Pacific Marine Science Report 77-5

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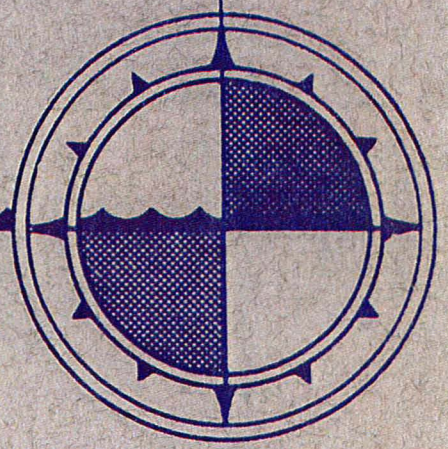

INSTITUTE OF OCEAN SCIENCES, PATRICIA BAY
Victoria, B.C.

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# TRACE ANALYSIS OF OIL IN SEA WATER BY FLUORESCENCE SPECTROSCOPY

by

W.J. Cretney

W.K. Johnson, C.S. Wong

Institute of Ocean Sciences, Patricia Bay

Victoria, B.C.

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Abstract

Several organic compounds were evaluated using fluorescence spectrophotometry as possible standards for the determination of trace amounts of crude oils or residual fuel oils in sea water. Chrysene was selected as the most suitable standard.

Dichloromethane was evaluated as a solvent for the extraction of oil in water at concentrations of a few micrograms per litre and was found suitable for the purpose.

Samples of sea water were collected on Cruise 73-006 of the weathership, CCGS Quadra. A portion of each sample was analyzed aboard ship with the remainder being retained for repeat analysis in the shore-based laboratory in Victoria. The average concentration of fluorescent extractable compounds in sea water collected at Ocean Station P (50°N, 145°W) was 0.038 µg/l when expressed as an equivalent concentration of chrysene. The average concentration, expressed as an equivalent concentration of a typical crude oil, would be about 0.4 µg/l.

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## Introduction

A method based on the work of Levy (1971) and Keiser and Gordon (1973) was adopted for the determination of low concentrations of petroleum oil in sea water. In this method oil is extracted from sea water with an organic solvent and its concentration in the sea water is determined by measuring the intensity of fluorescence of the extracted oil in a suitable organic solvent. The method has several attractive features. It is relatively simple to learn and can be carried out quickly, making it suitable for routine analysis. It can be used for both quantitative and qualitative work with the results being only minimally affected by weathering (Coakly, 1973). Because of the high sensitivity of fluorescence spectrophotometers, 1 to 2 litre samples of even the cleanest natural waters are sufficient for analysis, and because of the high selectivity inherent in the fluorescence technique, interferences tend to be less serious than they are in some other methods. A particular disadvantage of the fluorescence method, as herein described, is that only members of certain small groups of compounds present in oils are measurable the bulk of the compounds in oils being undetectable. Thus, a small measurable fraction of an oil is being used as a tracer for the whole and unfortunately, situations do occur where the fate of the tracer is different from the fate of the whole. Consequently, results must be interpreted cautiously.

The measurable groups of compounds in oils have in common the ability to fluoresce; that is, to emit light of longer wavelength than the incident or exciting light. Fluorescence takes place, moreover, generally within microseconds of excitation. Phosphorescence, a related phenomonen, is generally characterized by longer time intervals between excitation and emission.

The polycyclic aromatic hydrocarbons (PAH) constitute a major group of fluorescent compounds in petroleum oils. Many PAH and some related hetero-atom containing polycyclic aromatic compounds are carcinogenic. Teratogenic and/or mutagenic properties may also be displayed. Consequently, the fluorescence measurement of PAH and their hetero-atom containing relatives has a value which transcends its value in locating and tracing probable oil spills.

The spectra, emission or excitation, obtained from a fluorescent compound are characteristic of its electronic structure. Compounds having a different electronic structure give different spectra. Since oils differ in the relative concentrations of fluorescent compounds, the spectra obtained for individual oils, like the spectra obtained for individual compounds, will vary, each oil having distinct emission and excitation spectra. Since oils also vary in the concentration of their fluorescent compounds relative to their non-fluorescent compounds, the fluorescence method is inherently most sensitive for the detection of oils which have the largest relative concentration of fluorescent compounds.

The work described in this report was undertaken in part to familiarize ourselves with the fluorescent characteristics of different types of oils and to assess the applicability of the fluorescence method of analysis of some typical oils in sea water. We also sought a readily available organic compound as a calibration and reference standard so that other laboratories could compare their results with ours. After we had become familiar with the fluorescence method and had found a suitable standard, during August 4 to September 19, 1973, we undertook a study of sea water on Cruise 73-006 (Fig. 1) of the weathership CCGS Quadra.

## Selection of a Standard

There are many advantages in using a fluorescent compound rather than a fluorescent oil as a reference and calibration standard. A fluorescent compound can be chosen which is obtainable from many sources in unlimited supply. Purification to meet the criteria for a standard does not represent a serious problem. Once it is pure, a fluorescent compound need only be protected against chemical degredation, i.e., oxidation, photolysis, etc., to guarantee homogeneity between and within batches. In contrast, a standard fluorescent oil may be in limited supply from only one source, such as the American Petroleum Institute. Individual batches may vary unless strict attention to homogeneity is observed. Even within a batch homogeneity may be lost if strict handling precautions are not adhered to.

Since we felt that the oils most likely to be encountered would be crude oils, fuel oils or bunker oils, we looked for a standard for these oils rather than one for lighter refined oils. Since heavy oils generally show excitation maxima in the region 290-330 nm and emission maxima in the region 360-400 nm (Appendix 1), these characteristics were taken into consideration in the selection of the standard compound. Nearly two dozen compounds were examined before a choice was made (Appendix 2). For the selection of the trial compounds, a table, "Fluorescence Excitation and Fluorescence Emission Spectra of Model Compounds", compiled by McKay and Latham (1972), was very helpful and is reproduced in Appendix 3. There were, however, some discrepancies between their spectra and our spectra which could be attributed in part to the instruments used. Our instrument, a Perkin-Elmer Model 204, does not have the resolution of the more expensive Perkin-Elmer Model MPF-2A. Variation in detector response shape and source characteristics may also have contributed. It should be noted that the wavelengths of excitation peaks of solutions containing concentrations of over 250 µg/l were not even close to those reported by McKay and Latham. Dilution of these solutions resulted in more favorable comparisons. Intermolecular solute interactions or interference from undissolved solute particles were likely causes of the shift in the excitation peaks of the concentrated (possibly saturated) solutions.

The compound selected for our reference and calibration standard was chrysene. Although it is only slightly soluble in cyclohexane and hexane, it is otherwise particularly well suited as it has numerous peaks (Fig. 2) within the required ranges. Chrysene has excitation maxima at 308 nm and 318 nm (308 nm and 322 nm according to McKay and Latham) and emission maxima at 366 nm, 383 nm, and 400 nm (363 nm, 375 nm, 383 nm and 404 nm according to McKay and Latham). Six pairs of wavelengths could be used for comparison

with heavy oils. The 308 nm, 383 nm pair seemed the best choice since both wavelengths lie in the middle of the desired ranges. Further, our assignment of these wavelengths as the positions of the maxima agrees with that of McKay and Latham. The plot (Fig. 3) of emission intensity against chrysene concentration (in cyclohexane) shows good linearity over the concentration range, 10-50 µg/l, which corresponds to the concentration range, in chrysene equivalents, of the extracts of the oil-spiked water samples which we prepared for study. Good linearity was similarly achieved in the range 1-10 µg/l, which corresponds to the concentration range of extracts of deep ocean sea water samples which are presumably unpolluted.

## Oil and Chrysene Comparison

Chrysene was compared to available oils ranging from heavy fuel oils to outboard motor oils (Table 1 and Table 2). Since wavelengths used were more typical of the heavier oils than the lighter oils, the former gave much higher emission intensities. For example, crude and bunker oils studied exhibited intensities which were 20 to 80 times greater than that of Gulf outboard oil and 10 to 70 times greater than that of Esso diesel oil. The light fuel oils show excitation maxima between 280 nm and 290 nm and emission maxima between 310 nm and 330 nm. Chrysene comparisons, therefore, reflect only the tailing effects of the lighter oils. As chrysene was chosen as a standard for heavy oils, another standard would have to be found if the lighter oils were to be examined. Where possible appropriate spectra of the extracted oil should be used to identify the type of oil being measured and determine the credibility of the standard.

## Selection of Solvents

The decision to use dichloromethane as the extracting solvent was influenced by results obtained by Keiser and Gordon (1973) and verified in our own laboratory. Because dichloromethane is more dense than sea water, multiple extractions using a separatory funnel are easier to carry out than they would be with a lighter-than-water solvent such as hexane. Because it has a low boiling point (40°C), the danger of losing significant amounts of fluorescent compounds during evaporation is minimized. Recoveries of oil from spiked sea water are excellent, being in the range of 90-100% under most conditions (Table 3, Keiser and Gordon, 1973). One drawback, however, is dichloromethane's solubility in water (2% in fresh water). Larger volumes of dichloromethane must therefore be used in the first extraction to compensate for its solubility.

Cyclohexane was used to redissolve the residue after evaporation. It was chosen over pentane, hexane, benzene and dichloromethane. Cyclohexane has the highest boiling point, minimizing concentration changes due to evaporation. It also yielded the highest emission values for spiked samples. Benzene has similar characteristics in both cases, but was rejected because it is a strong absorber of short wavelength ultraviolet light. Cyclohexane proved satisfactory as a storage medium since dissolved oils showed little or no loss of fluorescence even after several months in the dark.

## Recovery of Oil from Water

The recoveries of different oils from waters of various origins were determined (Table 3). Recoveries averaging 92%, for example, were obtained for three different crude oils which had been added to previously extracted surface water from Station P. The loss of fluorescent oil components during roto-evaporation of dichloromethane and during redissolution of the residue in cyclohexane was determined for two oils (Table 4). Since this loss was approximately the same as the overall procedural loss observed for the crude oils in Table 3, the recovery of the crude oils in the extraction step of our procedure appears to have been nearly quantitative. The recovery of refined oil, however, was not quantitative. The reason for the poor recovery of the Esso motorboat oil was not pursued in the study. In this regard, however, it may be noted that the water used, which was surface water obtained from alongside the dock adjacent to the laboratories in Victoria's Inner Harbour, may have contained components, such as surfactants, which interfered with the extraction efficiency of the dichloromethane. Because of our experience with the extraction efficiency of Esso motorboat oil from harbour surface water, we were alerted to the necessity of testing water samples from a study area for the extractability of the pollutant oil, when it is known, in order that concentrations, which are determined by the fluorescence method, can be corrected for extraction efficiency.

Our findings generally agree with those of Keiser and Gordon (1971) who reported average percent recoveries for a No. 6 fuel oil of 99% for the roto-evaporation step and 90% for the extraction step. We found, however, that losses of oil were relatively greater in the roto-evaporation step than in the extraction step. This lack of agreement may reflect differences in the water and oils used in the two studies. In addition, there were procedural differences. We used a higher bath temperature for roto-evaporation of the solvent (55-60°C vs. 30°C) which may have accounted for our greater loss on roto-evaporation. For the extraction we used a 50 ml portion of dichloromethane followed by a 25 ml portion rather than two 40 ml portions. It should also be pointed out, the above arguments not withstanding, that Keiser and Gordon reported standard deviations of 11% and 7% for the roto-evaporation and the extraction steps, respectively. The differences between their results and our results, which come from a small number of experiments, may therefore be more apparent than real.

## Field Trial

The fluorescence method, as it had evolved by August, 1973, was given a field trial on weathership Cruise No. 73-006. Sea water samples were collected from the port bow with the ship steaming at approximately 5 knots. A solvent washed (distilled water, methanol, and dichloromethane, respectively) five-gallon stainless steel bucket suspended on a nylon line was swung forward at 45° to the ship. At the appropriate instant, determined with practice, the line was allowed to slacken momentarily so that the bucket dropped mouth first into the water. The first two grabs were used as rinses with the third being retained as the sample. (Current practice, however, involves taking the first grab as sample. The reason for this procedural

change is to minimize coating the bucket with material from the ocean's surface microlayer.) A subsample of 1.5 litres was transferred to a 2 litre separatory funnel and extracted with 65 ml of dichloromethane by vigorously shaking the funnel for two minutes. After allowing the layers to separate for approximately five minutes, the dichloromethane was drained into a 250 ml round bottomed flask. A second extraction using 25 ml of dichloromethane was then performed and the extract added to the first. The combined extracts were then evaporated to dryness using a water bath at 55°-60°C aboard ship and a roto-evaporator under reduced pressure with a similar bath at 55°-60°C at our shore laboratory. Care was taken to remove the evaporating flasks from the water bath immediately after evaporation of the solvent. The residue, rarely visible, was then redissolved in 15.0 ml of cyclohexane and compared with chrysene standards using the P.E. Model 204 fluorescence spectrophotometer. Appropriate blank corrections were made. Reagent blanks were prepared by evaporating 90 ml of dichloromethane from the same batch used for the extractions and taking up the residue in 15.0 ml of cyclohexane. All samples, standards, and blanks were compared at six different sets of wavelengths using 308 nm and 318 nm for excitation and 366 nm, 383 nm, and 400 nm for emission.

The average value in chrysene equivalents for all samples collected and analyzed (Table 5) at Station P was 0.038 µg/l with a standard deviation of 0.017 µg/l. Line P samples (Table 6) excluding Station 5 which was obviously contaminated had an average of 0.040 µg/l with a standard deviation of 0.025 µg/l. Of the Line P samples, those from Stations 1 and 2 had comparatively high concentrations. Stations 1 and 2 were over the continental shelf and were also stations of more than normal shipboard activity.

Using the sample (conc. 0.034 µg/l) collected on September 12 as being representative of the samples taken at Station P, the equivalent concentrations (Table 7) of five crude oils and five bunker oils for six wavelength pairs were calculated. The equivalent concentrations vary from oil to oil with the equivalent concentrations of the crude oils being about double those of the bunker or residual fuel oils. This result is a reflection of the greater relative quantities of fluorescent compounds in bunker or residual fuel oils. The amount of variation of the equivalent concentration of a given oil from one wavelength pair to another provides a measure of the similarity of the composition of the fluorescent components of the oil with the composition of the fluorescent components extracted from the sea water. The smaller the variation is, the greater the similarity. No variation would indicate that the oil in question was present, unweathered or otherwise changed, in the sea water. If the relative standard deviation (Table 7) is used as a measure of this variation, then, of the oils studied, B.C. light crude (0.16 R.S.D.) would be most like the sea water extract. With the exception of the Stewart bunker oil, moreover, the sea water extract resembles the crude oils more than it does the bunker oils. Inspection of Table 7 indicates that the equivalent concentration of a given oil may change in a systematic fashion with a change in excitation and emission wavelengths. A general decrease in equivalent oil concentration appears to be linked with an increase in excitation and emission wavelengths. This apparent relationship can be demonstrated more clearly if the equivalent oil concentration is plotted against a function which is the square

root of the sum of the squares of the excitation and emission wavelengths for which the value of the equivalent oil concentration was determined. This function can be regarded as a radius (Appendix 4) and for the purposes of this discussion is designated as r. In Figure 4 is shown the value of r for one pair of excitation and emission wavelengths ($\lambda$ ex = 308 nm and $\lambda$ em = 366 nm, respectively). Two plots of equivalent oil concentration against r are given in Figure 5. The plots show that the equivalent concentration of the "average" crude oil and the "average" bunker oil first decreases and then levels off or increases slightly as r increases.

Although the plots in Figure 5 are informative, a more useful comparison can be made if the equivalent concentration determined at each pair of wavelengths for a given oil is first divided by the average of all the equivalent concentrations. The ratios which are obtained in this manner can be plotted against r as shown in Figure 6. Should the equivalent oil concentrations determined at each wavelength pair be the same, then each of the ratios would be equal to 1 and the ratio against r plot would fall on the horizontal straight line shown in Figure 6. The closer the oil and sea water extract resemble each other (at the wavelengths used in the measurement of the fluorescence), the closer the curve of the equivalent concentration will be to the horizontal straight line. The sea water extract can therefore be seen to more closely resemble the "average" crude oil than the "average" bunker oil. The standard deviation of the ratios from the mean ratio gives a measure of the goodness of fit between the curve of ratios and the horizontal line in Figure 6. It can be easily shown (Appendix 5) that this standard deviation is in fact the relative standard deviation of the equivalent oil concentrations from the mean equivalent oil concentration. The relative standard deviations of the various oils studied are listed, as noted before, in Table 7.

In principle, it should be possible to perform the data analysis described above for a data set based on a much larger number of pairs of wavelengths. For identification purposes an increase in the data base would be much prefered. In this case, however, there may be more than one ratio determined for a given value of r so that the ratio against r plots would give a band of variable width and density rather than a line. A simplification can be achieved by using a set of fluorescent measurements determined for a set of points ( $\lambda$ ex, $\lambda$ em) which lie on a diagonal straight line such as the line shown passing through the origin and point (308, 366) in Figure 4. For the above set of points each value of r would correspond to only one ratio. It would not be difficult to obtain a set of fluorescent measurements for a set of points ( $\lambda$ ex, $\lambda$ em) along the diagonal line as shown in Figure 4. A fluorescence spectrophotometer can be easily modified to scan along such a diagonal line in a process called synchronous excitation of fluorescence emission (Lloyd, 1971a). This process is particularly useful in the analysis of oils and extracts of water which contain oil. More useful information regarding oil composition can be achieved in a single synchronous scan than can be achieved by a single scan with either the excitation or emission wavelength held constant (Gordon and Keiser, 1974; Lloyd, 1971 a, b, c).

On August 20, 1973, a series of sea water samples, which were collected at three hour intervals over a 24 hour period, were analyzed. The results

are given in Table 8. The average equivalent concentration for ( $\lambda$ ex, $\lambda$ em) = (308 nm, 383 nm) was 0.058 µg/l with a standard deviation of 0.021 µg/l. The relative standard deviation was 0.36 which corresponds favourably with the relative standard deviation of 0.63 and 0.45 for Line P and Station P samples, respectively. If the samples taken at 1700 hours, which contained zooplankton were omitted from the analysis, the average would drop to 0.052 µg/l and the relative standard deviation to 0.26. Although further verification would be necessary, the smaller relative standard deviation of samples collected hourly compared to that of samples collected daily indicates that there may be day-by-day variation in the concentration of fluorescent extractable materials at Station P.

## Storage of Samples

Samples were brought back to the laboratory for analysis in three different forms--as sea water, as dichloromethane solutions and as cyclohexane solutions (representing three stages in the analytical work-up). All samples were stored in glass containers with Teflon lined plastic caps. Both the containers and Teflon linings had been previously cleaned with dichloromethane. Some caps also had a liner of dichloromethane-rinsed aluminum foil covering the interior including the threads.

### Sea Water Samples (Table 9)

Samples of approximately three litres were preserved with 60 mg of mercuric chloride and stored in a freezer aboard ship and a cooler (<5°C) at the lab until time of analysis. The extractions performed in the lab on these samples yielded slightly lower results; 0.032 $\pm$ 0.019 µg/l compared to 0.038 $\pm$ 0.017 µg/l for the shipboard analyses. This difference may be due to cleaner experimental conditions in the laboratory rather than loss of fluorescene in the stored sample. Even the significance of the difference is questionable, however, since it is smaller than the standard deviation of either average.

### Dichloromethane

After extraction of the sea water the dichloromethane was stored in a cool, dark place until the time of analysis. Some samples having caps with only Teflon liners were definitely contaminated (Table 10) whereas none of the samples having caps with added aluminum foil were obviously contaminated. The latter samples had an average concentration of 0.046 $\pm$ 0.016 µg/l compared to 0.035 $\pm$ 0.010 µg/l found for the same sea water samples following analysis on shipboard. The results indicate that there was an increase in fluorescence on storage. A likely source of contamination would be the plastic caps. Simply rinsing the caps with solvent releases greater quantities of fluorescent materials than we have encountered in extracts of sea water

Cyclohexane

Upon completion of the extraction with dichloromethane and subsequent evaporation, the residue was taken up in cyclohexane and stored in a dark cooler. The consequences of storing extracts in cyclohexane were similar to those of storing extracts in dichloromethane. Samples in tubes with only Teflon liners became contaminated and those with aluminum foil gave mixed results (Table 10). Conclusions on this small amount of data are hard to make, but it does seem that meticulous care in preparing storage tubes or a more reliable type of sample bottle will be necessary if the samples are to be stored in organic solvents.

## Summary

After an extensive investigation chrysene was chosen as a reference and calibration standard for heavy oils, using its excitation maximum at 308 nm and its emission maximum at 383 nm as the pair of wavelengths to be used for comparison. No problems were encountered at sea that would make performance of the analyses impractical aboard ship except for the ever present possibility of contamination from the ship itself. Storage of samples did pose some problems. There may be some loss of fluorescence if samples of sea water are stored although our results are not definite enough to confirm this. If samples are to be stored in an organic solvent medium, a contaminant-free container must be found as many of our samples were contaminated.

Our results indicated that Station P sea water contained an equivalent of 0.4 µg/l crude oil or 0.2 µg/l bunker oil during the study period. This level is considerably lower than levels reported in the Atlantic. Levy (1972) published results for concentrations of residual oils observed in the northwest Atlantic off the coast of Nova Scotia (January, 1971) which averaged between 1.0 and 2.0 µg/l using bunker C from the ship Arrow as his standard. A few months later (April 1971) using the same standard, Gordon and Michalik (1971) found the average oil concentration in Chedabucto Bay area to be 1.5 µg/l. Further south, Brown, Searl and Elliot (1973) found nonvolatile or persistant hydrocarbons to be generally between 1 and 12 µg/l along well travelled tanker routes. Later Keizer and Gordon (1973), doing a survey from Halifax to Bermuda, found that 77% of their offshore above 200 metre depth samples contained less than 2 µg/l of oil using Venezuelan crude as a calibration standard. It should be noted that the samples from the Atlantic were taken closer to shore and in areas of higher ship activity than those collected for our study.

The concentrations we found must only be regarded as estimates of the maximum possible quantities of petroleum in sea water since there is no evidence to support the supposition that the fluorescent components in the extracts are petroleum derived. Even when an extract can be compared to standards from a probable oil source, the accuracy may be limited by weathering effects or the presence of other fluorescing materials in sea water.

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## FIGURES

1. Chart showing Line P station positions.

2. (a) Scan of fluorescence emission ( $\lambda$ ex = 308 nm) of a 114 µg/l solution of chrysene in cyclohexane and (b) Scan of fluorescence excitation ( $\lambda$ em = 383 nm) of 114 µg/l solution of chrysene in cyclohexane.

3. Linearity of response of Perkin Elmer Model 204 fluorescence spectrophotometer.

4. Rectangular coordinate system showing the distance r to the point (308, 366) and the section in which fluorescent measurements are made.

5. Plot of equivalent oil concentration against r.

6. Plot against r of the ratio of equivalent oil concentration (measured at the six pairs of wavelengths) to the average equivalent concentration.

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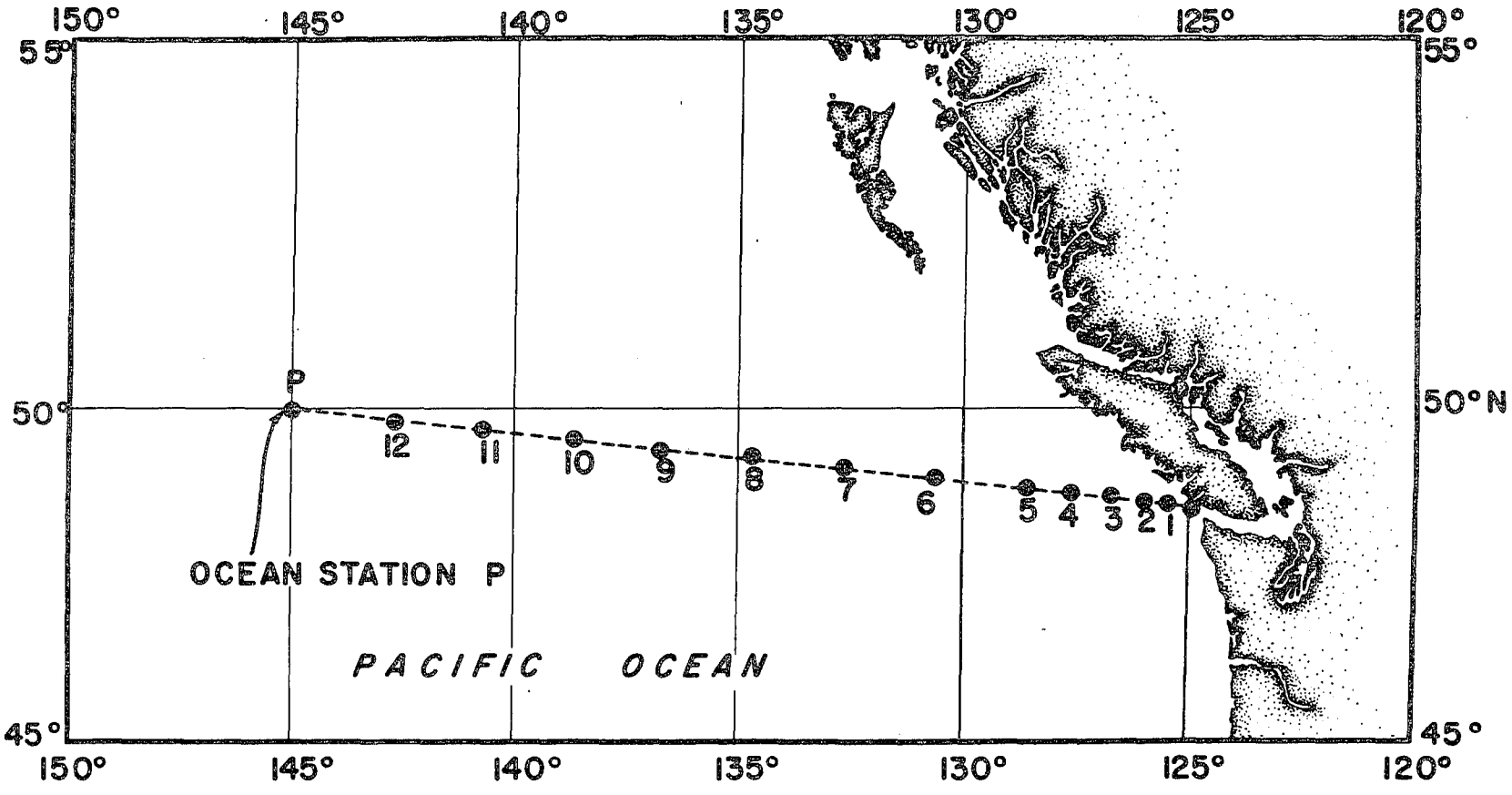


Figure 1. Chart showing Line P station positions.

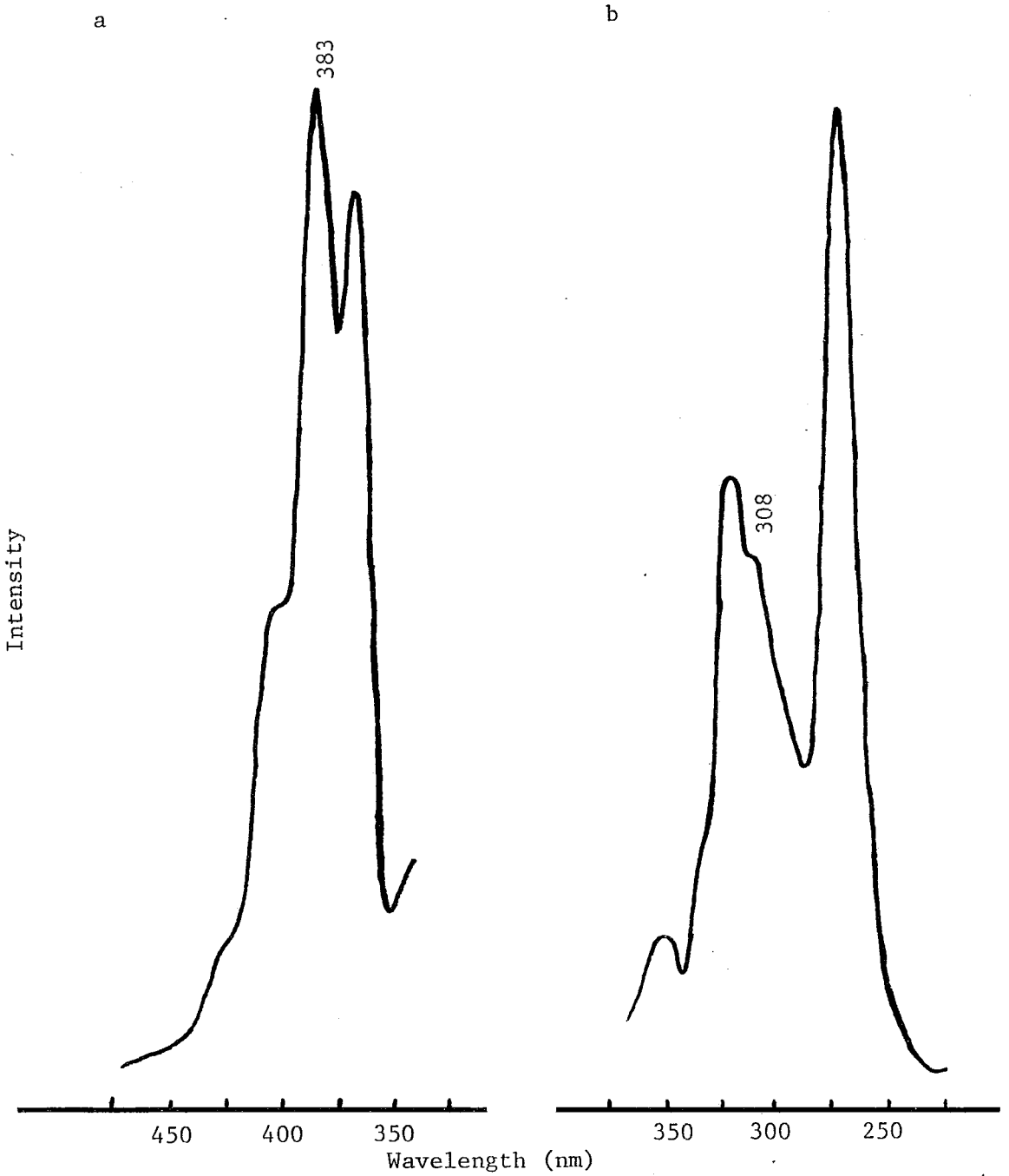


Figure 2. (a) Scan of fluorescence emission (λ ex = 308 nm) of a 114 µg/l solution of chrysene in cyclohexane and (b) Scan of fluorescence excitation (λ em = 383 nm) of 114 µg/l solution of chrysene in cyclohexane.

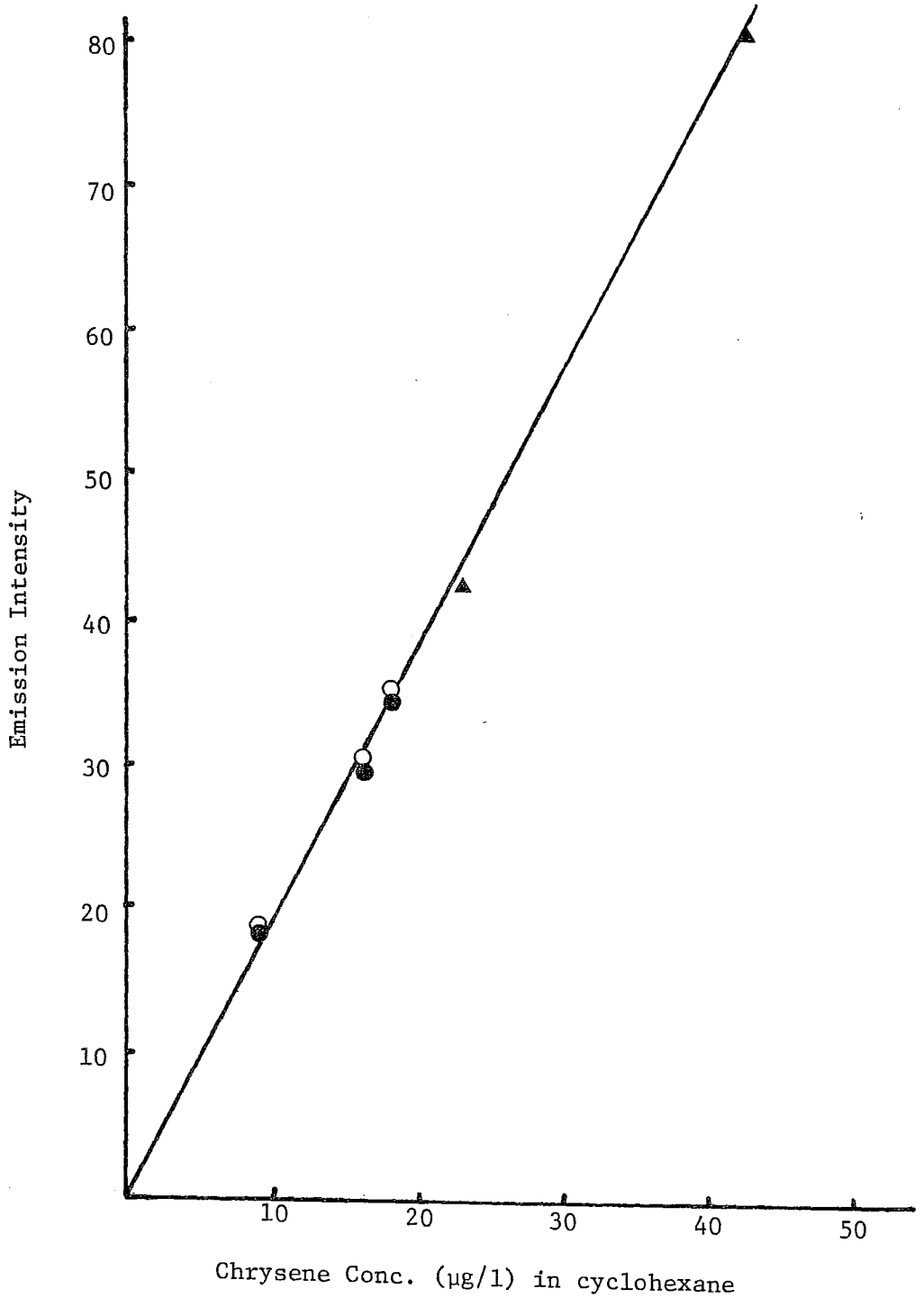


Figure 3. Linearity of response of P.E. Model 204 fluorescence spectrometer, λ ex = 308 nm, λ em = 383 nm, (●) 1st run, (▲) 2nd run, (○) 3rd run.

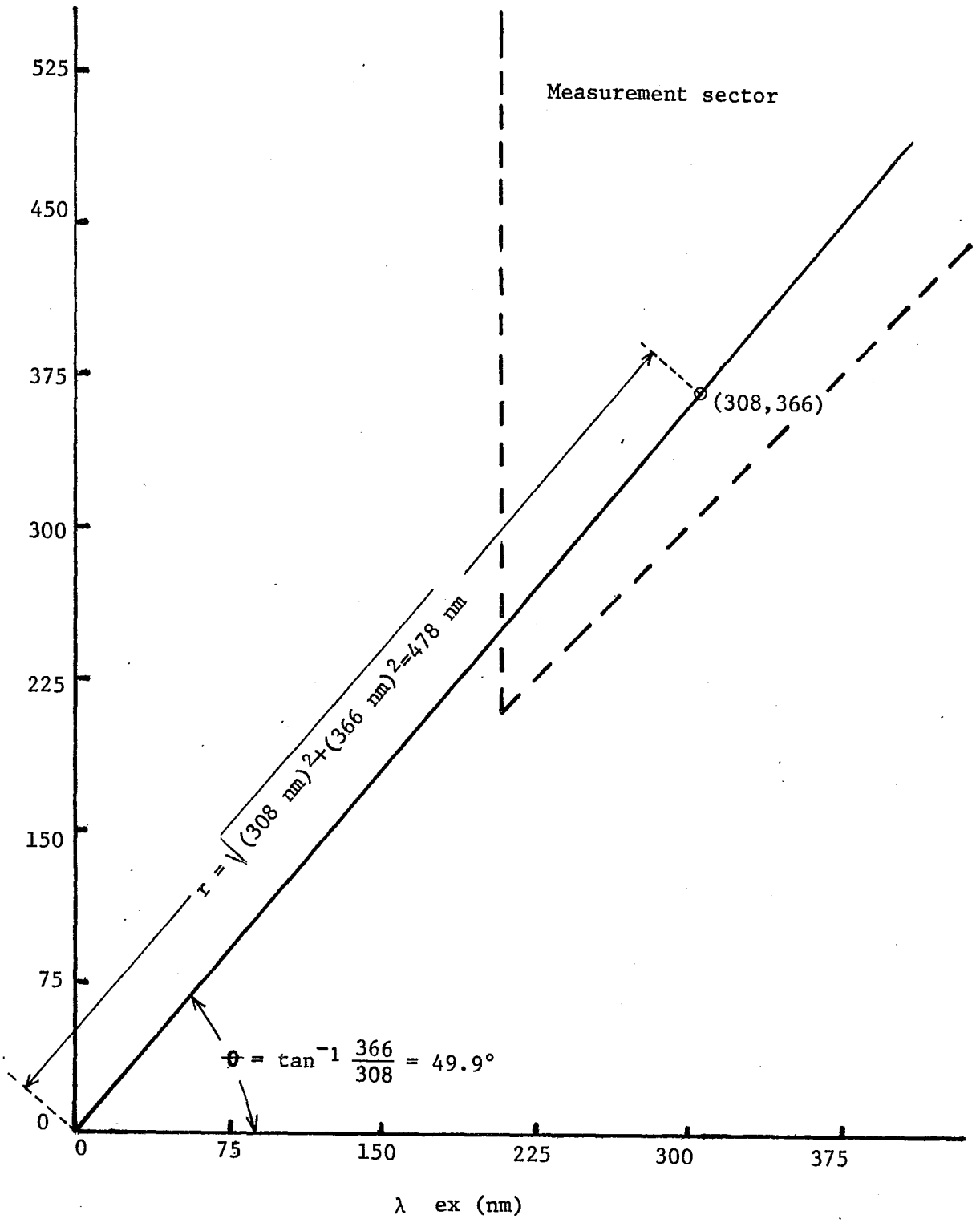


Figure 4. Rectangular coordinate system showing distance r to the point (308 nm, 366 nm) and the sector in which fluorescent measurements are constrained by the requirements of the instrument ( $\lambda$ ex $\geq$ 220 nm) and the fluorescence process ( $\lambda$ em $\geq$ $\lambda$ex).

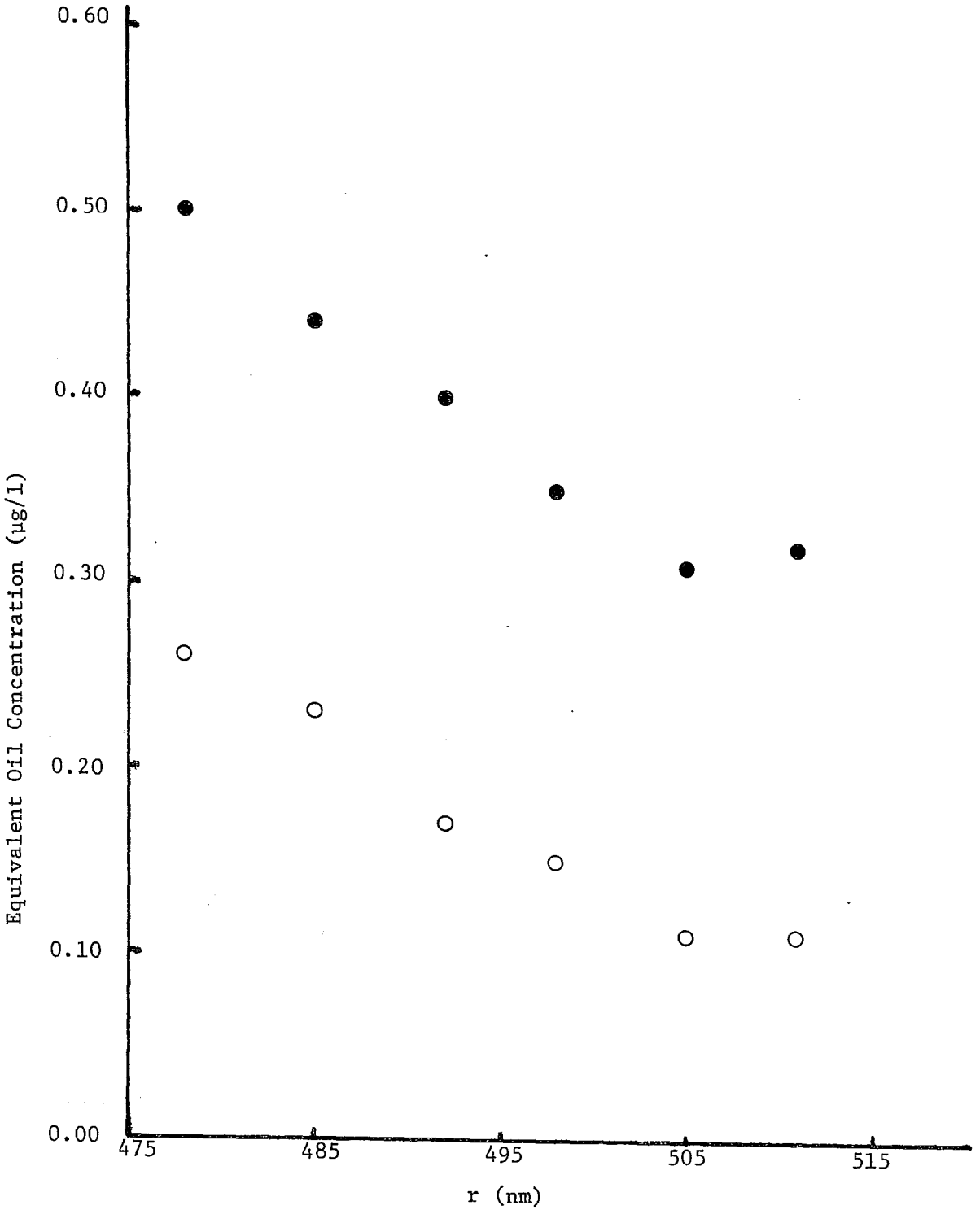


Figure 5. Plot of equivalent oil concentration against r. (●) "average" crude oil. (○) "average" bunker oil.

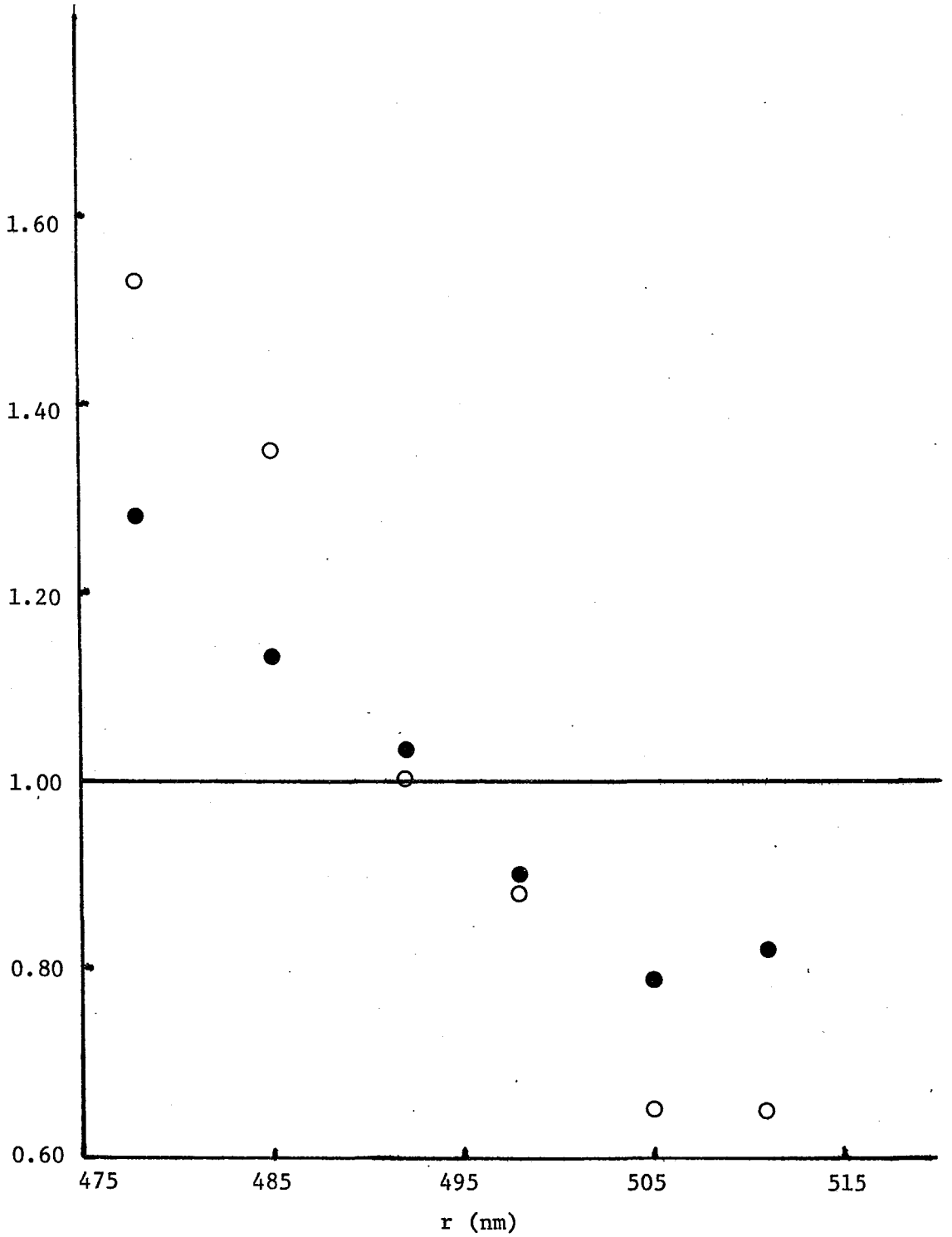


Figure 6. Plot against r of the ratio of equivalent oil concentration (measured at the six pairs of wavelength) to the average equivalent oil concentration. (●) "average" of crude oil (○) "average" bunker oil.

TABLES

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TABLE 1

The Concentration[a] of Chrysene which has the Same Fluorescent Intensity at Selected Pairs of Excitation and Emission Wavelengths as 1 mg/l[a] of a Given Oil[b]

| λ ex (nm) | 308 | 318 | 308 | 318 | 308 | 318 |
|---|---|---|---|---|---|---|
| λ em (nm) | 366 | 366 | 383 | 383 | 400 | 400 |
| Type of Oil | Equivalent Chrysene Conc., µg/l | | | | | |
| Cod liver | 0.0002 | 0.0002 | 0.0003 | 0.0003 | 0.0006 | 0.0005 |
| Gulf outboard | 0.0087 | 0.0066 | 0.0047 | 0.0038 | 0.0055 | 0.0046 |
| Esso diesel | 0.024 | 0.015 | 0.009 | 0.006 | 0.007 | 0.005 |
| B.C. light crude | 0.051 | 0.045 | 0.038 | 0.034 | 0.060 | 0.051 |
| Peace River crude | 0.078 | 0.068 | 0.063 | 0.056 | 0.104 | 0.091 |
| Esso crude | 0.119 | 0.103 | 0.095 | 0.084 | 0.163 | 0.144 |
| Red Water Gulf crude | 0.120 | 0.104 | 0.100 | 0.089 | 0.174 | 0.149 |
| Boundary Bay crude | 0.120 | 0.109 | 0.100 | 0.091 | 0.164 | 0.142 |
| Esso No. 46 | 0.185 | 0.168 | 0.183 | 0.166 | 0.366 | 0.318 |
| CCGS[c] Vancouver, bunker | 0.185 | 0.164 | 0.178 | 0.163 | 0.374 | 0.346 |
| CCGS[c] Quadra, bunker | 0.193 | 0.173 | 0.192 | 0.170 | 0.376 | 0.315 |
| Esso bunker C | 0.235 | 0.205 | 0.217 | 0.190 | 0.437 | 0.381 |
| CSS[d] Stewart, bunker | 0.279 | 0.268 | 0.231 | 0.221 | 0.407 | 0.389 |

[a] In cyclohexane.

[b] The crude oils were samples of pipeline feed oils obtained from the Imperial Oil Refinery, Ioco, B.C. The bunker oils obtained from the ships were not identified. The other oils were purchased.

[c] Canadian Coast Guard Ship.

[d] Canadian Survey Ship.

TABLE 2

The Concentration of a Given Oil which has the Same Fluorescent Intensity at Selected Pairs of Excitation and Emission Wavelengths as 1 µg/l[a] of Chrysene

| λ ex (nm) | 308 | 318 | 308 | 318 | 308 | 318 |
|---|---|---|---|---|---|---|
| λ em (nm) | 366 | 366 | 383 | 383 | 400 | 400 |
| Type of Oil | Equivalent Oil Conc., µg/l | | | | | |
| B.C. light crude | 19.6 | 22.6 | 26.3 | 29.4 | 16.7 | 19.6 |
| Red Water Gulf crude | 8.3 | 9.6 | 10.3 | 11.2 | 5.7 | 6.7 |
| Boundary Bay crude | 8.3 | 9.2 | 10.0 | 11.0 | 6.1 | 7.0 |
| Esso crude | 8.4 | 9.7 | 10.5 | 11.9 | 6.1 | 6.9 |
| Peace River crude | 12.8 | 14.7 | 15.9 | 17.8 | 9.6 | 11.0 |
| CCGS Quadra, bunker | 5.2 | 5.8 | 5.2 | 5.9 | 2.7 | 3.2 |
| CCGS Vancouver, bunker | 5.4 | 6.1 | 5.6 | 6.1 | 2.7 | 2.9 |
| CSS Stewart, bunker | 3.6 | 3.7 | 4.3 | 4.5 | 2.5 | 2.6 |
| Esso bunker C | 4.3 | 4.9 | 3.7 | 5.2 | 2.3 | 2.6 |
| Esso No. 46 | 5.4 | 6.0 | 5.5 | 6.0 | 2.7 | 3.1 |

[a] In cyclohexane

TABLE 3

Recovery of Oil from Water using Dichloromethane as Extractant

| Medium | Oil Added | Conc., µg/l | % Recovery[a] |
|---|---|---|---|
| Extracted Stn. P sea water | Peace River crude[b] | 3.1 | 90, 96 |
| | Esso crude[b] | 4.7 | 96, 94 |
| | Red Water Gulf crude[b] | 14.0 | 88 |
| Victoria Harbour sea water, 5 m[d] | Boundary Bay crude[b] | 4.0 | 99, 92 |
| | | 2.0 | 78 |
| | Boundary Bay crude[c] | 4.0 | 90, 86 |
| | | 2.0 | 84 |
| Distilled water | Boundary Bay crude[c] | 2.0 | 68, 102, 97, 99 |
| Victoria Harbour sea water, surface[d] | Esso outboard[c] | 19.5 | 55, 52, 38 |
| | | 39.0 | 56, 49 |

[a]Recovery after extraction of 1 liter of water with dichloromethane (50 ml, 25 ml), roto-evaporation and dissolution in [b]cyclohexane or [c]hexane.

[d]Filtered using Whatman No. 1 filter paper.

TABLE 4

Recovery of Oil from Dichloromethane following Roto-evaporation[a] and Redissolution in Hexane

| Oil | Recovery (%) | Average Loss (%) |
|---|---|---|
| Esso outboard | 93, 91, 87 | 10 |
| Peace River crude | 85, 91 | 12 |

[a]Water aspirator, water bath at 55°-60°C.

TABLE 5

Equivalent Chrysene Concentrations of Sea Water Samples Analyzed on Shipboard at Station P

| λ ex (nm) | 308 | 318 | 308 | 318 | 308 | 318 |
|---|---|---|---|---|---|---|
| λ em (nm) | 366 | 366 | 383 | 383 | 400 | 400 |
| 1973 Sample Date | Equivalent Chrysene Conc., µg/l | | | | | |
| August 8 | 0.11 | 0.11 | 0.080 | 0.080 | 0.080 | 0.11 |
| August 11 | 0.075 | 0.062 | 0.048 | 0.041 | 0.041 | 0.066 |
| August 14 | 0.031 | 0.024 | 0.019 | 0.014 | 0.014 | 0.024 |
| August 17 | 0.048 | 0.025 | 0.031 | 0.024 | 0.024 | 0.044 |
| August 20 | 0.082 | 0.067 | 0.054 | 0.046 | 0.046 | 0.077 |
| August 23 | 0.060 | 0.049 | 0.040 | 0.035 | 0.035 | 0.062 |
| August 26 | 0.076 | 0.056 | 0.039 | 0.031 | 0.031 | 0.043 |
| August 29 | 0.044 | 0.037 | 0.027 | 0.025 | 0.025 | 0.038 |
| September 1 | 0.026 | 0.022 | 0.016 | 0.013 | 0.013 | 0.026 |
| September 4 | 0.037 | 0.031 | 0.031 | 0.025 | 0.025 | 0.058 |
| September 7 | 0.049 | 0.039 | 0.031 | 0.025 | 0.025 | 0.045 |
| September 12 | 0.053 | 0.044 | 0.034 | 0.027 | 0.027 | 0.045 |
| September 15 | 0.066 | 0.053 | 0.045 | 0.039 | 0.039 | 0.074 |

TABLE 6

Equivalent Chrysene Concentrations of Sea Water Samples Analyzed on Shipboard Along Line P

| λ ex (nm) | | 308 | 318 | 308 | 318 | 308 | 318 |
|---|---|---|---|---|---|---|---|
| λ em (nm) | | 366 | 366 | 383 | 383 | 400 | 400 |
| Station | 1973 Sample Date | Equivalent Chrysene Conc., µg/l | | | | | |
| 2 | August 4 | n.d.[a] | n.d. | 0.069 | n.d. | n.d. | n.d. |
| 5 | August 4 | 0.19 | 0.17 | 0.17 | 0.14 | n.d. | 0.26 |
| 6 | August 4 | 0.048 | 0.074 | 0.035 | 0.031 | 0.047 | 0.046 |
| 7 | August 4 | 0.058 | 0.085 | 0.038 | 0.035 | 0.051 | 0.051 |
| 12 | August 6 | 0.045 | 0.033 | 0.026 | 0.022 | 0.034 | 0.030 |
| 12 | September 16 | 0.032 | 0.024 | 0.021 | 0.015 | 0.033 | 0.032 |
| 10 | September 17 | 0.042 | 0.033 | 0.028 | 0.024 | 0.049 | 0.046 |
| 9 | September 17 | 0.026 | 0.023 | 0.022 | 0.019 | 0.036 | 0.040 |
| 8 | September 17 | 0.055 | 0.043 | 0.034 | 0.026 | 0.046 | 0.045 |
| 7 | September 18 | 0.026 | 0.020 | 0.015 | 0.013 | 0.026 | 0.023 |
| 4 | September 18 | 0.076 | 0.060 | 0.044 | 0.039 | 0.060 | 0.057 |
| 3 | September 18 | 0.031 | 0.022 | 0.018 | 0.015 | 0.027 | 0.029 |
| 2 | September 19 | 0.12 | 0.10 | 0.089 | 0.085 | 0.14 | 0.13 |
| 1 | September 19 | 0.10 | 0.10 | 0.085 | 0.078 | 0.13 | 0.12 |

[a] not determined.

TABLE 7

Equivalent Oil Concentrations of Sea Water Samples Collected on September 12, 1973[a]

| λ ex (nm) / λ em (nm) | 308 / 366 | 318 / 366 | 308 / 383 | 318 / 383 | 308 / 400 | 318 / 400 | Avg. Conc. µg/l | S.D. | R.S.D. |
|---|---|---|---|---|---|---|---|---|---|
| Type of Oil | Equivalent Oil Conc., µg/l | | | | | | | | |
| B. C. light crude | 1.1 | 0.98 | 0.89 | 0.79 | 0.75 | 0.78 | 0.88 | 0.14 | 0.16 |
| Red Water Gulf crude | 0.44 | 0.44 | 0.34 | 0.30 | 0.26 | 0.27 | 0.34 | 0.081 | 0.24 |
| Boundary Bay crude | 0.44 | 0.40 | 0.34 | 0.30 | 0.27 | 0.28 | 0.34 | 0.079 | 0.23 |
| Esso crude | 0.45 | 0.43 | 0.36 | 0.32 | 0.27 | 0.28 | 0.35 | 0.076 | 0.22 |
| Peace River crude | 0.68 | 0.51 | 0.54 | 0.48 | 0.43 | 0.44 | 0.51 | 0.092 | 0.18 |
| "Average" crude | 0.50 | 0.44 | 0.40 | 0.35 | 0.31 | 0.32 | 0.39 | 0.074 | 0.19 |
| CCGS Quadra, bunker | 0.28 | 0.26 | 0.18 | 0.16 | 0.12 | 0.13 | 0.19 | 0.067 | 0.35 |
| CCGS Vancouver, bunker | 0.29 | 0.27 | 0.19 | 0.16 | 0.12 | 0.12 | 0.19 | 0.074 | 0.39 |
| CSS Stewart, bunker | 0.19 | 0.16 | 0.15 | 0.12 | 0.11 | 0.10 | 0.14 | 0.034 | 0.24 |
| Esso bunker | 0.23 | 0.22 | 0.13 | 0.14 | 0.10 | 0.10 | 0.15 | 0.058 | 0.39 |
| Esso No. 46 | 0.29 | 0.26 | 0.19 | 0.16 | 0.12 | 0.12 | 0.19 | 0.072 | 0.38 |
| "Average" bunker | 0.26 | 0.23 | 0.17 | 0.15 | 0.11 | 0.11 | 0.17 | 0.062 | 0.36 |

[a]Shipboard analysis (Table 5)

TABLE 8

Equivalent Chrysene Concentrations of Sea Water Samples during 24 Hour Time Series on August 20, 1973

| λ ex (nm) | 308 | 318 | 308 | 318 | 308 | 318 |
|---|---|---|---|---|---|---|
| λ em (nm) | 366 | 366 | 383 | 383 | 400 | 400 |
| Time | Equivalent Chrysene Conc., µg/l | | | | | |
| 0200 | 0.085 | 0.076 | 0.060 | 0.058 | 0.10 | 0.086 |
| 0500 | 0.10 | 0.081 | 0.067 | 0.054 | 0.10 | 0.078 |
| 0800 | 0.095 | 0.080 | 0.063 | 0.050 | 0.086 | 0.072 |
| 1100[a] | 0.058 | 0.045 | 0.037 | 0.031 | 0.052 | 0.047 |
| 1400 | 0.082 | 0.067 | 0.056 | 0.046 | 0.077 | 0.071 |
| 1700[b,c] | 0.16 | 0.12 | 0.10 | 0.085 | 0.12 | 0.12 |
| 2000[b] | 0.086 | 0.071 | 0.051 | 0.037 | 0.072 | 0.052 |
| 2300[b] | 0.053 | 0.040 | 0.031 | 0.028 | 0.041 | 0.040 |

[a] Paint chips in sample.

[b] Stored overnight in test tubes (cyclohexane medium) with Teflon lined plastic caps.

[c] Zooplankton in subsample.

TABLE 9

Equivalent Chrysene Concentrations of Sea Water Samples Brought Back to the Laboratory for Analysis

| λ ex (nm) | | 308 | 318 | 308 | 318 | 308 | 318 |
|---|---|---|---|---|---|---|---|
| λ em (nm) | | 366 | 366 | 383 | 383 | 400 | 400 |
| Station | 1973 Sample Date | Equivalent Chrysene Conc. µg/l | | | | | |
| 2 | August 4 | 0.86 | 0.068 | 0.069 | 0.057 | 0.13 | 0.11 |
| P | August 8 | 0.12 | 0.090 | 0.071 | 0.054 | 0.10 | 0.76 |
| P | August 11 | 0.065 | 0.049 | 0.035 | | 0.041 | 0.035 |
| P | August 14 | 0.050 | 0.038 | 0.031 | 0.026 | 0.043 | 0.041 |
| P | August 17 | 0.051 | 0.036 | 0.032 | 0.022 | 0.035 | 0.035 |
| P | August 20 | 0.048 | 0.032 | 0.027 | 0.021 | 0.036 | 0.031 |
| P[a] | August 23 | 0.086 | 0.072 | 0.060 | 0.052 | 0.11 | 0.10 |
| P | August 26 | 0.061 | 0.046 | 0.035 | 0.029 | 0.046 | 0.040 |
| P | August 29 | 0.033 | 0.023 | 0.018 | 0.013 | 0.021 | 0.022 |
| P | September 1 | 0.033 | 0.025 | 0.017 | 0.014 | 0.022 | 0.022 |
| P | September 1 Duplicate | 0.037 | 0.017 | 0.013 | 0.010 | 0.015 | 0.017 |
| P | September 4 | 0.030 | 0.017 | 0.013 | 0.010 | 0.018 | 0.014 |
| P | September 4 Duplicate | 0.025 | 0.014 | 0.011 | 0.007 | 0.013 | 0.010 |
| P | September 7 | 0.026 | 0.019 | 0.012 | 0.010 | 0.016 | 0.017 |
| P | September 12 | 0.044 | 0.029 | 0.025 | 0.019 | 0.034 | 0.032 |

[a]Aluminum foil liner corroded - flakes in sample.

TABLE 10

Comparison of Sample Storage Methods

| Place of Analysis: | Shipboard | Shore-based Laboratory | | |
|---|---|---|---|---|
| Storage Medium: | | Sea water, $HgCl_2$ | Organic solvent | Organic solvent |
| Container: | | Gallon jugs | Glass test tubes | Glass test tubes |
| Seal: | | Teflon lined screw caps | Teflon lined screw caps | Aluminum foil and Teflon lined screw caps |
| Sample Date 1973 | | Equivalent Chrysene Conc., µg/l | | |
| August 8 | 0.080 | 0.071 | n.d. | n.d. |
| August 11 | 0.048 | 0.035 | 0.34[b] | 0.064[c] |
| August 14 | 0.019 | 0.031 | 1.60[b] | 0.043[c] |
| August 17 | 0.031 | 0.032 | n.d. | 0.041[c] |
| August 20 | 0.054 | 0.027 | 0.16[c] | n.d. |
| August 23 | 0.040 | 0.060 | n.d. | 0.054[c] |
| August 26 | 0.039 | 0.035 | n.d. | 0.051[c] |
| August 29 | 0.027 | 0.018 | 0.037[b] | 0.024[c] |
| September 1 | 0.016 | 0.017 | 0.053[c] | n.d. |
| September 4 | 0.031 | 0.013 | 0.081[c] | 0.034[b] |
| September 7 | 0.031 | 0.012 | 0.13[c] | 0.084[b] |
| September 12 | 0.034 | 0.025 | n.d. | 0.026[c] |
| September 15 | 0.045 | n.d. | n.d. | 0.066[c] |

[a] Fluorescence measured at $\lambda$ ex = 308 nm, $\lambda$ em = 383 nm.
[b] Stored in cyclohexane.
[c] Stored in dichloromethane.

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Appendices

1. Fluorescence Excitation and Fluorescence Emission Spectra of Specific Oils

2. Fluorescence Excitation and Fluorescence Emission Spectra of Model Compounds--This Study

3. Fluorescence Excitation and Fluorescence Emission Spectra of Model Compounds--McKay and Latham

4. Transformation from Rectangular to Polar Coordinates

5. Relationship between Relative Standard Deviation of Equivalent Concentrations and Standard Deviation of Ratios

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Appendix 1

Fluorescence Excitation and Fluorescence Emission Spectra of Specific Oils[a]

| Oils | Fluorescence Excitation Wavelength, nm | Fluorescence Emission Wavelength, nm |
|---|---|---|
| Boundary Bay crude | 315 | 370 |
| Red Water Gulf crude | 300 | 363 |
| B.C. light crude | 300 | 337, 358 |
| Peace River crude | 308 | 366, 398 |
| CCGS Vancouver, bunker C | 310 | 350, 383, 410 |
| Tar Ball #15 Transpac 72 | 330 | 383 |
| Stewart, bunker | 323 | 370 |
| CCGS Quadra, bunker C | 310, 340 | 385 |
| Esso diesel | 290 | 333 |
| Esso fuel #46 | 340, 309, 359 | 387, 384, 405, 405 |
| Esso crude | 330, 305 | 369, 378, 363, 376 |
| Esso,bunker C | 338, 307, 358 | 386, 382, 405, 405 |
| Outboard motor oil | 237 | 342 |

[a]Underlined wavelength indicates maximum peak

Appendix 2

Fluorescence Excitation and Fluorescence Emission Spectra of Model Compounds[a]

| | Excitation Peaks (nm) | Emission Peaks (nm) | Solubility in Hexane (ppm range) |
|---|---|---|---|
| 1-Methylnapthalene (1020 ppm) | 316 | 336 | Miscible |
| 1-Ethylnapthalene (20 ppm) | 291 | 337 | Miscible |
| Dibenzothiophene | 287, 321 | 338, 344 | Fair |
| m-Terphenyl (582 ppm) | 306 | 339 | Fair |
| 2,3,5-Trimethylnapthalene | 300 | 340 | Fair |
| N-Ethylcarbazole (5.3 ppm) | 265, 290, 333, 340 | 350, 364 | Poor |
| N-Phenylcarbazole | 275, 300(s), 335 | 350(s), 358 | Fair |
| Phenanthrene (28 ppm) | 291 | 355(s), 366, 383(s) | Fair |
| Triphenylene (2.1 ppm) | 264(s), 284 | 357, 370 | Fair |
| Sym-Dibenzocyclooctatetraene | 316 | 358 | Fair |
| Chrysene (236 ppb) | 268, 308(s), 318 | 366, 383, 401 | Poor |
| 1,2,5,6-Dibenzanthracene (800 ppm) | 270, 317, 368 | 368, 398, 420 | Poor |
| 2-Phenyindole | 297(s), 330 | 355, 370(s) | Poor |
| 1,2,7,8-Dibenzphenanthrene (Picene) (2.4 ppm) | 285, 300, 325 | 380, 398, 419 | Poor |
| Bibenzyl (3 ppm & 150 ppm in MeOH) | 294, 319, 331(s), 350 | 397, 420 | |
| Decacyclene | 270, 300, 335, 380, 402 | 408, 432, 478, 506 | Fair |
| 2,3,6,7,-Dibenzanthracene(280 ppb) | 305, 324, 342 | 398, 421, 450, 487 | Poor |
| 9,10-Dimethyl- 1,2-benzanthracene | 336, 350, 388 | 413, 427 | Fair |
| Cod Liver Oil (240 ppb) | 330 | 480 | Miscible |
| Indene (in Benzene) | 298, 327, 384 | 481, 510 | Insoluble |
| Tetraphenylethylene | No spectra obtained | | |
| 9,9-Bifluorene | No spectra obtained | | Very poor |
| Biphenylene | No spectra obtained | | |

[a]A wavelength underlined was the most intense. Shoulders are indicated by (s).

# Appendix 3[a]

**Table I. Fluorescence Excitation and Fluorescence Emission Spectra of Model Compounds[a]**

| Compound | Fluorescence excitation spectra, wavelength, nm | Fluorescence emission spectra, wavelength, nm |
|---|---|---|
| Fluorene | *268* 275(s) 293 303 | *303* 310 |
| Naphthalene | 269 *278* 288 | *324* 338 350 |
| 9-Methylcarbazole (*N*-Methylcarbazole) | 249 *293* 322 | *334* 349 365(s) 378(s) |
| Carbazole | 249 *293* 320 | *335* 351 364(s) |
| 2-Methylcarbazole | 250 *297* 319 | *335* 350 365(s) 385(s) |
| 3-Methylcarbazole | 252 *296* 325 | *342* 358 372(s) 378(s) |
| 11*H*-Benzo[*b*]fluorene (2,3-Benzofluorene) | 270 288 306 *319* 326 342 | *342* 351 359 369 |
| 11*H*-Benzo[a]fluorene (1,2-Benzofluorene) | 255 *265* 296 306 318 | *347* 365 382(s) |
| Triphenylene | *262* 277 288 | *354* 364 373 381(s) 391(s) |
| 11*H*-Benzo[a]carbazole (1,2-Benzocarbazole) | 255 *279* 306 | *354* 372 392 413 |
| 7*H*-Benzo[c]carbazole (3,4-Benzocarbazole) | *263* 286 324 | *362* 369 381 402 |
| Chrysene | 261 *271* 297 308 322 | *363* 375 383 404 427 |
| Phenanthrene | 261 278 285 *296* | 348 357 *365* 385 |
| 7*H*-Dibenzo[*c*,*g*]carbazole (3,4,5,6-Dibenzocarbazole) | 278 303 350 *363* | *367* 386 405 |
| Dibenz[*a*,*c*]anthracene (1,2,3,4-Dibenzanthracene) | 269(s) 279 *289* | *377* 388 398 409(s) |
| Picene (1,2,7,8-Dibenzphenanthrene) | *287* 304 328 | *377* 398 421 449 |
| Anthracene | *260* 312 325 341 358 377 | *380* 401 424 451 |
| 13*H*-Dibenzo[*a*,*i*]carbazole (1,2,7,8-Dibenzocarbazole) | *290* 321 334 348 | *380* 401 425 |
| Pyrene | 308 322 *337* | 374 379 *384* 389 395 |
| Benz[*a*]anthracene (1,2-Benzanthracene) | 255 271 280 *290* 317 329 344 360 | *387* 408 435 462 |
| Benzo[*b*]chrysene (2,3,7,8-Dibenzphenanthrene) | 255 *289* 305 | *394* 418 444 470(s) |
| Dibenzo[*g*,*p*]chrysene (1,2,7,8-Dibenzochrysene) | 280 292 *303* 340 353 | *395* 409 |
| Dibenz[*a*,*h*]anthracene (1,2,5,6-Dibenzanthracene) | *301* 324 335 351 | *396* 407 418 446 473 |
| Naphtho[1,2,3,4-*def*]chrysene (1,2,4,5-Dibenzopyrene) | 276 293 *305* 330 342 358 376 | *397* 408 420 446 |
| Benz[*c*]acephenanthrylene (3,4-Benzofluoroanthene) | 255 299 *310* | *403* 413 429 459 489(s) |
| Benzo[*a*]pyrene (1,2-Benzopyrene) | 256 269 286 *300* 333 349 365 385 | *405* 410 429 457 |
| Dibenzo[*c*,*g*]phenanthrene (3,4,5,6-Dibenzophenanthrene) | 239 272 *312* 332 | *405* 424 446(s) |
| Benzo[*ghi*]perylene (1,12-Benzoperylene) | 291 *302* 331 348 364 385 | 399 408 *420* 446 |
| Dibenzo[*def*,*mno*]chrysene (Anthanthrene) | 260 296 *308* 384 401 407 422 430 | *432* 459 494 |
| Benzo[*rst*]pentaphene (3,4,9,10-Dibenzopyrene) | 247 274 285 *297* 316 332 355 373 395 | *434* 450 462 480 494 |
| Perylene | 255 370 388 410 438 | *440* 466 500 540(s) |
| Coronene | 292 *303* 324 340 | 411 422 428 435 *446* 455 475 485 508 |
| Dibenzo[*b*,*def*]chrysene (3,4,8,9-Dibenzopyrene) | 272 300 *312* 399 422 448 | *451* 480 518 |
| Fluoranthene | 241 256 266 280 *290* 311 326 344 360 | 409(s) 418(s) 436 *463* |
| Ovalene | 314 328 *342* 399 422 448 | 450 462 475 482 490 *503* 509 514 539 |

[a] The most intense peak in each spectrum is in italic type. Shoulders are indicated by (s).

[a] McKay and Latham (1972)

## Appendix 4

### Transformation from Rectangular to Polar Coordinates

A rectangular coordinate system is shown in Figure 4 with emission wavelengths ( $\lambda$ em) marked along the ordinate and with excitation wavelengths ( $\lambda$ ex) marked along the abscissa. Each point in the plane has rectangular coordinates ( $\lambda$ ex, $\lambda$ em) and represents a single pair of wavelengths. The distance r from the origin (0,0) to the point ( $\lambda$ ex, $\lambda$ em) is given by the Theorem of Pythagoras:

$$r = \sqrt{\lambda\ ex^2 + \lambda\ em^2}$$

The distance r may also be regarded as the radius of a circle with centre (0,0) passing through the point ( $\lambda$ ex, $\lambda$ em). Whether r is regarded as a distance or a radius, however, a given value of r does not correspond to a unique wavelength pair or point ( $\lambda$ ex, $\lambda$ em). For example, in Figure 4 the point (308 nm, 366 nm) has a value of r equal to 478 n, but this value of r could correspond as well to any other point of the same distance from the origin. The point (308 nm, 366 nm) is uniquely determined by the value 478 nm and the value and angle ~~$\theta$~~ shown in Figure 4. This value of ~~$\theta$~~ is 49.9° and is determined using the formula:

$$\text{~~}\theta\text{~~} = \tan^{-1} \frac{\lambda\ em}{\lambda\ ex}$$

Thus, the point (308 nm, 366 nm) can be also represented as (478 nm, 49.9°) which are called the polar coordinates of the point.

Appendix 5

Relationship between Relative Standard Deviation of Equivalent Concentrations and Standard Deviation of Ratios

$$s = \sqrt{\frac{1}{n-1} \sum_{i=1}^{n} (x_i - \bar{x})^2} \quad \text{by definition}$$

$$\frac{s}{\bar{x}} = \sqrt{\frac{1}{n-1} \sum_{i=1}^{n} \frac{(x_i - \bar{x})^2}{\bar{x}^2}}$$

$$= \sqrt{\frac{1}{n-1} \sum_{i=1}^{n} \left(\frac{x_i}{\bar{x}} - 1\right)^2}$$

$$= \sqrt{\frac{1}{n-1} \sum_{i=1}^{n} (R_i - 1)^2}$$

but $$\bar{R} = \frac{1}{n} \sum_{i=1}^{n} R_i$$

$$= \frac{1}{n} \sum_{i=1}^{n} \frac{x_i}{\bar{x}}$$

$$= \frac{\frac{1}{n} \sum_{i=1}^{n} x_i}{\bar{x}}$$

$$= \frac{\bar{x}}{\bar{x}} = 1$$

$$\therefore \quad \frac{s}{\bar{x}} = \sqrt{\frac{1}{n-1} \sum_{i=1}^{n} (R_i - \bar{R})^2} = s^1$$

where

$s$ = standard deviation of equivalent concentrations about the mean equivalent concentration

$n$ = number of equivalent concentrations

$x_i$ = $i^{th}$ equivalent concentration

$\bar{x} = \frac{1}{n} \sum_{i=1}^{n} x_i$ = mean equivalent concentration

$\frac{s}{\bar{x}}$ = relative standard deviation of the equivalent concentrations about the mean equivalent concentration

$R_i = \frac{x_i}{\bar{x}}$ = $i^{th}$ ratio

$\bar{R}$ = mean ratio

$s^1$ = standard deviation of ratios about the mean ratio.