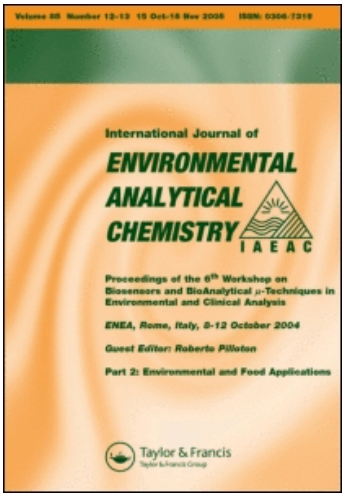

International Journal of Environmental Analytical Chemistry
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713640455

Evaluation of Direct Fluorescence Spectroscopy for Monitoring Aqueous Petroleum Solutions

K. østgaard[a]; A. Jensen[a]
[a] Institute of Marine Biochemistry, University of Trondheim, Trondheim, NTH, Norway

To cite this Article østgaard, K. and Jensen, A.(1983) 'Evaluation of Direct Fluorescence Spectroscopy for Monitoring Aqueous Petroleum Solutions', International Journal of Environmental Analytical Chemistry, 14: 1, 55 — 72
To link to this Article: DOI: 10.1080/03067318308071607
URL: http://dx.doi.org/10.1080/03067318308071607

Intern. J. Environ. Anal. Chem., 1983, Vol. 14, pp. 55–72
0306-7319/83/1401-0055 $06.50/0

Printed in Great Britain

Evaluation of Direct Fluorescence Spectroscopy for Monitoring Aqueous Petroleum Solutions

K. ØSTGAARD and A. JENSEN
Institute of Marine Biochemistry, University of Trondheim, N-7034 Trondheim—NTH, Norway

(*Received March 10, 1982; in final form July 7, 1982*)

The fluorescence of carefully prepared solutions of Ekofisk crude oil in sea water was studied directly in the water phase. Significant fluorescence was observed in the 290–460 nm range for oil solutions well below the concentration level of 10 ppb. On the basis of spectra of single components, the influence of pH on these, and data from gas chromatography/mass spectrometry, it was established that fluorescence at 335 nm (excitation wavelength 230 nm) was dominated by petroleum naphthalenes, while the fluorescence at 300 nm (excitation wavelength 265 nm) could be ascribed to the phenols of the crude oil. For both groups of compounds the intensity of the fluorescence was strongly dependent on the alkylation pattern of the components, making the choice of reference substances in quantitative applications problematic. Quantitative differences in fluorescence itself, could, however, easily be detected with significance, and studies of the preparation and stability of petroleum solutions in sea water are included as examples of application. Properly evaluated, direct fluorescence spectroscopy constitutes a very simple and rapid method for monitoring aqueous petroleum solutions.

KEY WORDS: Fluorescence, crude oil, petroleum, sea water.

INTRODUCTION

The dissolution of petroleum in sea water is a complex process, dependent on physical, chemical and biological factors. A complete description of the resulting solution or dispersion is a formidable analytical problem,[1] at present unresolved. Although the analytical methodology is improving, this is generally leading to increased complexity, and expensive and time-consuming procedures. The lability of aqueous petroleum solutions creates on the other hand a need for analysis of a large number of samples. This concerns particularly detection of oil contamination of the environment and control of the dose in studies of oil toxicity, from natural conditions to laboratory scale. A simple and rapid screening method will

in these cases be helpful in selecting the right samples for thorough analysis. It is important that the screening methods are critically evaluated, particularly the use of reference substances for calibration. In toxicity studies there is no reason to assume any *a priori* correlation between the biological impact of petroleum dissolved in water and the concentration of "oil", without considering the actual physicochemical composition of the oil dispersion.

Of several methods applied for monitoring purposes, infrared spectroscopy is particularly applicable to extracts of oil dispersions due to its sensitivity towards saturated hydrocarbons.[2] In aqueous petroleum solutions, however, characterized by absence of long-chain alkanes and a high content of aromatic compounds,[3] methods based on fluorescence represent a more interesting approach. Although generally applied to extracts,[4–6], Schwarz and Wasik[7] found fluorescence measurements to be highly sensitive also when applied directly to pure water solutions of single aromatic compounds. Direct fluorescence therefore appears promising also for monitoring aqueous solutions of petroleum.[8,9] This approach does, however, require reasonable knowledge of the relationship between the fluorescing and the non-fluorescing parts of the oil solutions involved, i.e., the actual physicochemical conditions of the samples.

The purpose of the present work was therefore to evaluate direct fluorescence spectroscopy applied as a practical method for monitoring aqueous petroleum solutions, using Ekofisk crude oil as the source of petroleum.

EXPERIMENTAL

Preparation of solutions

Solutions of single petroleum components were generally prepared by adding excessive amounts to distilled water. After shaking in closed Ehrlenmeyer flasks for 1–2 days, undissolved material was removed by filtration (solids) or decantation (liquids). For quantitative studies, known amounts of material were treated in the same way. Due to the particularly high volatility and low solubility of the naphthalenes, standard concentrations were obtained by dissolving the compound (0–2 mg) in 0.5 ml CH_2Cl_2, and diluting the solution to 500–1000 ml in water immediately before the fluorescence measurements. The small additional fluorescence background introduced by the solvent was corrected for in the treatment of the data. The use of ethanol (1.0 ml) instead of the CH_2Cl_2 had no influence on the linear part of the standard curves.

In the laboratory, sterile solutions of Ekofisk crude oil were prepared in 5 l glass bottles closed with silicone stoppers. One part of oil (200 ml) was carefully layered on top of 20 parts of sterile sea water† (4 l) and stirred gently by a magnetic stirrer, so that no deformation of the oil-water interface could be observed. This was achieved by reducing the voltage to 120–170 V instead of the normal 220 V, and is considered essential to avoid formation of emulsions.[11] For comparison, dispersions formed by turbulent stirring for 18 h were also studied. The temperature during preparation was in all cases 14°C.

Sterile samples for testing were pumped through a silicone rubber tube located below the level of the oil layer and collected in sterile brown glass medical flasks of 50 ml size. The first 20–30 ml were used for washing. The flasks were completely filled before closing with aluminium foil and screw caps. When larger volumes were needed, they were treated similarly. Storage until analysis took place at 4°C.

In open, outdoor tanks, unpolluted sea water from approx. 10 m depth of the Trondheimsfjord was used directly. Ekofisk crude oil (20 l) was carefully layered on top of 1,500 l sea water. The surface area was approx. 1 m^2. The oil slick was not visually affected by the slow stirring (16 rpm) of a propeller located near the bottom of the tank. A transparent plastic roof was used for protection against rain. Apart from this, the system was open to natural light, temperature, evaporation and microbial activity.

Test samples from the tank were treated as described above, after adjustment to pH 2 with conc. H_2SO_4 to prevent bacterial activity. This treatment did not affect the fluorescence of the samples. Common bacteriocides such as $HgCl_2$ and cetylpyridinium chloride were found to interfere strongly with the fluorescence signal.

Fluorescence analysis

Prior to analysis, the samples were brought to room temperature. A pre-cleaned quartz cuvette was washed with the test sample, completely filled and closed.

Analysis was performed in a Perkin Elmer Model 3000 fluorescence spectrometer, with excitation slit 15 nm and emission slit 5 nm. Emission spectra were scanned at 120 nm/min. With the exception of the qualitative spectra of single components, all recordings were made at the same calibration setting. The fluorescence unit is thus arbitrarily chosen, but identical in all the results presented.

†More precisely; the *f*/10 culture medium of Guillard and Ryther,[10] diluted to 2.5% salinity.

Chemical analysis

Chemical analysis was performed by F. Oreld and R. G. Lichtenthaler at the Central Institute of Industrial Research, Oslo. The more volatile components were determined by headspace analysis.[12] Briefly, the compounds were evaporated at 60°C by a stream of nitrogen and the volatiles adsorbed on finely granulated active charcoal in a capillary tube that was placed in the injector of the gas chromatograph. The components were identified by GC/MS, and quantified using benzene-d_6 as internal standard. Less volatile components were extracted with CH_2Cl_2, identified by GC/MS, and quantitated using deuterated internal standards (diphenyl-d_{10} and anthracene-d_{10}).[12] The total content of components boiling higher than toluene in the same extract was determined by GC (packed column) using known amounts of Ekofisk crude oil for calibration. Although commonly referred to as "total hydrocarbon content", this fraction is in fact neither total nor strictly composed of hydrocarbons. The designation "C_{7+}-fraction" is therefore used here.

RESULTS AND DISCUSSION

Practical detectability

Aqueous petroleum solutions contain numerous components with individual fluorescence characteristics, having different optimal excitation wavelengths. Widely different fluorescence emission spectra can therefore be produced for the same sample by small changes in the excitation wavelength (λ_{ex}).[4] In the present study two excitation wavelengths, 230 nm and 265 nm, were selected for closer inspection, partly because they gave simple spectra with dominating maxima, and partly because the spectra obtained were distinctly different (see Figures 1 and 2).

Figure 1A illustrates the emission spectrum excited at 230 nm, of a sea water solution of Ekofisk crude oil having a C_{7+}-fraction of 5.7 ppm, obtained after 10 days in the open tank system. The solution shown in Figure 1B also contained 5.7 ppm of the C_{7+}-compounds, but was prepared in the laboratory by slow stirring for 10 days in complete darkness. Both solutions had a significant fluorescence in the wavelength range 270–460 nm. A dominating double peak was observed at 330–340 nm.

The emission spectra of the above samples excited at 265 nm are given in Figure 2A and B. The fluorescence of the oil samples occurred in the 290–460 nm range, with maxima at 300 nm and 322 nm.

Since emission is measured 90° to the incident excitation beam, scattered light may also be included if the spectra are recorded close to the

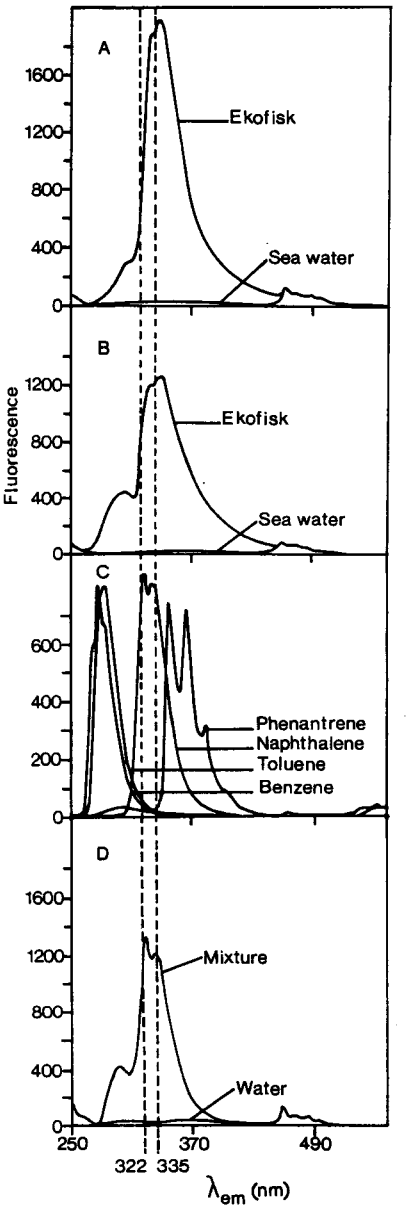

FIGURE 1 Fluorescence emission spectra of water solutions at excitation wavelength λ_{ex} = 230 nm of: (A) Ekofisk crude oil solution prepared in sea water in open tank system for 10 days, C_{7+}-fraction = 5.7 ppm; (B) Ekofisk crude oil solution prepared in sea water in lab. system for 10 days in darkness, C_{7+}-fraction = 5.7 ppm; (C) Some compounds commonly found in crude oil solutions; (D) A mixture of 0.75 ppm naphthalene and 2.1 ppm phenol in water.

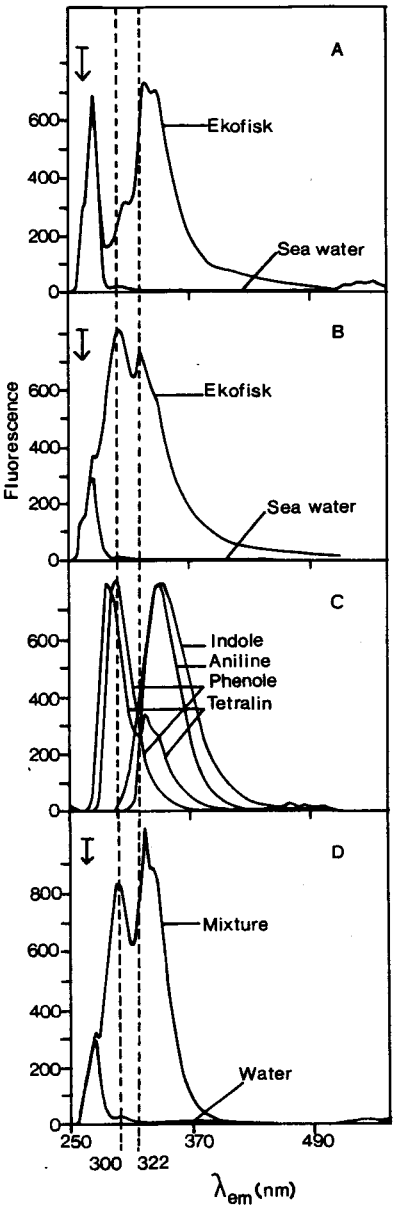

FIGURE 2 Fluorescence emission spectra of water solutions at excitation wavelength λ_{ex} = 265 nm (indicated by arrows). A–D: As in Figure 1.

excitation wavelength. Due to the Raman effect the scattered light will be of somewhat lower energy. In Figure 2 the maxima in scattered light was observed at 272 nm. The background spectra of Figure 2A and B illustrate the difference in the particle scattering between unfiltered and filtered sea water.

When oil was purposely emulsified in sea water by vigorous stirring for 18 h, and the mixture left in the dark for 10 days, the fluorescence spectra obtained from the aqueous phase were very similar to those measured in solutions produced by slow stirring in the dark (Figures 1B and 2B). The scattering at 272 nm was, however, significantly increased in the former case, remaining 2.5–3.0 times higher than the background. This peak was completely removed by treatment with dichloromethane (CH_2Cl_2/water, 1/10), in accordance with the assumption that the effect was due to oil droplets in the sample. The measurements showed that the main contribution to the fluorescence of oil dispersed in sea water came from dissolved components (see also ref. 8). Since oil droplets must leach components to the surrounding water quite rapidly, direct fluorescence is expected to be a sensitive indicator of both dispersed and dissolved oil. The complexity of oil emulsions makes quantitative approaches doubtful. The rest of this study was therefore limited to solutions of Ekofisk crude oil.

A sample of the solution giving the spectra in Figures 1B and 2B was diluted in steps, and the intensity of the fluorescence measured repeatedly. The recorded values for the solution itself were $1{,}200 \pm 4$ at $\lambda_{ex} = 230\,nm/\lambda_{em} = 335\,nm$ and 806 ± 5 at $\lambda_{ex} = 265\,nm/\lambda_{em} = 300\,nm$, with a corresponding background of the sea water solvent of 18.8 ± 1.1 and 27.2 ± 0.5, respectively ($\pm$ s.d., $n = 4$). The signal-to-noise level approached 1 for dilutions of $1:10^4$, showing that concentrations of the C_{7+}-fraction of 0.6 ppb could be detected. Increased sensitivity may be obtained for particular purposes by strict control of the solvent and the use of regression analysis.[7] In real cases of oil in natural sea water, however, the proportion of fluorescing material to total amount of dissolved oil is highly variable, and so is the quality of the water used as a solvent or reference. The practical lower working range is therefore estimated to be 10 ppb. This is similar to the limit reported by others.[13] Saturated solutions of different crude oils have been found to contain between 2 and 20 ppm at 25°C.[14] The sensitivity obtained in direct fluorimetry should therefore be satisfactory for most purposes.

The two samples involved in Figures 1 and 2 were selected because of their apparently similar petroleum content (C_{7+}-fraction). The very different fluorescence spectra observed illustrate the need for a more detailed knowledge of the composition before the quantitative aspects can be approached.

Identification

The fluorescence emission spectrum of a compound will be affected by interactions with the solvent. Fluorescence spectra of some aromatic hydrocarbons dissolved in water, recorded at the standard instrument setting, have been included in Figure 1C for comparison. Only naphthalene exhibited maxima close to those of the Ekofisk crude oil sample. However, the naphthalene fraction of crude oils generally consists of a complex mixture of alkylated isomers and homologs.[3,15,16] As shown in Table I, methylation generally shifts the maxima to longer wavelengths. The effect was too small to make a complete separation possible. By selecting the combination $\lambda_{ex} = 230\,nm/\lambda_{em} = 335\,nm$ it should, however, be possible to obtain some information on the amount of these naphthalenes as a group.

Sea water solutions of crude oils contain compounds in addition to aromatic hydrocarbons.[3,11] Figure 2C shows the spectra of tetralin, and the non-hydrocarbons aniline, indole and phenol. Phenol gave a fluorescence spectrum with a maximum at 293 nm, which closely resembled the peak of the Ekofisk sample (Figure 2B). As was the case

TABLE I

Location of intensity maxima (λ_{max}) in emission spectra of aqueous solutions of some petroleum components

Compound	λ_{max}	(nm)	
Hydrocarbons:			
Tetralin	284	324	
Aromatic compounds:			
Phenanthrene	346	364	388
Naphthalene	321	334	
1-Methylnaphthalene	324	334	
2-Methylnaphthalene	320	335	
2,3-Dimethylnaphthalene	328	336	
2,3,5-Trimethylnaphthalene	330	338	
Toluene	282		
Benzene	277		
Non-hydrocarbons:			
Indole	339		
Aniline	338		
Phenol	293		
2, 6-Dimethylphenol	295		
3, 5-Dimethylphenol	296		
2, 3, 5-Trimethylphenol	299		
2, 4, 6-Trimethylphenol	304		

with the naphthalenes, methylation shifted the maxima to higher wavelengths (Table I). The combination $\lambda_{ex} = 265\,nm/\lambda_{em} = 300\,nm$ was therefore expected to give an indication of the phenols in the solution.

Phenol has a pK_a of 10.0. By increasing the pH above this value the fluorescence spectrum changed, as illustrated in Figure 3. The scattering peak at 272 nm increased because a larger fraction of the material became particulate. The effect of increasing pH on the 300 nm peak of the Ekofisk sample was very similar to that on the fluorescence spectrum of phenol (Figure 3). At pH 11.6, the Ekofisk crude oil sample had a double peak left at 322–335 nm (Figure 3) which resembled that of naphthalene (Figure 2). The fluorescence at 322 nm ($\lambda_{ex} = 265$ nm) was routinely recorded in all

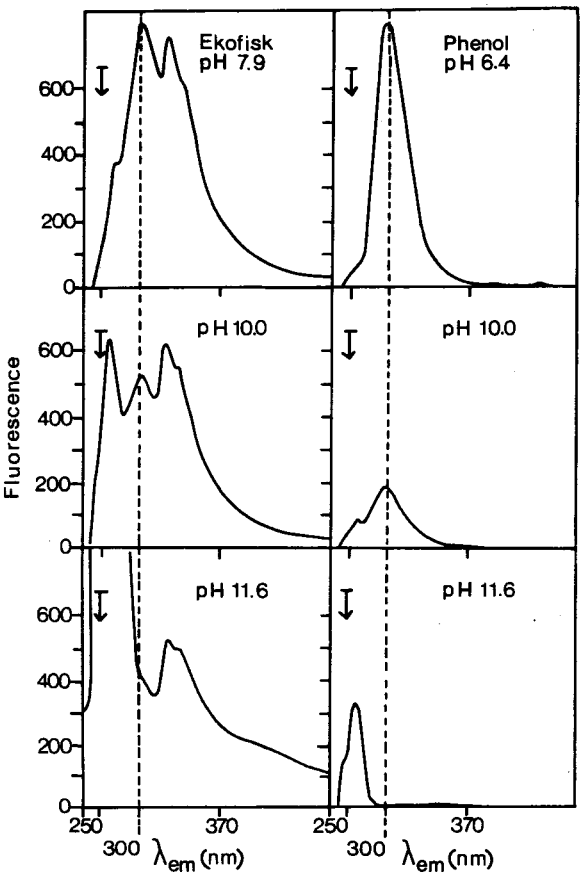

FIGURE 3 Fluorescence emission spectra of sea water solutions at $\lambda_{ex} = 265$ nm (indicated by arrows). Ekofisk crude oil solution and phenol solution compared at different pH values.

experiments. It was, however, always clearly correlated to the signal of the naphthalenes measured at 335 nm ($\lambda_{ex} = 230$ nm). The peak at 322 nm was therefore related to the naphthalenes, but not well separated from the fluorescence of the phenols. Containing no additional information, the fluorescence data obtained at $\lambda_{em} = 322$ nm have been omitted.

Figures 1D and 2D shows the spectra of a synthetic mixture of 0.75 ppm naphthalene and 1.2 ppm phenol, indicating that compounds other than naphthalenes and phenols are not needed to give the fluorescence spectra observed for the Ekofisk solution in sea water.

The chemical composition of two crude oil samples (corresponding to "A" and"B" in Figures 1 and 2) obtained by GC/MS is given in Table II. Due to partial thermolability of the heaviest compounds during MS, these could not be identified. A more complete list of compounds found in sea water solutions of Ekofisk crude oil is given elsewhere.[17] The aromatic compounds benzene, toluene, xylene and naphthalene, together with their alkylated derivatives, were the dominating compounds, while the Ekofisk crude oil itself contains only about 2% aromatics. In addition to the

TABLE II

Chemical composition of different sea water solutions of Ekofisk crude oil

Compound	Prepared in	
	Lab. system, 10 days in complete darkness	Open tank system, 10 days
Compounds lighter than benzene	4,000 ppb	24 ppb
Benzene	6,000 ppb	2 ppb
Toluene	1,000 ppb	11 ppb
Ethylbenzene	23 ppb	12 ppb
Xylenes	270 ppb	70 ppb
C_3-alkylbenzenes	72 ppb	220 ppb
C_4-alkylbenzenes		120 ppb
Naphthalene	43 ppb	187 ppb
Methylnaphthalenes	37 ppb	182 ppb
C_2-alkyl-naphthalenes		81 ppb
C_3-alkyl-naphthalenes		7 ppb
Phenol	3 ppb	
Sum of C_1–C_3-alkylated phenols	214 ppb	31 ppb
C_{7+}-fraction of sample	5,700 ppb	5,700 ppb
C_{7+}-fraction of control		150 ppb
Total:	17 ppm	6 ppm

hydrocarbons, also phenol and its C_1- to C_6-alkylated derivatives were observed. These phenols partially overlap in the GC/MS-analysis, and are therefore given as a sum only. As expected, the concentration of volatile compounds not included in the C_{7+}-fraction was much higher in the sample from the closed laboratory system compared to that from the open tank (Table II). The real concentration of compounds was thus 3 times higher than the value of the C_{7+}-fraction.

Both of the above samples were thus highly enriched in aromatic hydrocarbons and phenols. This is generally observed in sea water extracts of crude oil.[3,11] Quantitatively, however, the sample from the open tank had a much higher concentration of naphthalenes and a lower concentration of phenols, compared to the sample prepared in the laboratory. This is in accordance with the higher fluorescence at 335 nm ($\lambda_{ex}=230$ nm. Figure 1) and the lower value observed at 300 nm ($\lambda_{ex}=265$ nm, Figure 2).

It is concluded that while the combination $\lambda_{ex}=230\,nm/\lambda_{em}=335\,nm$ may give an indication of the content of naphthalenes, the combination $\lambda_{ex}=205\,nm/\lambda_{em}=300\,nm$ may be used for the estimation of phenols.

Quantitation

The selection of a proper standard for the calibration in the analysis of crude oil components is a general problem. A quantitative estimation of the naphthalene and phenol fractions of crude oil solutions by fluorescence spectroscopy must take into account the diversity in alkylation of these compounds.

Figure 4A shows standard fluorescence curves for some methylated naphthalenes recorded at 335 nm ($\lambda_{ex}=230$ nm). The response for naphthalene itself showed the same curved shape, probably due to quenching, as that of the other compounds, but was linear below 3 ppm (results not included). The differences shown were not caused by the small shifts in the location of the various intensity maxima (Table I). The specific fluorescence of 2,3,5-trimethylnaphthalene was more than 10 times that of naphthalene.

Since the proportion of the various alkylated naphthalenes in the aqueous solution of the crude oil is not known, the naphthalene fraction measured by fluorescence can only be empirically calibrated against methods which allow quantitation of at least some of the actual compounds. Moreover, since the alkylation also will affect such properties as evaporation, solubility and reactivity, the empirical correlations cannot be expected to be generally valid even for a given oil. These limitations do

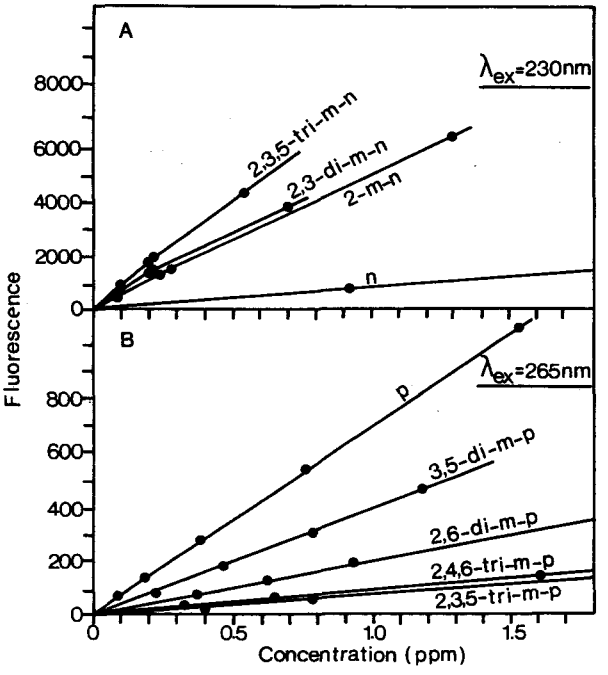

FIGURE 4 Fluorescence *vs.* concentration of some methylated phenols and naphthalenes in water. Abbreviations: m, methyl; n, naphthalene; p, phenol. A: Naphthalenes ($\lambda_{ex}=230/\lambda_{em}=335$ nm). B: Phenols ($\lambda_{ex}=265$ nm/$\lambda_{em}=300$ nm).

not, however, make quantitative comparisons of fluorescence spectra less interesting.

Figure 4B shows the fluorescence *vs.* concentration curves for several methylated phenols. At higher concentrations, the increase in fluorescence with concentration deviated from linearity (results not included). Contrary to what was found for naphthalenes, methylation reduced the specific fluorescence in the phenol series. The effect was very clear also on a molar basis, and will make attempted calibrations problematic also for the phenols.

It is clear from the results presented above that no exact correlation between fluorescence data and GC/MS can be expected. The sum of naphthalene and monomethylnaphthalenes determined by GC/MS and the naphthalene fraction determined by fluorescence have been compared for some samples in Figure 5A. The analysis were performed over a period of 1 year, and some variation in the analytical equipment and in water quality cannot be excluded. The values recorded for the content of naphthalenes by the two different methods were clearly related.

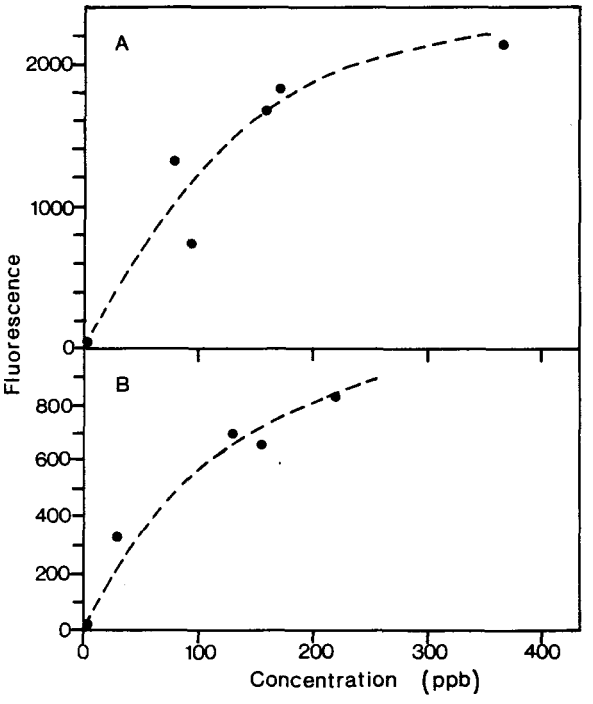

FIGURE 5 Correlation between concentration of crude oil compounds estimated by fluorescence and GC/MS. A: Naphthalenes. Fluorescence ($\lambda_{ex}=230$ nm/$\lambda_{em}=335$ nm) plotted as a function of the sum of identified naphthalene + monomethylnaphthalenes. B: Phenols. Fluorescence ($\lambda_{ex}=265$ nm/$\lambda_{em}=300$ nm) plotted as a function of the sum of identified phenol + C_1–C_3-alkylated phenols.

The extraction step and the GC/MS-analysis have been adapted to the quantitation of oil components in general, and are therefore far from optimal for polar compounds such as phenols. However, since the shortcomings are mainly systematic, the GC/MS method was compared with flourescence spectroscopy also for phenols (Figure 5B). A correlation was observed also in the case of phenols.

In total, the results presented in Figure 5 verify that the fluorescence of the samples was related to the contents of naphthalenes and phenols, supporting the interpretation of the fluorescence spectra given earlier. The relationships are, however, quite complex. It is therefore stressed that the empirical calibration curves tentatively suggested in Figure 5 can be used only for rough estimates of naphthalenic or phenolic content in the actual experimental systems, and should not be considered of general validity.

From the results presented above, it should be evident that any attempt

to calibrate fluorescence to the total amount of oil components must be purely empirical, and documented for each experiment.

Application

As examples of application of the method some studies of the kinetics of the preparation of oil solutions and their stability in the experimental systems described have been included.

Water soluble fractions (WSF) of crude oil and oil products for toxicity testing are commonly prepared by slow stirring in closed, sterile systems.[15,18,19] Figure 6A illustrates this dissolution process performed in

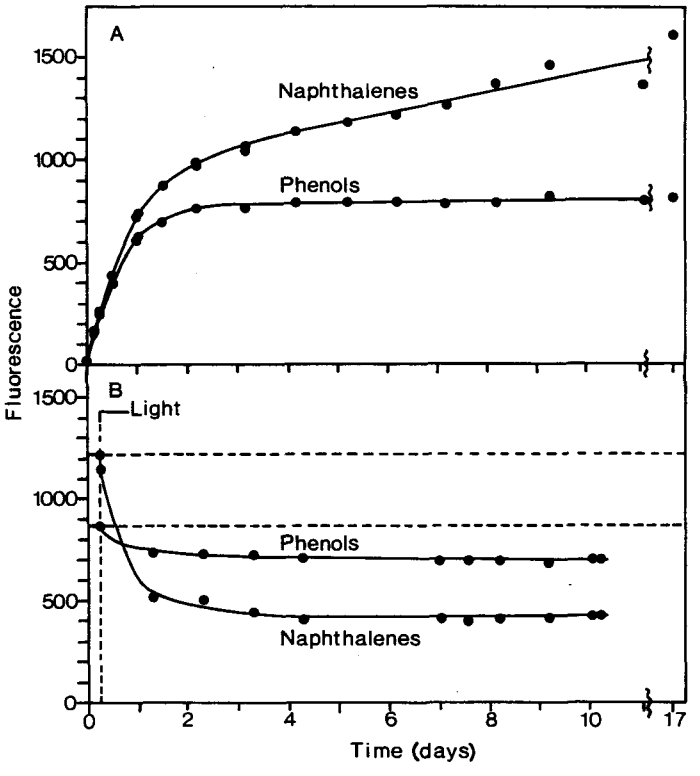

FIGURE 6 Ekofisk crude oil solutions in sea water in laboratory conditions. Fluorescence at $\lambda_{ex}=230\,nm/\lambda_{em}=335\,nm$ (naphthalenes) and $\lambda_{ex}=265\,nm/\lambda_{em}=300\,nm$ (phenols) given as a function of time. A: Preparation of a sterile solution by slow stirring under normal indoor illumination ($20\,\mu E/m^2s$, day:night = 14 h:10 h). B: Storage of a sterile solution in closed, completely filled flask under illumination ($360\,\mu E/m^2s$, day:night = 14 h:10 h). Dotted lines: Initial value reference lines corresponding to storage in darkness.

normal indoor illumination. No equilibrium level was reached within 18 days. Even in total darkness,[20] the preparation time normally used, 12–18 h, is probably too short to give a stable level. The possible consequences for the determination of the toxicity of crude oil and petroleum components will be dealt with elsewhere.

After 10 days of stirring in darkness in the closed laboratory system, the water phase was transferred to another flask, which was completely filled and closed. Figure 6B shows the effects of increased illumination on this solution, now without the cover of the oil layer. In this particular experiment, samples were taken by pumping in a corresponding volume of sea water and the introduction of a gaseous phase thus avoided. The results have been corrected for the dilution due to sampling. When stored in the dark under similar conditions, fluorescence was stable for 1–2 months. As illustrated in Figure 6B, light-induced reactions apparently led to a rapid reduction in the concentration of naphthalenes, and had a somewhat smaller effect on the phenols. The nature of the stable levels reached after 4 days is not known. Figure 6B clearly illustrates the problems of producing constant concentrations of crude oil components in sea water even in sterile systems without evaporation.

Figure 7 shows the fate of the naphthalenes and phenols in an experiment in the open tank system. The temperature remained between

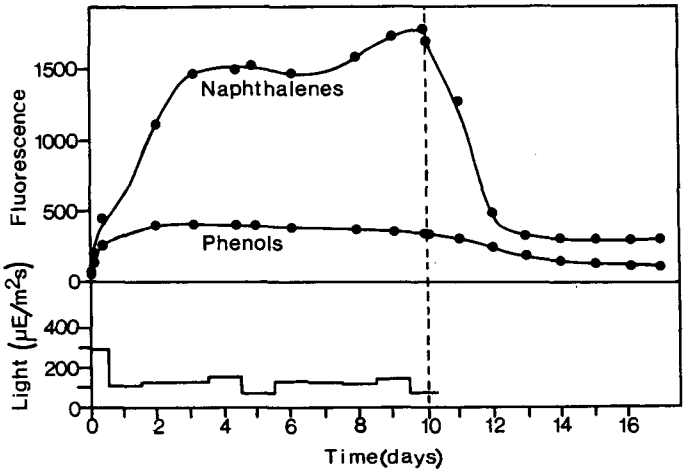

FIGURE 7 Ekofisk crude oil solution in sea water in open tank system. Fluorescence at $\lambda_{ex} = 230\,nm/\lambda_{em} = 335\,nm$ (naphthalenes) and $\lambda_{ex} = 265\,nm/\lambda_{em} = 300\,nm$ (phenols) given as a function of time. 0–10 days: Preparation of sea water (with oil layer) by slow stirring illuminated as indicated (calculated as the mean value of a theoretical 14-h day). Dotted line: Transfer of solution. After 10 days: Storage of solution (without oil layer) in open tank.

10 and 12°C during the recorded period. The variations in light intensity during the dissolution process are also given in the figure. It is not yet clear whether the light intensity can be expected to correlate with the fluorescence data as in the laboratory system. Uncontrolled factors such as evaporation and microbial activity could be important in the open tank. Since the quality of the natural and the artificial light was different, light intensities of the two experimental systems cannot be directly compared. Still, it is interesting to note that according to the fluorescence values the naphthalene concentration was higher in the open tank relative to that obtained in the laboratory (cf. also Table II), despite the lower oil-to-water ratio.

After 10 days of stirring the oil solution was transferred to an open test tank. The rapid loss of fluorescence which took place after the solution was separated from the oil layer is shown in Figure 7. Although this system was open and evaporation should be significant, the drop in fluorescence was qualitatively similar to that observed in the closed laboratory system (Figure 6B).

CONCLUSIONS

Direct fluorescence spectroscopy evidently constitutes a very simple and rapid means for monitoring aqueous petroleum solutions. Since no special treatment of the samples is necessary, large numbers can be easily handled. Concentration levels of 10 ppb are estimated to be within the practical working range.

Although generally applicable as a qualitative indicator of oil dispersions in water, quantitative analysis should be attempted only in cases where emulsification is not extensive. Light scattering measured in the fluorescence spectrometer may possibly be used to reveal the presence of oil droplets. Based on the combination $\lambda_{ex} = 230$ nm/$\lambda_{em} = 335$ nm the concentration of petroleum naphthalenes can be estimated. Similarly, the fluorescence at 300 nm ($\lambda_{ex} = 265$ nm) can be used to indicate the concentration of the phenols in the sample. These two classes of compounds are generally highly enriched in aqueous petroleum solutions.[3,11]

Since the specific intensity of the fluorescence of the aromatic compounds studied was highly dependent on alkylation, selection of suitable calibration standards is difficult. For a given experimental situation and a defined oil, calibration curves for the content of naphthalenes or phenols may be established on the basis of independent methods (GC/MS). Even in this case, however, the proportion of these

compounds is highly variable. Any calibration of direct fluorescence to total content of dissolved components should be restricted to interpolation between values obtained by more thorough methods, and not be expected to correlate to dispersed petroleum.

The need for reliable monitoring during preparation and tests involving aqueous petroleum solutions was clearly demonstrated in the examples of application given in the present report. Quite unexpected changes in the chemical composition of oil solutions were observed even under rigidly controlled laboratory conditions (Figure 6). Against this background it seems obvious that close characterization and control are weak points in the toxicity testing of oil components in aquatic systems. Since the fluorescence analysis gives immediate results it is particularly suited to guide the sampling during experiments and for the selection of samples for quantitative analysis by more advanced methods. The fluorescence technique should furthermore be particularly useful, eventually in an automatic version, for monitoring purposes in continuous flow systems used for the preparation of aqueous oil solutions.[21,22] Various commercial instruments are now available for the detection and tracing of oil pollution in water.[9,13,23,24] It is hoped that our findings may be of help also in the evaluation of such measurements.

Acknowledgements

We are grateful to A. Andrewes, Y. Folkedahl and E. Nøst Hegseth for skilful assistance in the experiments, and to F. Oreld and R. G. Lichtenthaler at the Central Institute of Industrial Research, Oslo, for the chemical analysis performed.

This work is part of the Norwegian Marine Pollution Research and Monitoring Programme.

References

1. L. Petrakis and F. T. Weiss (eds.), *Petroleum in the Marine Environment* (American Chemical Society, Washington DC. 1980).
2. National Academy of Sciences, *Petroleum in the Marine Environment* (National Academy of Sciences, Washington DC, 1975 pp. 19–41.
3. K. Winters, R. O'Donnel, J. C. Batterton and C. V. Baalen, *Mar. Biol.* **36,** 269 (1976).
4. U. Frank, *US Coast Guard Prevention & Control of Oil Pollution Conf. Proc.* (Am. Petroleum Inst. and Environ. Protect. Agency, Washington DC, 1975) pp. 87–91.
5. K. Shigehara, K. Kimura, J. Ohoyama and N. Kubo, *Oceanogr. Mag.* **30,** 61 (1979).
6. H. Awad, *Rev. Trav. Inst. Pêches marit.* **41,** 375 (1979).
7. F. P. Schwarz and S. P. Wasik, *Analyt. Chem.* **48,** 524 (1976).
8. A. W. Hornig, *Proc. NBS Symposium May 13–17, 1974* (NBS Spec. Publ. 409, Marine Pollution Monitoring, 1974) pp. 135–144.
9. G. Turner, *Sea Technol.* **20,** 30 (1979).

10. R. R. L. Guillard and J. H. Ryther, *Can J. Microbiol.* **8,** 229, (1962).
11. D. G. Shaw and S.K. Reidy, *Environ. Sci. Technol.* **13,** 1259 (1979).
12. N. Berg, K. Gustavsen, N. Gjøs, R. G. Lichtenthaler, F. Oreld, K. Vadum and T. Øfsti, *Chemical analysis of water soluble petroleum fraction* (SI report no. 780702–1, SI, P.B. 350 Blindern, Oslo 3, 1980).
13. E. Bender, *Sea Technol.* **19,** 28 (1978).
14. L. C. Price, *AAPG Bull,* **60,** 213 (1976).
15. J. W. Anderson, J. M. Neff, B. A. Cox, H. E. Tatem and G. M. Hightower, *Mar. Biol.* **27,** 75 (1974).
16. C. McAuliffe, *AAPG Bull.* **63,** 761 (1979).
17. K. Østgaard, I. Eide and A. Jensen, submitted to *Mar. Env. Res.* (1982).
18. P. Kauss, T. C. Hutchinson, C. Soto, J. Hellebust and M. Griffiths, *Proc. Joint Conf. on "Prevention and Control of Oil Spills"* (American Petroleum Institute, Washington DC, 1973) pp. 703–713.
19. R. Blundo, *Contr. Mar. Sci.* **21,** 25 (1978).
20. J. M. Giddings and J. N. Washington, *Environ. Sci. Technol.* **15,** 106 (1981).
21. S. Krugel, D. Jenkins and S. A. Klein, *Water Res.* **12,** 269 (1978).
22. P. E. Benville Jr., T. G. Yocum, P. Nunes and J. M. O'Neill, *Water Res.* **15,** 1197 (1981).
23. Anonymous, *Pollution Monitor* (No. 45), 13 (1978).
24. Anonymous, *Oil in the environment* (Turner Designs, 2247 Old Middlefield Way, Mountain View, CA 94043).