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Cleanup Procedure for Contaminated Oils Prior to Fingerprinting by Gas Chromatography and Infrared Spectroscopy

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The Chemistry Branch of the U.S. Coast Guard Research and Development Center has developed and refined a system of four mutually supporting instrumental analytical methods to match waterborne petroleum oil spills to their source oils (CHEMISTRY BRANCH 1977). In the course of studying several hundred actual spill case sample sets, we have found that two of the methods, gas chromatography (GC) with dual flame ionization detection (FID) and sulfur flame photometric detection (FPD), and infrared spectroscopy (IR), occasionally suffer from interfering contaminants which mask the characteristic petroleum oil patterns. The common contaminants are animal or vegetable oils or their breakdown products, as well as other biological materials from sewage or sediment. These substances remain mixed with the petroleum oil even after our standard pentane deasphalting procedure.

There are two well established ways of removing such contaminants. The mixtures can be adsorbed on beds of silica gel and/or alumina in a liquid chromatographic column. The petroleum oil components are eluted, and the eluting solvent removed (KAHN et al. 1977). This is the procedure of ASTM Method D3326 for preparation of sample for identification of waterborne oils (ASTM D19 COMMITTEE 1978). Alternatively the mixtures can be subjected to acid or base-catalyzed saponification reactions, followed by extraction of the non-saponifiable portion into a solvent of low polarity and evaporative removal of the solvent (HARRINGTON and TRIP 1975) (HILPERT et al. 1978). In either case, the entire mixture of aliphatic and aromatic hydrocarbons and heterocyclic compounds which comprises the petroleum oil will not be reconstituted in the exact proportions found in the original uncontaminated oil. Therefore, it will be necesary to apply the cleanup procedure to all possible source samples in the case as well as the contaminated spill samples in order to demonstrate a match or non-match of the spectra or chromatograms.

In some spill cases, there may be more than 100 oils to be treated, a large analytical load for any laboratory. Reproducible application of the adsorption column cleanup procedures requires close, continuous operator attention to the preparation and deactivation of each column and to the progress of each elution. CHESLER et al. (1978) found it necessary to

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substitute a column cleanup procedure employing μBondapak-NH_2 columns in an HPLC instrument to overcome non-reproducible separations due to variable water deactivation of silica gel/alumina columns with which they attempted to extract trace level petroleum hydrocarbons from marine biota for GC analysis.

The usual "saponification" cleanup procedure is performed on a semi-micro or macro scale requiring the use of round-bottom flasks, heating mantles, reflux condensers, and separatory funnels. The necessary equipment occupies large volumes of fume hood space, and it requires excessive amounts of solvents and glassware cleaning to handle the sample load of a large spill case. We encountered these problems in attempting to apply either cleanup procedure to spill case sample sets containing contaminated spill samples. Consequently, we have developed a small-scale "saponification" cleanup procedure for GC and IR analysis, which uses small quantities of easily cleaned glassware. With it, we have simultaneously treated large numbers of samples in a short time using minimal fume hood space. The purpose of this paper is to describe the procedure and to present the results of experiments designed to show whether the spectra and chromatograms obtained after employing it are suitable for purposes of oil identification.

METHODS

Apparatus

Figure 1 displays the apparatus necessary to perform the cleanup procedure on 12 samples simultaneously. Refluxing of the KOH-methanol reagent occurs satisfactorily in the tall test tubes when they are heated in a block heater with cooling of their upper portion by a current of air from the action of the fume hood. Extractions are performed by vortexing in the tall tubes and transferring most of the upper layer with a disposable Pasteur capillary pipet. The assembly on the left, constructed from a block heater and other readily available components, is designed to remove pentane from the oil by evaporation with heat and a stream of nitrogen. Details of its construction are outlined by FRAME and FLANIGAN (1978). Conical-bottomed 10 ml centrifuge tubes are used for the concentration step, and the design of the evaporation assembly allows for easy monitoring and control of the last stages of pentane removal in each individual tube. This is critical since the volume of recovered oil can be small ($\sim$ 0.1 ml), and it is important to stop the evaporation and take out the tube once pentane has been removed in order to avoid excessive depletion of the most volatile oil components.

Cleanup Procedure

Add 0.2 g of oil to a 25 mm x 200 mm test tube using a disposable capillary pipet. Add 20.0 ml of 0.5 M KOH in methanol to the tube. Add a carborundum boiling stone. Vortex tube for 30 sec, and place in block heater at 70°C to reflux for 2 hours. Cool

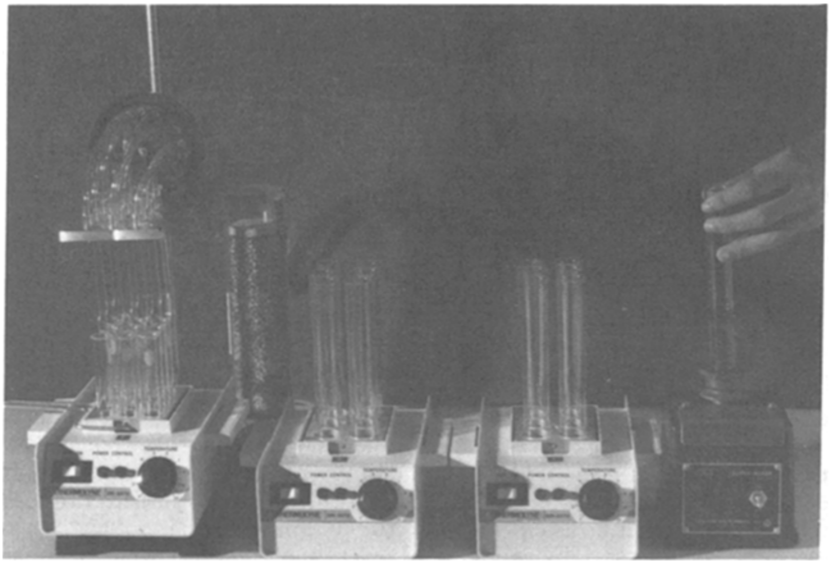

Figure 1 Apparatus for applying cleanup procedure:

a) The two block heaters with tall tubes reflux 12 samples.
b) The evaporation assembly on the left simultaneously removes solvent from another 12 samples.
c) The vortex mixer on the right is used to perform all extractions in the tall tubes.

the tube, and add 10 ml of n-pentane followed by 0.5 ml of distilled water. Vortex tube for 30 sec, let stand until phases separate, and transfer pentane layer to another 25 mm x 200 mm test tube. Add 10 ml of a solution 1% w/v of NaCl in 50:50 distilled water:methanol to the second tube. Vortex second tube 30 sec, let stand until phases separate, and transfer pentane layer to a 10 ml conical-bottom centrifuge tube. Remove pentane under a flow of dry nitrogen with heat, taking care to remove tube when evaporation of pentane ceases. Cover tube with aluminum foil and save recovered oil for subsequent GC and IR analysis.

GC Analysis

Solvent-free oil samples of 0.4 microliter volume were injected into a Perkin-Elmer 990 gas chromatograph using an AS-41 automatic capsule injector system. Peaks from a flame ionization detector (FID) were displayed on a strip chart recorder and peak areas were obtained using a PEP-1 data system from Perkin-Elmer. The injector temperature was 250°C and the detector temperature 350°C. Two GC columns were used:

a) A 3 m x 3 mm od stainless steel column packed with 10% OV101 on 60/80 mesh Chromosorb W(AW), temperature programmed from 75^{o}C to 325^{o}C at 8^{o}C/min with helium carrier gas flow controlled at 20 ml/min.

b) A 15.25 m x 0.5 mm id stainless steel OV101 support coated open tubular (SCOT) column from Perkin-Elmer, programmed from 75^{o}C to 250^{o}C at 8^{o}C/min with helium carrier gas pressure controlled at a head pressure of 3.5 psig.

IR Analysis

IR spectra were obtained using a Perkin-Elmer 467 grating IR spectrometer scanning at 400 cm^{-1}/min. The oil was applied as a smear in 0.05 mm thick sealed demountable cells equipped with KBr windows. In addition to chart recorder depiction of the spectra, the data were digitized on a specially modified PCM-12 microprocessor and transferred to a PDP-8 mini-computer, where they were processed by a special pattern recognition program for oil identification. This program compared the absorbances at 18 specially selected wavelengths and used a weighted version (ANDERSON et al. 1982) of the "log ratio" comparison method of BROWN et al. (1976) to determine the degree of match of pairs of spectra.

RESULTS AND DISCUSSION

The result of the cleanup procedure is illustrated by its application to several contaminated tar balls washed up on Cape Cod in the months after the grounding of the tanker Argo Merchant. The major contaminants were mixtures of 14-, 16-, and 18-carbon saturated and unsaturated free fatty acids. These are likely to be the hydrolysis products of triglycerides which may also be present and which would contribute to the IR spectrum but not the GC pattern as they do not come through the column under the conditions used. Additionally, GC/MS analysis showed the presence of di-octyl phthalate and a number of other phthalate esters.

In Figure 2, the lower curve B represents the contaminated IR spectrum while upper curve A is that of the oil after cleanup. Curve A shows a residual carbonyl peak at about 740 cm^{-1} indicating that small portions of the contaminants remain. The small doublet at about 920 cm^{-1} shows the presence of residual pentane, indicating that the evaporation was not quite completed. In most of the actual and artificial spill samples to which the cleanup procedure has been applied, these artifacts are absent. In any event they do not affect the critical region between 700 cm^{-1} and 900 cm^{-1} which is most useful for petroleum oil comparison.

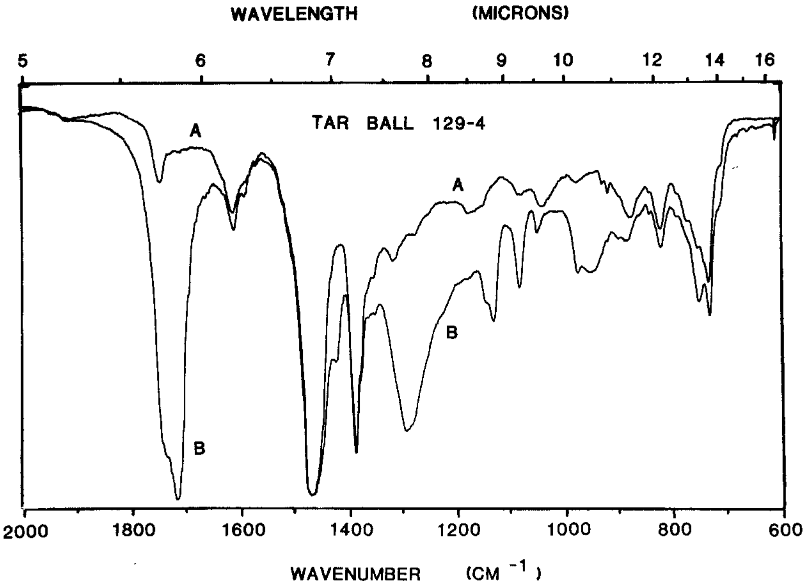

Figure 2 Infrared spectra (% transmission from 2000cm^{-1} to 600cm^{-1}) of Cape Cod tar ball.
Curve A: Spectrum of tar ball after cleanup
Curve B: Spectrum of contaminated tar ball

Figure 3 illustrates the effect of the cleanup on gas chromatograms. Curve 3B is the chromatogram of a pentane-deasphalted sample of the original contaminated tar ball. Curve 3A is that of an injection of a pentane-reconstitution of a dried methanol extract of the contaminants. Experiments have shown that simple methanol extraction is insufficient to remove all such contaminants from the oil. Curve 3C is that of the sample after application of the proposed cleanup procedure, and curve 3D is that of the same sample treated by the ASTM method D3326 adsorption column cleanup method (ASTM D19 COMMITTEE 1978).

In several additional cases of contaminated oil spills, from both salt and fresh water, the cleanup procedure has yielded GC and IR data which are adequate for the purposes of oil identification. In all of these cases, however, the cleaned up spill sample was shown to be distinctly different from any of the proposed source samples. Even a definitive negative result of this sort is of value to the forensic chemist.

This experience still left us with a need to demonstrate that a contaminated oil sample could be matched to the original uncontaminated oil after application of the cleanup procedure. The following series of experiments was undertaken to demonstrate this. Each of three different oils was mixed separately with each of three different contaminants in a ratio of 2 to 1.

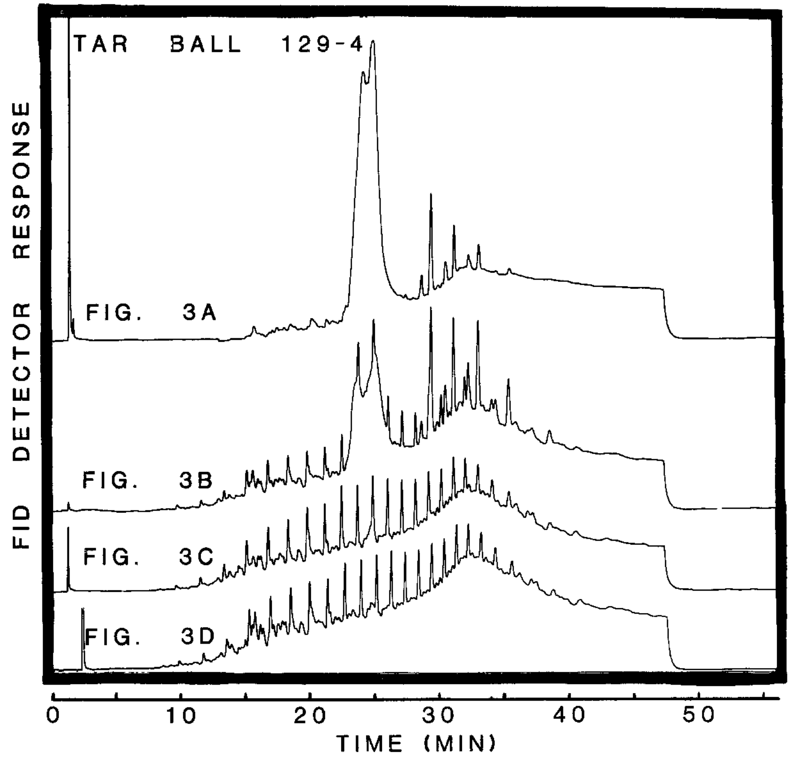

Figure 3 FID gas chromatograms of Cape Cod tar ball on packed column
Curve A: Methanol-extracted contaminants
Curve B: Contaminated tar ball
Curve C: Tar ball after proposed cleanup procedure
Curve D: Tar ball after ASTM column cleanup procedure

The oils were:

a) a light API No. 2 Fuel Oil
b) a light Kuwait Crude Oil
c) a heavy No. 6 Fuel Oil

The contaminants were:

a) a vegetable oil (Mazola brand corn oil)
b) used cooking grease (animal fats)
c) sewage sludge

Three replicates of each uncontaminated oil as well as each oil-contaminant mixture were subjected to the cleanup procedure. The original uncontaminated oils and all the treated samples were analyzed by IR using visual and computerized (ANDERSON et al. 1980) comparison and by GC on 15.25m SCOT columns using visual pattern matching and comparison of n-alkane peak area ratios.

Comparison of the spectra and chromatograms of oils with and without the cleanup procedure showed different patterns. Methanol extraction of oils is known to remove portions of heavier aromatic hydrocarbons (SANER et al. 1976). Since these are the compounds observed by the other two methods in the Coast Guard system, UV fluorescence spectroscopy and TLC using UV fluorescence visualization, the cleanup procedure cannot be used with these techniques. However, in our experience, the contaminants for which the method is intended do not interfere with these techniques. FRAME et al. (1979) observed that methanol extraction removes some sulfur and nitrogen heterocycles, and therefore GC-FPD and GC-NPD chromatograms are not usable after the procedure has been applied.

The GC-FID patterns of the treated oils differed from those of the original oil primarily in a slight loss of the volatile components due to the pentane evaporation step and subtle changes in the unresolved envelope which were probably due to losses of heavier aromatics by methanol solution. The n-alkane peak area ratios (ratioed to the area of the C-19 peak) were unchanged for hydrocarbons heavier than pentadecane. The IR spectra showed small changes in absorbances of bands in the 700 cm^{-1} to 900 cm^{-1} region which reflect aromatic-aliphatic ratios. These GC and IR differences confirm the requirement to apply the cleanup procedure to all samples in a case.

The IR spectra and GC-FID chromatograms of replicate treated oils were indistinguishable. This demonstrated that the procedure could be performed reproducibly. Further the spectra and chromatograms from all treated, contaminated oils matched each other and those of their treated parent oils. The IR spectra were almost always exact overlays, and the results of the computerized 18 peak matching program showed all peaks matching within 2% vs a $<5\%$ criterion for a good match. All methods of visual comparison of GC peaks as well as the n-alkane peak area ratios matched to the same degree of precision as was observed in replicate analyses of untreated, uncontaminated oils.

We conclude that oils subjected to the cleanup procedure can be matched by GC and IR techniques with the same precision as untreated, uncontaminated oils. The only problem encountered with the procedure is occasional low recoveries of oil from very heavily contaminated samples (more than 60% non-petroleum content). In this event it may be necessary to repeat the procedure to obtain more sample or to employ a micro-cell to obtain the IR spectra.

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