Notes

# Flow Injection Analysis of C-Fuel Oil-Contaminated Samples Based on the Fluorescence Detection of Polycyclic Aromatic Hydrocarbons

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More than 6000 kl of C-fuel, a type of fuel oil, was spilled from the Russian tanker "Nakhodka" into the Sea of Japan on January 2, 1997, which drifted toward eight prefectural sea coasts from Shimane to Yamagata in Japan.  Fuel oil contains various organic compounds.[1]  Among them, polycyclic aromatic hydrocarbons (PAHs) are considered to be an oil-contamination indicator, because PAHs are not easily degraded in the environment, and some of them, such as benzo[*a*]-pyrene, are mutagenic and/or carcinogenic.  Compared with PAHs having less than 4 rings, PAHs having 4 or more rings are less volatile and less water soluble. These compounds remain in the environment for a long time.[2,3]  Therefore, it is important to monitor PAHs having 4 or more rings in oil-contaminated environmental samples.

Previous studies[4,5] have used a spectrofluorometer for the analysis of fuel oil because many PAHs are fluorescent.  After the Nakhodka spill, we determined PAHs in the spilled oil, sea sand and water samples using a high-performance liquid chromatograph equipped with a fluorescence detector ( HPLC/FLD).[6,7]  Although the HPLC method is accurate and quantitative, it takes too much time for the rapid analysis of many oil-contaminated environmental samples.  In this paper we report on a flow injection analysis (FIA) method as a rapid screening test of C-fuel oil contamination.  We also report on the results of applying this method to the analysis of sea sands contaminated with the oil from the Nakhodka.

## Experimental

*Reagents*

PAH standard compounds used were: naphthalene and fluorene (Kanto Chemical, Tokyo, Japan); acenaphthylene, anthracene, benz[*a*]anthracene, benzo[*k*]-fluoranthene and benzo[*a*]pyrene (Wako Pure Chemical, Osaka, Japan); fluoranthene (Nacalai Tesque, Kyoto, Japan); phenanthrene, pyrene and benz[*a,h*]anthracene (Tokyo Kasei, Tokyo, Japan); chrysene, benzo[*b*]fluoranthene and benzo[*ghi*]perylene (Aldrich, Milwaukee, WI, USA); indeno[1,2,3-*cd*]pyrene (National Cancer Institute, Kansas City, MO, USA).  Authentic A- and C-fuel oil samples were from Nihonsekiyu (Tokyo, Japan).  All other chemicals were of analytical grade.

*FIA conditions*

The FIA system consisted of a JASCO (Tokyo, Japan) 880-PU pump, a Rheodyne (Cotati, CA, USA) 7125 injector (20 μl loop), a JASCO FP920S fluorescence detector (Ex 260 nm, Em 370 nm) and a Shimadzu (Kyoto, Japan) C-R6A integrator.  The flow line from the injector to the detector was made of polytetrafluoroethylene tubing (0.25 mm i.d.×10 cm).  The carrier solution was acetonitrile at a flow-rate of 1.0 ml min$^{-1}$.

*Sample preparations*

Unspilled C-fuel oil sampled from the Nakhodka on February 21, 1997 was provided by Ishikawa Prefecture.  Sand core samples collected at Katano beach, Kaga-shi, Ishikawa, Japan on April 8, 1997 were kindly provided by Professor Eiichi Tamiya of Japan Advanced Institute of Science and Technology.  An adequate volume of hexane was added to each sample according to Method 1664 of the U. S. Environmental Protection Agency (EPA), and the mixture was ultrasonicated.  The solution was filtered through an HLCDISK 13 (Kanto Chemical Co.) membrane filter cartridge (pore size, 0.45 μm).  The filtrate was diluted with acetonitrile and then injected into the FIA system.

## Results and Discussion

*Detection wavelengths*

Both the oil from the Nakhodka and authentic C-fuel oil showed similar fluorescence spectra with an excitation maximum at 260 nm and an emission maximum

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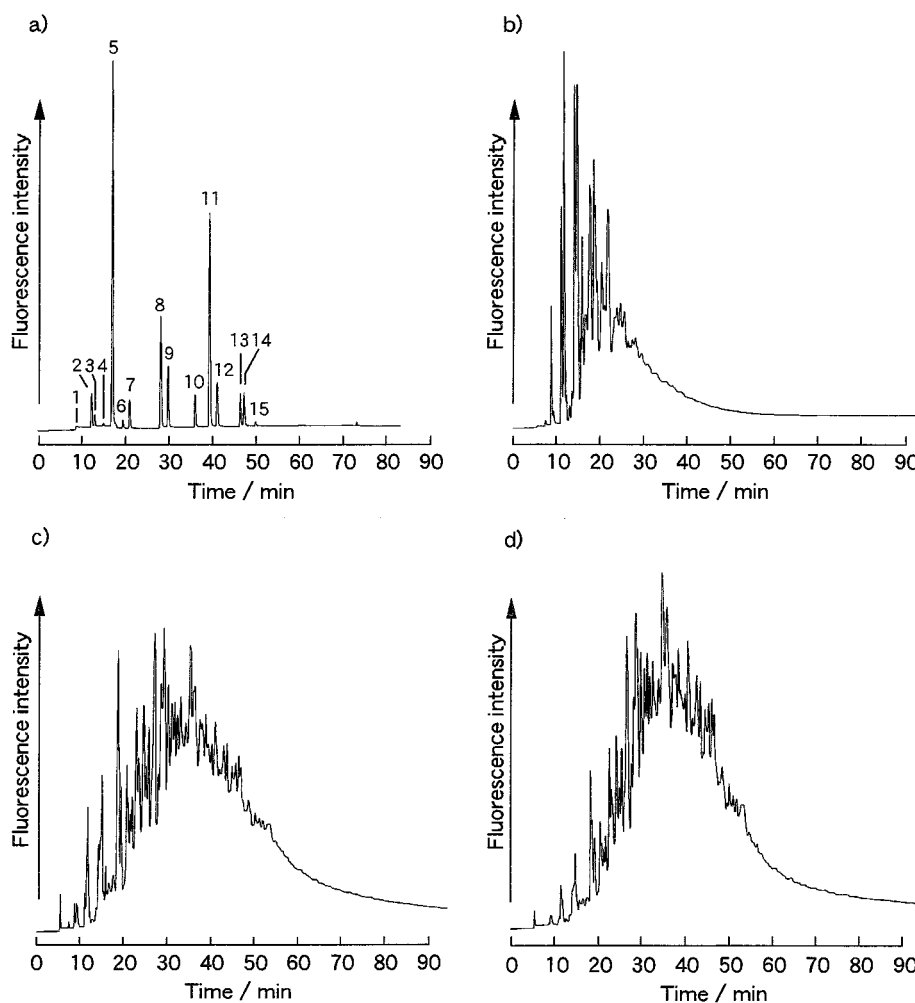[†] To whom correspondence should be addressed.

Fig. 1   Chromatograms of fuel oil from the Nakhodka, A- and C-fuel oil samples, and authentic standards. Analytical column, Vydac 201TP54 (4.6 i.d.×250 mm); guard column, Cosmosil 5C18 (4.0 i.d.×10 mm); injector volume, 20 μl; column temperature, 20°C; mobile phase, water–acetonitrile. Acetonitrile concentration was increased from 50% (0 min) to 100% (50 min) at 1% min⁻¹. The flow-rate was 1 ml min⁻¹. a) authentic PAHs (each 2 fmol): 1, naphthalene; 2, acenaphthylene; 3, fluorene; 4, phenanthrene; 5, anthracene; 6, fluoranthene; 7, pyrene; 8, benz[a]anthracene; 9, benzo[b]fluoranthene; 10, chrysene; 11, benzo[k]fluoranthene; 12, benzo[a]pyrene; 13, benz[a,h]anthracene; 14, benzo[ghi]perylene; 15, indeno[1,2,3-cd]pyrene. Fluorescence excitation/emission wavelengths (nm) were set at 280/340 (0 – 16.8 min); 250/400 (16.8 – 20.0 min); 286/433 (20.0 – 21.6 min); 331/392 (21.6 – 25.0 min); 266/402 (25.0 – 35.0 min); 294/430 (35.0 – 40.5 min); 300/430 (40.5 – 46.0 min); 294/430 (46.0 – 51.8 min); 294/482 (51.8 – 90.0 min), respectively. b) A-fuel oil (100 ng). Fluorescence excitation/emission wavelengths (nm) were set at 280/340 (0 – 90 min). c) C-fuel oil (50 ng). Fluorescence excitation/emission wavelengths (nm) were set at 260/370 (0 – 90 min). d) oil from the Nakhodka (28 ng). Fluorescence excitation/emission wavelengths (nm) were set at 260/370 (0 – 90 min).

at 370 nm, suggesting that the oil spilled from the Nakhodka was C-grade and that these excitation and emission wavelengths were suitable for use in the detection of the Nakhodka oil.

If the excitation and emission wavelengths indicated mainly PAHs having less than 4 rings, the method would not be suitable for the monitoring of sites that had been contaminated with oil. In order to know what compounds were detected at the above excitation and emission wavelengths, the Nakhodka oil and A- and C-fuel oil samples were separated using reversed-phase

chromatography. PAHs were generally eluted in increasing order of the ring number, as shown in a typical chromatogram of PAH standards (Fig. 1a). Sixteen 2-to-6-ring PAHs have been identified as priority pollutants by the EPA. The first 4-ring PAH in this group to elute was fluoranthene, which eluted at 20 min. The area of the peaks between 20 min and 77 min accounted for more than 82% of the total peak area between 0 min and 77 min in both C-fuel oil (Fig. 1c) and the Nakhodka oil (Fig. 1d). This result suggested that the contribution of PAHs having 4 or more rings was large
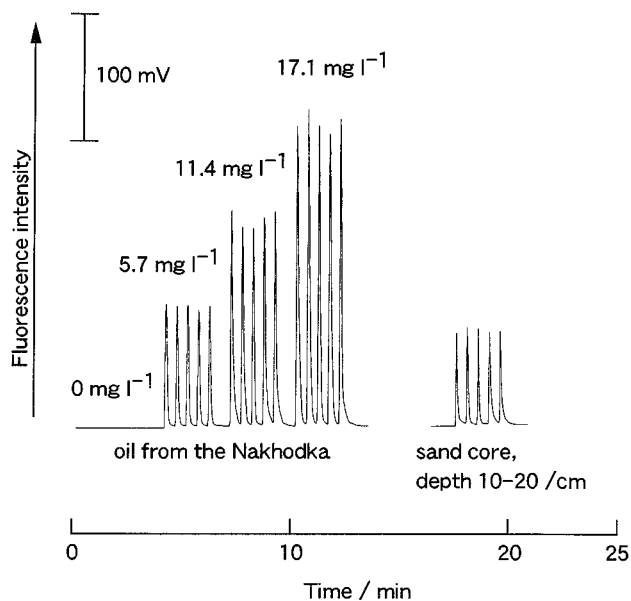
Fig. 2   Flow signals for oil from the Nakhodka and for a practical sample (sand core sample).  One hundred grams of sand core (depth 10 – 20/cm) was extracted with 100 ml of hexane. The extracted solution was diluted 8000 times with acetonitrile.

Table 1   Analytical results of sand core samples at Katano Beach, Ishikawa, Japan

| Depth/cm | RFI[a]$\times 10^5$ | B[a]P concentration by FIA[b], ppm | B[a]P concentration by HPLC[c], ppm |
|---|---|---|---|
| 0 – 10 | 6.5±0.1 | 4.6 | 3.7 |
| 10 – 20 | 6.3±0.3 | 4.5 | 3.4 |
| 20 – 30 | 3.8±0.0 | 2.7 | 2.8 |
| 30 – 40 | 9.4±0.1 | 6.7 | 5.2 |
| 40 – 50 | 4.9±0.1 | 3.5 | 2.9 |
| 50 – 60 | 8.4±0.5 | 6.0 | 5.7 |
| 60 – 70 | 0.1±0.0 | 0.1 | 0.1 |
| 70 – 80 | 0.0±0.0 | 0.0 | 0.0 |

a. RFI means the relative fluorescence intensity of a test solution to that of $10^{-6}$ M of B[a]P (mean±SD, $n=5$).
b. Present method. It was calculated from the mean value of RFI.
c. $n=1$.

in the proposed FIA.  However, A-fuel oil showed different fluorescence spectra (excitation maximum at 280 nm, emission maximum at 340 nm) and the contribution of peaks eluted after 20 min was smaller (Fig. 1b), because A-fuel oil contains mainly PAHs having fewer than 4 rings.

There was a good linearity between the fluorescence intensity and the concentration of the Nakhodka oil (Eq. (1)) in the range from 0.06 to 17 mg l⁻¹ with a correlation coefficient of 0.997.

$$\text{RFI}=18.9\times[\text{concentration of oil/mg l}^{-1}]-3.86, \qquad (1)$$

where RFI is the fluorescence intensity of the test solution relative to that of $10^{-6}$ M B[a]P.

*Application to oil-contaminated sea sands*

As an application of the proposed FIA method, we analyzed a sand core at Katano beach that was sampled after removal of the beached oil (Fig. 2).  The core was sliced into eight 10-cm bands starting at the surface. Strong fluorescence intensities were observed down to 60 cm from the surface, suggesting the existence of oil contamination even at this depth (Table 1).

B[a]P has been used as an indicator of the carcinogenicity and/or mutagenicity of mixtures of PAHs.  The concentration of B[a]P in the Nakhodka oil determined by HPLC/FLD was 134 ppm.[6,7]  Utilizing this value, the B[a]P concentration could be roughly estimated from the fluorescence intensity by using

$$[\text{B}[a]\text{P}]=7.13\times\text{RFI}+27.5. \qquad (2)$$

A good correlation coefficient ($r=0.987$) was observed between the present method and the HPLC/FLD method, as shown in Table 1.  By using Eq. (1), the initial concentration of C-fuel oil could also be calculated.

Although the proposed FIA method could not determine individual PAHs, it took only 30 s.  As a conclusion, the proposed method based on the fluorescence of PAHs is a simple and quick screening method for use prior to HPLC analysis.

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