Specific Dual Beam Fluorometry Analysis of Benzo[a]pyrene and Benzo[k]fluoranthene in Diesel Exhaust Particulate Samples

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We developed a specific analysis method for benzo[a]pyrene (B[a]P) and benzo[k]fluoranthene (B[k]F) using dual beam fluorometry. B[a]P and B[k]F exhibited two specific fluorescence intensities (up to 10-fold) compared with 22 other polycyclic aromatic hydrocarbons (PAHs) at 370 nm excitation and 429 nm emission wavelengths, and similarly at 395 nm excitation and 429 nm emission wavelengths. The concentrations of these compounds in diesel exhaust particles were calculated from two calibration curves determined using a mixture of 24 PAH standards. The precision of this detection method was compared with that of conventional high-performance liquid chromatography/fluorescence detector (HPLC/FLD), and our method gave accurate values within the analytical standard deviations. The monitoring method developed in this study will enable more rapid and easier detection.

1. Introduction

Polycyclic aromatic hydrocarbons (PAHs) are one group of organic compounds with considerable environmental effect owing to their widespread distribution; this is because they are generated both by natural causes (e.g., forest fires and volcanic activity) and by incomplete combustion of fossil fuels and other organic materials. In particular, air pollutants from diesel exhaust particles including PAHs seriously damage human health and this has become a problem. Benzo[a]pyrene (B[a]P) and benzo[k]fluoranthene (B[k]F) are PAHs exhibiting strong mutagenicity, carcinogenicity, and endocrine disruptor func-
Moreover, B[a]P and B[k]F cause immune disturbances that result in allergic diseases such as asthma and hay fever in developed countries. Tomiya et al. studied among the PAHs, eight major compounds in diesel exhaust particles for activity in inducing thymic involution. Among these eight compounds, seven, with the exception of benzo[g,h,i]perylene, are significantly effective in decreasing the number of thymocytes upon in vivo exposure. In particular, B[a]P, B[k]F and B[b]F are most potent in inducing the loss of thymocytes with median effective doses (ED_{50}'s) of less than 0.1 mg per mouse. The concentration of B[b]F in exhaust particles is lower than that of the other two PAHs, but these three PAHs are particularly important because they disturb the immune system, as shown by their study. Because B[a]P and B[k]F accumulate inside the body, the concentration of 0.1 mg is not low.

The real-time analysis of B[a]P and B[k]F in the atmosphere is important. In general, a separation process is necessary to measure the concentration of B[a]P and B[k]F present in diesel exhaust particles. For this reason, B[a]P and B[k]F concentrations are usually measured using high-performance liquid chromatography (HPLC) and gas chromatography/mass spectroscopy (GC/MS). However, for the purpose of on-site monitoring, there are the problems of cost and device size. Moreover, the above measurement systems are complex and are not applicable to continuous measurement in real time. Therefore, the development of an on-site monitoring device for B[a]P and B[k]F is a priority.

To measure B[a]P and B[k]F concentrations easily, the development of a separation method different from that used in GC/MS and HPLC is necessary. A key to solving this problem is absorption spectroscopy in which a dual beam wavelength is used. As an advantage of this system, the absorption spectrum can be confirmed when two or more absorption peaks overlap because there is little difference in wavelengths. In addition, a weak-absorption spectrum masked when the absorbance is strong compared with wavelength can be confirmed. Also, the single absorption maximum wavelength of the spectrum of underlying absorption can be confirmed. However, a disadvantage is that its sensitivity is low.

In this study, absorption spectroscopy, in which a dual-beam wavelength and a dual-wavelength fluorescence were used, was carried out. Using dual-wavelength fluorescence solves the problem regarding the time of separation of PAHs, and the advantages of μ-TAS and lab-on-a-chip techniques can be exploited to the fullest.

2. Materials and Methods

2.1 Apparatuses

Fluorescence spectra were recorded on a Shimadzu RF-5300PC spectrofluorometer. A modular component liquid chromatographic system consisting of a JASCO PU-1580 ternary pump, a JASCO DG-2080-53 degasser, a Shimadzu fluorescence detector, and a JASCO RF-10AXL integrator was used. In experiments, 20 μL of a sample was injected through a JASCO AS-1555 autoinjector. An LC-PAH column from SUPELCO, 150×4.6 mm in length and 5 μm in particle size, was used.

2.2 Reagents and samples

Dichloromethane (DCM) of analytical grade from Kanto Chemicals was used. The PAH standards were purchased from different companies: acenaphthylene (Alene), acenaphthene
(Ace), fluorene (Flu), anthracene (Ant), phenanthrene (Ph), pyrene (Pyr), B[a]P, B[b]F, B[k]F, benzo[g,h,i]perylene (B[ghi]P), and DB[a,h]A were purchased from Wako Pure Chemicals. Fluoranthenes (Fluor), 2,3-benzofluorene (2,3BF), benz[a]anthracene (B[a]A), naphthalene (Nap), triphenylene (TPh), benzo[e]pyrene (B[e]P), perylene (Pery), and indeno[1,2,3-cd]pyrene (I[1,2,3-cd]P) were obtained from Kanto Chemicals. 2,3-Benzotriphenylene (2,3BTPh), pentacene (Pen), coronene (Cor), and naphtho[2,3-a]pyrene (N[2,3-a]P) were purchased from Tokyo Chemical Industry. Chrysene (Chry) was purchased from Aldrich Co.

The standard stock solution (100 μg/L) was prepared in DCM and stored in a refrigerator before use. PAH solutions were prepared by diluting the stock solution to the required concentrations.

Exhaust gas collected from a diesel car was processed using a fixed constant volume sampler (CVS) device, and diesel exhaust particles containing PAHs were sampled through a quartz filter using a high-volume air sampler. Road conditions for the diesel car were set using a chassis dynamometer. Using a dilution tunnel made of the stainless steel, 6.5 m in length and 0.6 m in internal diameter, diesel exhaust particles were sampled at a flow rate of 40–120 m³/min. Only a small fraction was sampled isokinetically and then allowed to pass through the filter. The particles adsorbed onto the filter were extracted using benzene: methanol = 3:1. The PAHs in this solution were separated from other organic compounds using a C₁₈ cartridge (Mega Bond Elute C18, VARIAN) with acetonitrile. The filtrate containing PAHs was concentrated to 1 mL using DCM solution.

2.3 Procedures

Twenty-four PAHs were obtained from commercial sources and each of them was dissolved in DCM. All solutions were subjected to spectrofluorometry for the optimization of excitation and measurement wavelengths. On the basis of the results, the conditions were determined whereby B[a]P and B[k]F could be detected separately from PAHs.

The new detection method was compared with high-performance liquid chromatography/fluorescence detector (HPLC/FLD) using diesel exhaust particles.

3. Results

3.1 Unique fluorescence spectra of B[a]P and B[k]F

The fluorescence spectra of B[a]P and B[k]F are shown in Fig. 1. When all PAH solutions were subjected to fluorescence spectroscopy, we found two excitation-emission wavelengths (370 nm-429 nm and 395 nm-429 nm), whereas only B[a]P and B[k]F gave high fluorescence intensities.

On the basis of fluorescence spectra, the other 22 PAHs were classified into the following three types in terms of these wavelengths. Type 1 PAHs did not exhibit excitation or emission at these wavelengths. Type 1 PAHs were Flu, Ph, Ace, Alene, 2,3BF and TPh. The spectrum of 2,3BF is shown in Fig. 2 as an example. Type 2 PAHs emitted fluorescence at a wavelength of 429 nm, but were not excited at wavelengths of 370 nm and 395 nm. Type 2 PAHs were Pyr, Ant, Chry, B[a]A, DB[a,h]A, Nap, 2,3BTPh, B[e]P, N[2,3-a]P and Cor.
The spectrum of Chry is shown in Fig. 3 as an example. Finally, type 3 PAHs exhibited excitation and emission at these wavelengths, but their fluorescence intensities were very low. Type 3 PAHs were Fluor, Pery, B[b]F and B[g,h,i]P. The spectrum of Fluor is shown in Fig. 4 as an example. Considering the concentrations of these PAHs in diesel exhaust particles, these types of PAH did not influence the measurement of B[a]P and B[k]F concentrations.

3.2 Dual-beam fluorometric detection method for B[a]P and B[k]F in PAH mixture

Figure 5 shows that B[a]P and B[k]F have two specific fluorescences with intensities up to 10-fold those of the other 22 PAH species. From the calibration curves obtained using these two wavelength ranges, the other 22 PAHs did not exhibit any fluorescence intensities (Fig. 6): A sum of the calibration curves of B[a]P and B[k]F is almost equal to the combined intensities of the 24 species of PAHs under these conditions. From these results, the concentrations of B[a]P and B[k]F using the method we developed was calculated from the...
following equations:

\[ I_{370} = I_{A\ 370} + I_{K\ 370} = 2.52 c_A + 1.30 c_K \] (1)
\[ I_{395} = I_{A\ 395} + I_{K\ 395} = 0.71 c_A + 1.55 c_K \] (2)

where \( I_{370}, I_{A\ 370}, I_{K\ 370}, I_{395}, I_{A\ 395}, \) and \( I_{K\ 395} \) are the intensities of the 24 species of PAHs, B[a]P and B[k]F for the 370 nm or 395 nm excitation wavelength, and \( c_A \) and \( c_K \) are the B[a]P and B[k]F concentrations. The detection limit of B[a]P was 1.2 \( \mu g/L \) (\( \alpha = 10 \)), and that of B[k]F was 1.4 \( \mu g/L \). The sensitivity of this monitoring device was satisfactory for determining B[a]P and B[k]F concentrations in diesel exhaust particles.

The method we developed was compared with HPLC/FLD in the evaluation of diesel exhaust particle samples containing PAHs (Table 1). The particles were emitted from the
exhaust of a diesel car for which the road conditions in Tokyo were simulated using a chassis dynamometer, and three running modes were used. The coefficients of variation were determined to be 5.93% for B[a]P and 7.58% for B[k]F by the method we developed, and 6.77% for B[a]P and 6.91% for B[k]F by the HPLC/FLD method (Sample A). It was reported by Shiozaki et al. that the coefficients of variation are 5.0% for B[a]P and 5.9% for B[k]F from an extract of airborne particles analyzed using HPLC/FLD.\textsuperscript{(6)} The values were in good agreement with our results. The sensitivity of this method was satisfactory for the determination of B[a]P and B[k]F concentrations in diesel exhaust particles.

B[a]P concentration measurement was completed in several minutes using this method because no separation process was required. In general, the B[a]P and B[k]F analysis time using GC/MS is about 1 h, and about 30 min using HPLC.
4. Discussion

The proposed fluorimetric method was successfully applied to the rapid and easy detection of B[a]P and B[k]F in diesel exhaust particles containing PAHs. Furthermore, B[a]P and B[k]F concentrations in diesel exhaust particles could be determined. The detection limit of B[a]P was 1.2 μg/L and that of B[k]F was 1.4 μg/L. This method facilitates miniaturization of the detection system. In this study, two particularly important compounds were chosen. However, if some other conditions are met, this method is likely applicable.
to other PAHs. Some experimental conditions are specific to target compounds, and contaminants cannot be quenched. Using a miniaturized monitoring device and the detection method we developed, the analysis time can be shorter than conventional detection methods for PAHs. Furthermore, this device requires the use of a lower load than HPLC and GC/MS devices. For example, HPLC analysis requires large quantities of organic eluting solution such as methanol, ethanol, and acetonitrile. However, if the measuring method we developed is applied to a flow injection system or a lab-on-a-chip system, this method might require organic solution at the μL level. In conclusion, pollutant analysis may help retard environmental pollution if this monitoring method is used.

Fig. 5. Relative fluorescence intensities of PAHs. PAHs were at a concentration of 100 mg/L (a) 370 nm excitation and 429 nm emission wavelengths (b) 395 nm excitation and 429 nm emission wavelengths.
Fig. 6. B[a]P, B[k]F and calibration curves of 24 species of PAHs. (a) 370 nm excitation wavelength and 429 nm emission wavelength (b) 395 nm excitation wavelength and 429 nm emission wavelength.

Table 1

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<th>Dual-beam fluorometric method (μg/L)</th>
<th>HPLC/FLD (μg/L)</th>
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<tr>
<td>Sample A</td>
<td>B[a]P 76.66</td>
<td>78.41</td>
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<tr>
<td></td>
<td>B[k]F 90.37</td>
<td>90.56</td>
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<td>Sample B</td>
<td>B[a]P 70.07</td>
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<tr>
<td></td>
<td>B[k]F 81.34</td>
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<tr>
<td>Sample C</td>
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<td>68.37</td>
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<td>B[k]F 102.78</td>
<td>95.80</td>
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n = 3
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References


