oils had $S_D < 0.21$. Two known matches lay between 0.22-0.26 as did two known mismatches. The values in this region were designated as inconclusive. All other mismatches had values exceeding 0.26. Therefore, based on these initial empirical results:

a. $S_D < 0.22$ is defined as a match.

b. $S_D > 0.26$ is defined as a mismatch.

c. $0.22 \leq S_D \geq 0.26$ is defined as inconclusive.

10.0 EXPOSURE TIME IN HOURS

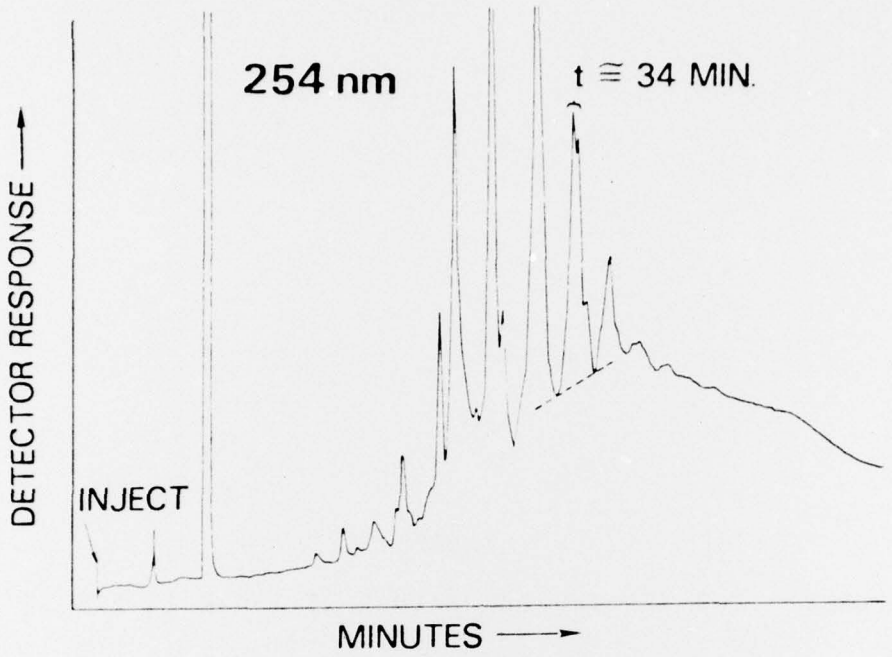
10.1 Based on a linear correlation between S_D and exposure time to weathering for 9 out of 11 oils tested, an equation can be written for an approximate exposure time in hours:

$$t_{(exp)} = \frac{(S_D - 0.1)\ 10^3}{1.25}$$

TABLE I

RATIO SERIES FOR THE TWO CHROMATOGRAMS SHOWN IN FIGURE 2

CHROMATOGRAM X RATIO (spill)	CHROMATOGRAM Y RATIO (suspect)
1.13	1.39
2.57	2.57
.07	.07
.99	1.01
2.94	2.88
.93	.87
.76	.75
1.07	1.07
3.31	3.39
.83	.84
1.16	.94



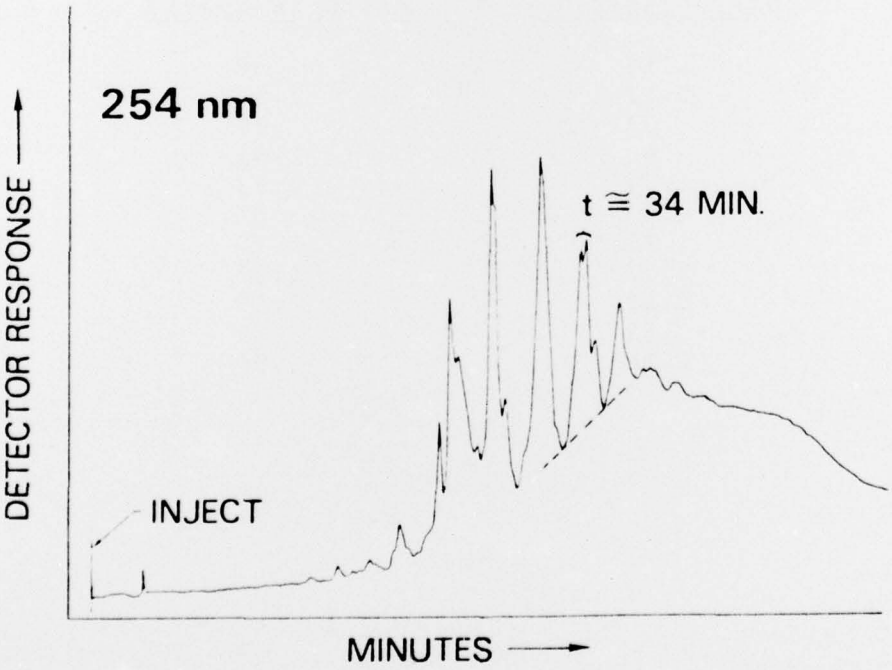


Figure 1. Chromatograms of two No. 6 fuel oils from the same feedstock.

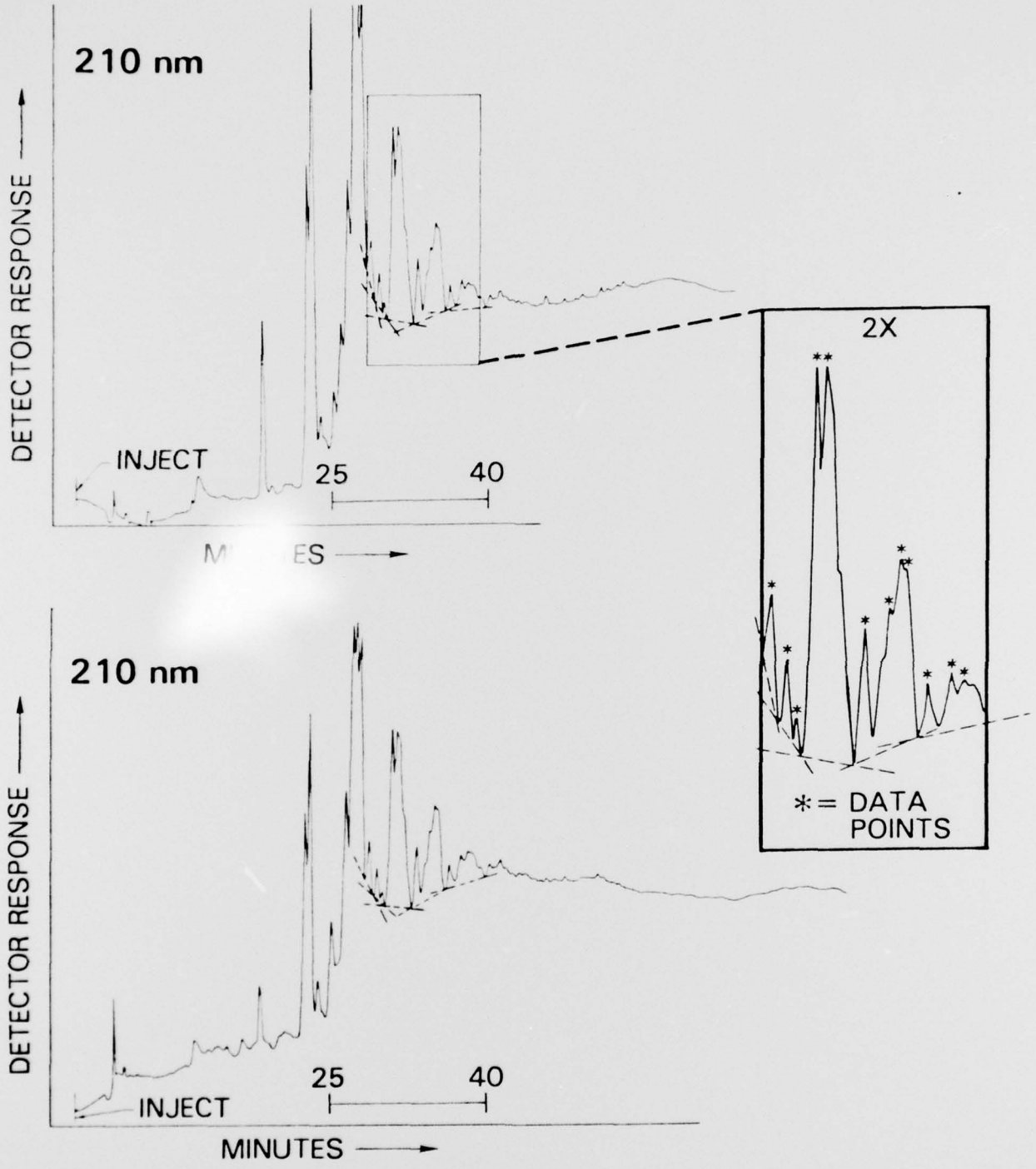


Figure 2. Chromatograms of a spill (upper curve) and a suspect (lower curve).

APPENDIX L
SAFETY IN OIL SPILL IDENTIFICATION

I. INTRODUCTION

Safety is an integral part of any operation. Ultimately, the individual worker is responsible for his own safety, and for those around him. This requires that he be informed of the hazards likely to be encountered in his work and be able to cope with them.

This appendix is not an all-purpose chemical safety manual. Rather, it addresses safety aspects peculiar to the preceding appendices which present several analytical techniques for oil spill identification.

It is assumed that there is an operative safety manual at the laboratory, or facility, where these methods are being used, and that the laboratory personnel have been instructed in general safety procedures regarding the physical handling of materials, precautions against electric shock, basic elements of first aid, etc.

It is further assumed that the laboratory is properly designed with appropriate equipment such as: explosion-proof refrigerators; fume hood with separately controlled exhaust and makeup vents; safe gas cylinder storage area; approved solvent storage cabinets, explosion-proof exhuast fans on the hood; eyewash station and safety shower; fire extinguishers.

Smoking is prohibited in laboratory areas. Eating and drinking is likewise prohibited, except for areas so designated.

II. PERSONAL PROTECTIVE EQUIPMENT

A. Safety Glasses. All personnel working in laboratory areas must wear eye protection. For this purpose, approved safety glasses are usually adequate; special cases might require face shields.

Use of safety glasses is absolutely essential when handling solvents or liquid nitorgen. At the discretion of the laboratory supervisor, certain work areas, or operations may be designated as exceptions. For example, while an electronic instrument is in operation, it may not be necessary to wear the glasses.

In practice, it is better for the laboratory personnel to acquire the habit of wearing the glasses at all times in the laboratory. However, to insure compliance in particular areas - such as the hoods, or when handling solvents over the sinks, etc. - it is beneficial to differentiate clearly the "absolute must" situations from other less critical ones in the laboratory.

B. Gloves. Gloves are particularly important when handling large volumes of solvents, or if an individual has particularly sensitive hands. Rubber, or solvent-resistant gloves should be used.

C. Safety Bottle Carriers. Bottle carriers should be used when transporting glass bottles containing hazardous chemicals. This includes acids (glacial acetic acid) as well as flammable solvents and is particularly important for the 5 lb (3L) bottles (these are not to be transported by holding the neck of the bottle).

III. SOLVENTS

A. Handling. The handling of solvents should be done carefully with respect for their potential fire and health hazards. In oil identification, a number of solvents are used to prepare solutions for analysis, or for cleaning sample cells and equipment. Table 1 lists the individual solvents and the appendices in which they appear.

Two general rules should be followed whenever possible: (1) work in a fume hood when rinsing glassware or transferring solutions; (2) avoid contact with the eyes and any unnecessary contact with the skin. Particularly bad in this regard is toluene which is a primary skin irritant and absorbed directly through the skin. Avoid breathing solvent vapors by maintaining adequate ventilation in the room when working with solvents.

B. Storage. Keep a minimum supply of solvents on hand in the laboratory work space. Do not store solvents on shelves at eye level in the work area. Bulk storage should be in an approved storage cabinet in a well ventilated area.

C. Disposal. In the laboratory, small quantities of waste solvent should be placed in an approved solvent container. This container may be plastic or metal - preferably wide-mouthed - with a flame arrestor. Do not dispose of inorganic acids in the same container! (See 5E., p. 4.)

When the small container is filled, it should be emptied into a suitable drum. The filled drum, in turn, is to have its contents disposed of by a qualified outside contractor.

IV. COMPRESSED GASES

The compressed gases used for oil identification are, except for hydrogen, not chemically hazardous. Helium and nitrogen are inert. Compressed air used for the hydrogen flames in the chromatograph can be obtained by means of a compressor - eliminating the need for a high pressure cylinder. Likewise, a hydrogen generator can also be substituted for a cylinder (with its attendant problems). A carbon dioxide cylinder may be used for sub-ambient cooling in gas chromatography, or for making dry ice for cooling purposes.

The primary gases themselves (helium and nitrogen) are innocuous, but the main hazard potential is from the high pressure under which these gases are kept. To handle cylinders safely, they must be stored and transported with shipping caps on. They should be moved in the laboratory only by means of approved wheeled carts to which the cylinder is secured safely.

The cylinders must be kept away from heat, and always secured in an upright position by clamping to a firm support. The appropriate reducing valve must be used (without adaptors) for each type of gas. Never lubricate or tamper with a cylinder valve - always send them to the supplier for repair if necessary.

V. MISCELLANEOUS

A few special precautions are noted below which apply to particular methods.

A. Xenon Lamps. Xenon lamps are used as light sources in fluorescence and low temperature luminescence (Appendices E and F) and the following precautions should be strictly observed:

1. Xenon lamps generate ozone which is an irritant and which is also toxic on chronic exposure. It is therefore imperative that ozone be exhausted from the room when operating in a confined area. This is accomplished by means of a small fan and duct located over the lamp housing leading to the outside.

2. Xenon lamps emit ultraviolet-rich light. Under no circumstances should anyone look directly at the operating lamp since damage to the eyes could result.

3. When it is necessary to change a lamp, ALWAYS wear safety glasses and heavy gloves when handling the lamp. The lamps are under extremely high pressure and may explode if dropped or twisted. To change a xenon lamp, remove the new one from its protective case only when ready to install it - then immediately place the old lamp back into the case.

B. Liquid Nitrogen. Liquid nitrogen is a non-flammable, non-toxic material with a boiling point of 77.3°K (-195.7°C). Because of its very low temperature, liquid nitrogen must be handled with extreme care. Another hazard is the extreme pressure buildup due to evaporation if liquid nitrogen is kept in a confined space.

Also, if stored uncovered or poorly covered it will slowly be converted (by condensation) to liquid air (b.p. 85°K) which is reactive. This, in turn, may become slowly enriched in liquid oxygen (b.p. 90.2°K) to form a very hazardous oxidizing mixture. Therefore, always store liquid nitrogen in a loosely stoppered container, so that the pressure of nitrogen gas can be released while maintaining a positive pressure to prevent admission of atmospheric air or water vapor to be condensed (ice crystals from the water vapor serve as nucleation centers for bubbles which would cause light scatter in low temperature luminescence).

Glass Dewars are commonly used as containers for small quantities of liquid nitrogen. They are evacuated and implode violently if struck or dropped - shattering the glass into many small shards. The Dewar should therefore be wrapped (except for optical surfaces) with strong tape (preferably cloth adhesive) before use. Quartz or glass Dewars should be kept away from heluim which can diffuse through them and destroy the vacuum.

If it is necessary to dispose of liquid nitrogen in order to clean and/or dry a Dewar, pour the liquid nitrogen slowly onto the floor or the ground. Never pour it into sinks, as it could crack the sink and/or the drain pipes.

Liquid nitrogen should never be handled carelessly. A few drops on the skin will evaporate before doing permanent damage. However, if any becomes entrapped between a hand and glove, or foot and shoe, it can cause painful frostbite. When transferring large amounts of liquid nitrogen, use asbestos gloves.

C. Silica Gel Dust. When thin-layer chromatographic (TLC) plates are scraped, (Appendix J) precautions should be taken to avoid having the silica gel dust become airborne and breathed into the lungs. Avoidance of unnecessary jarring and gentle handling of the plates, particularly during scraping and disposal of the loosened silica powder will keep airborne dust to a minimum.

D. Liquid Chromatograph Solvent. The liquid chromatographic column effluent (Appendix K) contains methanol. The column effluent can be emptied directly into a sink drain with a continual simultaneous flushing with water in order to avoid contaminating the laboratory atmosphere. The vessels used to flush the water pump with methanol and to catch injector loop flow should be emptied into a waste solvent container and rinsed with water after contact with methanol.

E. Acids. Acids have irritating fumes, are corrosive and should be handled with care. Safety glasses and rubber gloves should be worn when handling them. Accidental spills on the skin should be flushed immediately with copious amounts of water. When diluting, always add the acid to water not the reverse.

The acids used in oil identification are acetic acid and nitric acid. They are used in different procedures, but it should be noted here that they are not compatible and should never be placed in the same waste container.

1. Acetic acid. The acetic acid is used as an extractant for chromatographic methods and is diluted during use. It is safe to pour into a sink and flush with water.

2. Nitric Acid. Nictric acid is hazardous because of its oxidizing and nitrating properties in addition to its acidity. It should be kept away from organic materials and metals. Store the waste acid in glass bottles and have a contractor dispose of accumulated waste. Small amounts (10 ml) can be diluted (always add acid to water) and poured down the sink with copious flushing. This acid should only be handled in the hood.

TABLE I

SAFETY PRECAUTIONS REQUIRED

SECTION	SUBJECT	PERTINET APPENDIX	RELATIVE TOXICITY*
III	SOLVENTS		
	Cyclohexane	E	1
	Methylcyclohexane	F	1
	Methylene chloride	D,G,H	2
	Toluene	D,I	2
	Methanol	J,K	1
	Hexane	G,H,J,K	1
IV	COMPRESSED GASES		
	Helium	D	
	Nitrogen	D,E,F	
V	MISCELLANEOUS		
	A. Xenon Lamps	E,F	
	B. Liquid Nitrogen	F	
	C. Silica Gel Dust	J	
	D. Liquid Chromatograph Solvent	K	
	E. Acids		
	Acetic	J,K	2
	Nitric	E,P	2

*Toxicities

1. Exposure causes irritation; only minor residual injury if untreated.

2. Continued or intense exposure can cause temporary incapacitation; possible residual injury unless promptly treated.

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