

# Development of HPLC Determination Method for Trace Levels of 1-, 2-Nitropyrenes and 2-Nitrofluoranthene in Airborne Particulates and Its Application to Samples Collected at Noto Peninsula

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## ABSTRACT

1-Nitropyrene (1-NP), 2-NP and 2-nitrofluoranthene (2-NFR) are useful markers for studying the atmospheric behaviors of polycyclic aromatic hydrocarbons (PAHs) and nitropolycyclic aromatic hydrocarbons (NPAHs). However, present methods for measuring trace levels of these compounds are less-sensitive and laborious. Here we describe several improvements to a previously reported high-performance liquid chromatography-chemiluminescence detection system that allows it to determine trace levels of 1-, 2-NPs and 2-NFR. The proposed system was equipped with a reducer column packed with Pt/Rh instead of zinc whose life-time was limited. The combination of Cosmosil MS-II (monomeric ODS) and AR-II (polymeric ODS) columns was used instead of polymeric ODS columns as the separator column to improve the separation. An ethanol mixture with acetate buffer (pH 5.5) was used in place of an acetonitrile mixture with the same buffer to activate the reducer column. The same ethanol mixture was used as the mobile phase for the clean-up column. The switching time of the column switching valve was optimized to concentrate the amino-derivatives of above NPAHs quantitatively on the concentrator column. The concentrations of bis(2,4,6-trichlorophenyl) oxalate and hydrogen peroxide in the chemiluminescence reagent solution were optimized to 0.4 mM and 30 mM, respectively, to increase the sensitivity. Under the above conditions, the detection limits ( $S/N=3$ ) of 1-, 2-NPs and 2-NFR were 1 fmol (0.25 pg), 10 fmol (2.5 pg) and 4 fmol (1 pg), respectively. The proposed system was effectively used to determine trace levels of 1-, 2-NPs and 2-NFR in airborne particulates collected at Noto Peninsula. The atmospheric concentrations of 1-, 2-NPs and 2-NFR were not more than sub  $\mu\text{g m}^{-3}$  levels. They were higher in

winter (January) than in summer (July). In both seasons, the concentrations were in decreasing order,  $[2\text{-NFR}] > [1\text{-NP}] > [2\text{-NP}]$ .

**Key words:** 1-Nitropyrene, 2-Nitropyrene, 2-Nitrofluoranthene, Trace analysis, Airborne particulate matter

## 1. INTRODUCTION

Among the air pollutants exhausted from the combustion of fossil fuels such as petroleum and coal, polycyclic aromatic hydrocarbons (PAHs) show carcinogenicity and/or mutagenicity (Tokiwa *et al.*, 1980), endocrine disrupting activity (Kizu *et al.*, 2000) or reactive oxygen species producing activity (Motoyama *et al.*, 2009). Nitropolycyclic aromatic hydrocarbons (NPAHs) such as 1-nitropyrene (1-NP) and 1,3-dinitropyrene are also exhausted from the combustion of fossil fuels, and their mutagenicity was much stronger than that of benzo[*a*]pyrene. Additionally, several NPAHs such as 2-NP and 2-nitrofluoranthene (2-NFR) are subsequently formed by the reaction of PAHs and NO<sub>x</sub> in the air (Arey *et al.*, 1986), suggesting that 1-, 2-NPs and 2-NFR are markers for studying the atmospheric behaviors of PAHs and NPAHs. Therefore, a method for simultaneously determining these three NPAHs would be useful. The authors developed a highly sensitive determination method for NPAHs in environmental samples such as airborne particulates and particulates exhausted from automobiles by using high-performance liquid chromatography (HPLC) with chemiluminescence detection (CLD) (Hayakawa *et al.*, 1995, 1991; Imaizumi *et al.*, 1990). Although NPAH concentrations in urban air were high enough to be determined by HPLC-CLD, the concentrations were much lower in the air at suburban and background sites that were not

near any contributors such as factories or high-traffic roads. We collected one-week airborne particulates with a high-volume air sampler at Kanazawa University Wajima Air Monitoring Station in the Noto Peninsula, Japan, which was a background monitoring site. The data showed a seasonal variation of PAH concentrations caused by long-range transport from China (Yang *et al.*, 2007). However, no NPAH were detected.

We previously determined trace levels of 1-, 2- and 4-NPs, 2-NFR and 6-nitrochrysene (6-NC) in airborne particulates and precipitation collected in urban area by introducing reducer and concentrator columns into our conventional HPLC-CLD system (Murahashi and Hayakawa, 1997). However, the life time of the reducer column packed with zinc was limited and the analysis time took more than 90 minutes since three sample injections were necessary to analyze the NPAHs. Recently, we developed an HPLC-CLD system for the simultaneous determination of more than 20 NPAHs by introducing the reducer column packed with Pt/Rh instead of zinc, since Pt/Rh catalyzed the reduction of NPAHs. In the system, an ethanol-aqueous solution containing ascorbic acid was used to activate the reducer column instead of an acetonitrile-aqueous solution (Tang *et al.*, 2005, 2003; Hayakawa *et al.*, 2001). However, the resolution and sensitivity were not high enough to detect trace levels of above NPAHs.

In this report, we improved an HPLC-CLD system with lower detection limits and higher resolutions of 1-, 2-NPs and 2-NFR by improving our previous system (Murahashi and Hayakawa, 1997). The proposed method was used for determining trace levels of 1-, 2-NPs and 2-NFR in airborne particulate samples collected in winter and summer at Noto Peninsula.

## 2. MATERIALS AND METHODS

### 2.1 Chemicals

1-, 4-NPs and 2-NFR were purchased from Aldrich Company Inc. (Milwaukee, WI, U. S. A.). 2-NP was kindly supplied by Professor Akihisa Hirayama, Kyoto Pharmaceutical University. Deuterated 1-NP (1-NP- $d_9$ ) was purchased from CDN Isotopes Inc. (Pointe-Claire, Quebec, Canada). All other chemicals used were of analytical reagent grade.

### 2.2 HPLC System and Conditions

Three on-line reducers, including an electrochemical reactor and columns packed with metals have been reported for reducing NPAHs to their corresponding amino-derivatives (Hayakawa *et al.*, 2001, 1993; Imaizumi *et al.*, 1990). The electrochemical reducer showed the lowest reduction efficiency and the other two showed quantitative reduction efficiency. The life time of

the zinc reducer column was limited, while the life time of the Pt/Rh reducer column was not (Hayakawa *et al.*, 2001, 1993). Considering these advantages of the Pt/Rh reducer column, we set up the HPLC-CLD system with a Pt/Rh reducer column according to our previous report (Tang *et al.*, 2003) with several modifications (Fig. 1). The system consisted of four HPLC mobile phase pumps, a chemiluminescence reagent solution pump, a chemiluminescence detector, a system controller, an integrator, a degasser, two column ovens for the reducer column (80°C) and for the clean-up and separator columns (20°C), an auto sample injector (100  $\mu$ L), a switching valve, a guard column, a concentrator column, a clean-up column, a reducer column (4.0 i.d.  $\times$  10 mm, packed with Pt/Rh, Shimadzu) and two separator columns (Cosmosil 5C18-MS-II, 4.6 i.d.  $\times$  150 mm, Nacalai Tesque and 5C18-AR-II, 4.6 i.d.  $\times$  250 mm, Nacalai Tesque) connected in series.

The mobile phases for the clean-up column and reducer column was ethanol-acetate buffer (pH 5.5) (3:1, v/v) at a flow rate of 0.3 mL min<sup>-1</sup>. The mobile phase for the concentrator column was the same ethanol-acetate buffer with 30 mM ascorbic acid at a flow rate of 1.8 mL min<sup>-1</sup>. The mobile phase for the separator columns was a mixture of acetonitrile and 10 mM imidazole buffer (pH 7.6) (1:1, v/v) at a flow rate of 1.0 mL min<sup>-1</sup>. The chemiluminescence reagent solutions were acetonitrile solutions containing 0.4 mM bis(2,4,6-trichlorophenyl)oxalate (TCPO) and 30 mM hydrogen peroxide, respectively, each cooled with ice-water. Both solutions were mixed (1:1, v/v) in the system, and the flow rate of the mixture was 1 mL min<sup>-1</sup>. Other conditions were the same as in our previous reports (Tang *et al.*, 2005, 2003).

### 2.3 Preparation of Standard Solutions

Stock standard solutions of 1-, 2-, 4-NPs, 2-NFR and 1-NP- $d_9$  were independently prepared by dissolving each crystal in ethanol. The stock standard solutions were mixed, and then the mixture was diluted adequately with acetonitrile-water (3:1, v/v) to give calibration curve solutions: 0.1-100 pmol L<sup>-1</sup> for 2-NFR; 0.4-400 pmol L<sup>-1</sup> for 2-NP; 0.05-50 pmol L<sup>-1</sup> for 1-NP. 1-NP- $d_9$  standard solution was at the concentration of 0.2 pmol L<sup>-1</sup> in ethanol.

### 2.4 Sampling and Sample Preparations

Airborne particulate samples were collected at Kanazawa University Wajima Air Monitoring Station at Noto Peninsula (Nisifutamata-machi, Wajima City, Ishikawa Prefecture, Japan). Airborne particulates were collected by a high volume air sampler (AH-600, Shibata Japan) with a quartz fiber filter (8  $\times$  10 inch, 2500QAT-UP, Pallflex Products, Putnam, CT, U. S. A.) at a flow rate of 0.7 m<sup>3</sup> min<sup>-1</sup> in January and July, 2007.

The filter was newly changed every week. Airborne particulates were collected from January 4 to 11, 2008, too. One-fourth of the filter (corresponding to  $3.53 \times 10^3 \text{ m}^3$  air) was cut into small pieces in a flask and 100  $\mu\text{L}$  of 1-NP- $d_9$  standard solution was added to the flask as an internal standard. Then, NPAHs were extracted ultrasonically twice with benzene/ethanol (3:1, v/v). The solution was washed successively with sodium hydroxide, sulfuric acid solution and water. After filtering the organic solution with an HLC-DISK membrane (pore size 0.45  $\mu\text{m}$ , Kanto Chemical Co., Tokyo, Japan), the solution was evaporated to dryness. The residue was dissolved in 1.0 mL of ethanol, and then an aliquot (100  $\mu\text{L}$ ) of the solution was injected into the HPLC-CLD system.

### 3. RESULTS AND DISCUSSION

#### 3.1 Comparison of Separator Columns

1-NP is one of the major NPAHs exhausted directly from combustion processes of fossil fuel, and both 2-NFR and 2-NP are major NPAHs which are secondarily formed in the air (Arey *et al.*, 1986). These facts

suggest that the simultaneous determination of 1-, 2-NPs and 2-NFR is useful for determining their atmospheric behaviors. In our previous HPLC-CLD system using the reducer column packed with Pt/Rh, however, the retention time of 2-NFR was close to that of 2-NP (Tang *et al.*, 2005). Moreover, the retention time of 4-NP was similar to the retention times of 2-NFR and 2-NP, and the concentration of 4-NP was much lower (Murahashi *et al.*, 1999). These observations suggest that the resolution of the NPAHs should be improved, resulting in more accurate determination.

Polymeric ODS columns are known to give better resolution of PAHs than monomeric ODS columns. However, the difference of those two-type ODS columns in the separation of amino-derivatives of PAHs (APAHs) has not been examined. We compared the retentions of amino-derivatives of 1-, 2-, 4-NPs (1-, 2-, 4-aminopyrenes, 1-, 2-, 4-APs) and 2-NFR (2-amino-fluoranthene, 2-AFR) on Cosmosil MS-II (monomeric ODS) and AR-II (polymeric ODS) columns by introducing them into the HPLC-CLD system as shown in Fig. 1 without the two guard columns and the clean-up column. A mixture of acetonitrile and aqueous buffer is suitable for the peroxyoxalate chemiluminescence

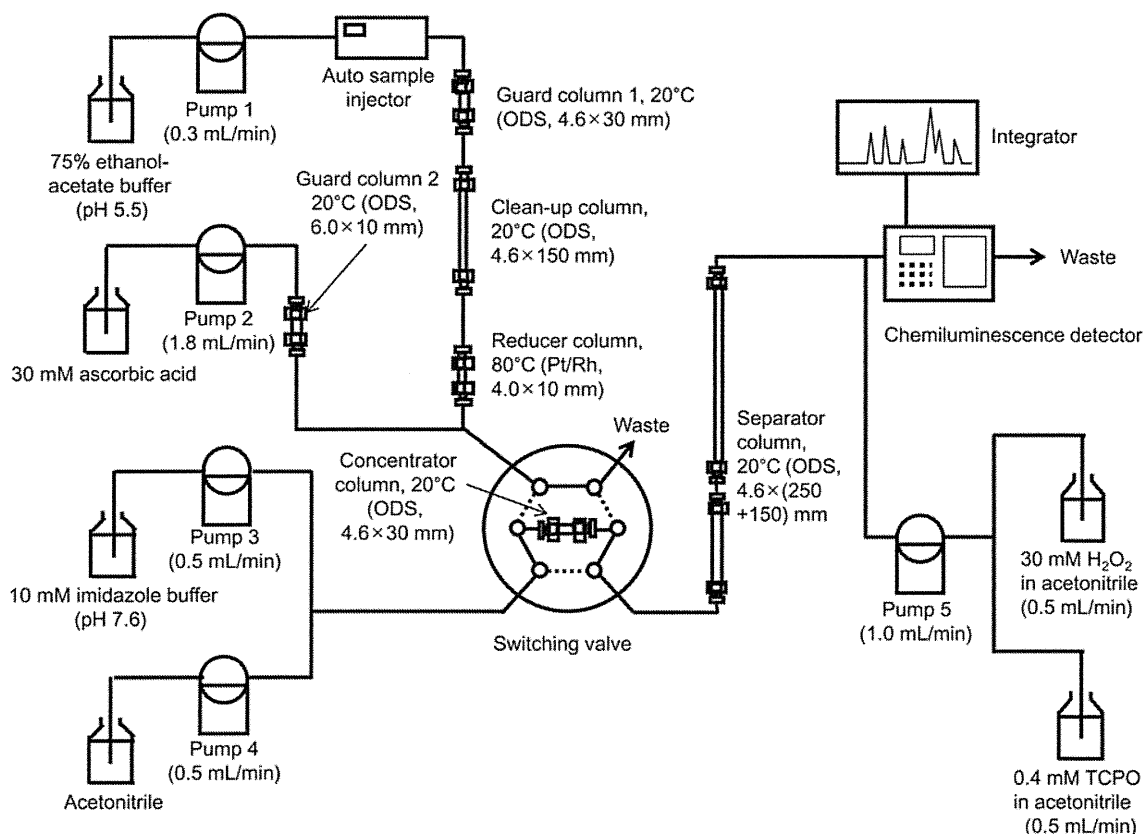


Fig. 1. Schematic diagram of the proposed HPLC-CLD system.

**Table 1.** Retention times (minute) of 1-, 2-, 4-NPs and 2-NFR<sup>1)</sup>.

Compound	MS-II (400 mm) <sup>2)</sup>	AR-II (400 mm) <sup>2)</sup>	MS-II (150 mm)+AR-II (250 mm) <sup>2)</sup>
2-NFR	48.5	54.5	52.5
2-NP	48.5	58	54.5
4-NP	51	58	57
1-NP	55.5	63.5	60.5

<sup>1)</sup>1-, 2-, 4-NPs and 2-NFR were reduced to their corresponding amino-derivatives in the reducer column and then introduced into the separator columns.

<sup>2)</sup>Inner diameter of the columns was 4.6 mm.

detection of amino-derivatives of PAHs (Hayakawa *et al.*, 2001, 1995). However, the reduction activity of Pt/Rh occurs in hot ethanol but not in acetonitrile, suggesting that the carrier solution going into the reducer column must contain ethanol but not acetonitrile. Therefore, a mixture of ethanol and acetate buffer (pH 5.5) was used as the mobile phase for the reducer column. On the other hand, amino-derivatives of PAHs were unstable in the system. Ascorbic acid was added to the effluent from the reducer column to keep the amino-derivatives stable in our previous HPLC-CLD system (Murahashi and Hayakawa, 1997). Thus, ascorbic acid was added to the mobile phase for the concentrator column in the proposed system as described in Experimental.

When NPAH standard solutions were injected into the system, NPAHs were all reduced quantitatively to their corresponding amino-derivatives in the reducer column. However, the separation of 2-AFR and 2-AP was impossible on a Cosmosil MS-II column, although the separation of 1- and 2-APs was complete. On the other hand, a Cosmosil AR-II column completely separated 2-AFR and 2-AP but did not completely separate 2- and 4-APs. By using a combination of Cosmosil MS-II and AR-II columns, the four APAHs were separately determined as amino derivatives (Table 1). The mean concentration ratio of 4-NP ( $0.57 \text{ fmol m}^{-3}$ ) was around 1/100 or 1/6 of those of 2-NFR and 2-NP, respectively, in the urban air of Kanazawa (Murahashi and Hayakawa, 1997), suggesting that 4-NP is not a good primary or secondary formation marker such as 1-, 2-NPs and 2-NFR. Accordingly, 1-, 2-NPs and 2-NFR were selected as target compounds in the following experiments and the combination of Cosmosil MS-II column (4.6 i.d.  $\times$  150 mm)+Cosmosil AR-II column (4.6 i.d.  $\times$  250 mm) was used for the following experiments.

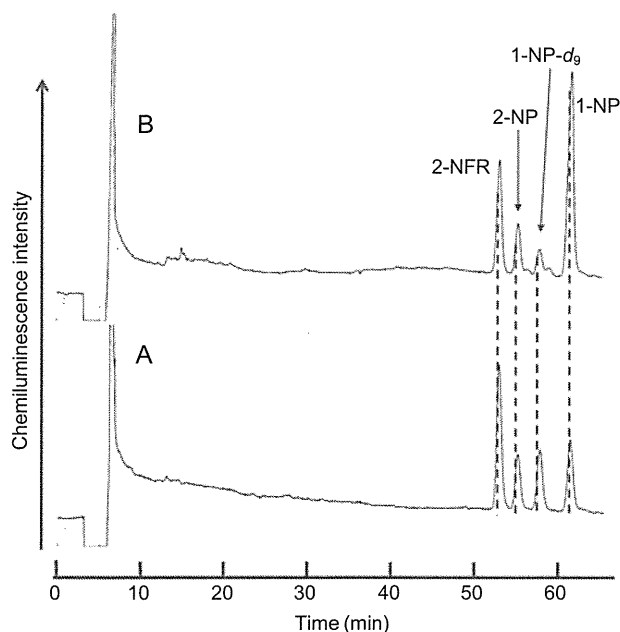
### 3.2 Optimum Composition of Chemiluminescence Reagent Solution

Next, we examined the optimal time for switching the switching valve after loading APAHs on the concentrator column of the HPLC-CLD system as shown in Fig. 1. When the standard mixture of 1-, 2-NPs, 2-

NFR and 1-NP-*d*<sub>9</sub> was injected into the system, they were eluted from the reducer column in the period from 17 to 29 minutes with the following elution order; 1-NP-*d*<sub>9</sub>=1-NP < 2-NFR < 2-NP. From this result, the concentration time (switching time of the switching valve) was set at 17-29 minutes.

The optimum composition of chemiluminescence reagent solution is known to be different for different analytes, and the chemiluminescence intensity is known to be affected by the concentration of organic solvent in the mobile phase (Hayakawa *et al.*, 1991). In view of the fact that the optimum acetonitrile concentration in the mobile phase was 50% for the elution and separation of 1-, 2-APs and 2-AFR, we examined the optimum concentrations of TCPO and hydrogen peroxide in the chemiluminescence reagent solution. The signal to noise (S/N) ratio of 1-AP increased with increasing TCPO concentration at concentrations above 0.02 mM and peaked at 0.15 mM TCPO. Furthermore, the S/N ratios of 2-AFR and 2-AP increased with increasing TCPO concentration, peaking at 0.4 mM TCPO. The S/N ratios of 2-AFR and 1-AP increased with increasing hydrogen peroxide concentration at concentrations above 4 mM and peaked at 15 mM hydrogen peroxide. On the other hand, the S/N ratios of 2-AP increased with increasing hydrogen peroxide concentration, peaking at 30 mM hydrogen peroxide. In view of the fact that the concentration of 2-NP in airborne particulates is usually lower than the concentrations of 2-NFR and 1-NP, the concentrations of chemiluminescence reagents should be optimum to obtain the highest sensitivity of 2-AP. Thus, we used 0.4 mM TCPO and 30 mM hydrogen peroxide in the following experiments.

Under the above conditions, the detection limits (S/N = 3) of 2-NFR, 2-NP and 1-NP by the proposed HPLC-CLD system were 4, 10 and 1 fmol, respectively. These values were better than the values obtained by the previous system. For example, the previous detection limit of 1-NP was 10 fmol (Tang *et al.*, 2005). The calibration curves were straight in the ranges from 10 fmol to 10 pmol (2-NFR), from 50 fmol to 50 pmol (2-NP) and from 5 fmol to 10 pmol (1-NP), respectively, with correlation coefficients over 0.999 and RSDs less than 5%.



**Fig. 2.** Typical chromatograms of (A) standard mixture of 1-, 2-NPs, 2-NFR and 1-NP- $d_9$  and (B) extracts from airborne particulates collected at the Noto Peninsula. (A) Airborne particulates were collected by a high-volume air sampler at a flow rate of  $1,500 \text{ L min}^{-1}$  from January 4 to 11, 2008. One-fourth of the filter was used. (B) Injected amounts of 1-, 2-NPs, 2-NFR and 1-NP- $d_9$  were 5, 50, 50 and 5 fmol, respectively.

### 3.3 Determination of 1-, 2-NPs and 2-NFR Extracted from Airborne Particulates

Fig. 2 shows typical chromatograms of (A) the standard mixture of 1-, 2-NPs and 2-NFR and (B) the extracts from airborne particulates collected at Kanazawa University Wajima Air Monitoring Station on the Noto Peninsula, Japan from January 4 to 11, 2008. 2-NFR, 2-NP and 1-NP were chemiluminescently determined as their corresponding amino-derivatives in chromatogram B. This result suggests that the proposed HPLC-CLD system is suitable for the determination of NPAHs in air samples collected at background monitoring sites. The calculated concentrations of 1-NP, 2-NP and 2-NFR were  $1.8 \text{ fmol (450 fg) m}^{-3}$ ,  $0.65 \text{ fmol (160 fg) m}^{-3}$  and  $6.0 \text{ fmol (1,500 fg) m}^{-3}$ , respectively. The stable baseline of chromatogram A suggests that the proposed system can determine these NPAHs at even much lower concentration.

### 3.4 Atmospheric Concentrations of 1- and 2-NPs and 2-NFR at Noto Peninsula

1-, 2-NPs and 2-NFR in airborne particulates collected in January and July, 2007 at Noto Peninsula were determined by the proposed method. The atmospheric concentrations were not more than sub  $\text{pg m}^{-3}$  levels.

**Table 2.** Atmospheric concentrations ( $\text{fg m}^{-3}$ )<sup>1)</sup> of 1-, 2-NPs and 2-NFR at Noto Peninsula.

Month <sup>2)</sup>	1-NP	2-NP	2-NFR
January	$840 \pm 280$	$230 \pm 100$	$1900 \pm 520$
July	$80 \pm 33$	$56 \pm 31$	$510 \pm 290$

<sup>1)</sup>Each data means mean  $\pm$  SD of four different week samples.

<sup>2)</sup>Samplings were performed in January and July, 2007. The filter was newly changed every week.

They were compared in Table 2. The concentrations of 1-, 2-NPs and 2-NFR were higher in winter (January) than in summer (July) by the factors of 1/3.7, 1/10.5 and 1/4.1, respectively. In both seasons, the concentrations were in decreasing order,  $[2\text{-NFR}] > [1\text{-NP}] > [2\text{-NP}]$ . It has been reported that PAHs exhausted from coal-burning systems especially in winter season in North-East China were long-range transported to Noto Peninsula over the Japan Sea (Yang *et al.*, 2007). The concentration level of 1-NP at Noto Peninsula was much lower than that at Shenyang (annual average concentration  $107 \pm 81 \text{ pg m}^{-3}$  (Hattori *et al.*, 2007)) by the factor of 3 or 4 orders of magnitude. This is the first report that the atmospheric concentrations of 1-, 2-NPs and 2-NFR at Noto Peninsula were determined. The proposed method is useful to know the behaviors of trace levels of NPAHs at background monitoring sites such as Noto Peninsula. The long-range transport of NPAHs from Asian Continent to Japan clarified by the proposed method will be reported elsewhere.

## 4. CONCLUSIONS

A HPLC-CLD system has been developed for the determination of trace levels of 1-, 2-NPs and 2-NFR in airborne particulates by modifying our previous system. The new system uses a reducer column packed with Pt/Rh, a chemiluminescence detector and a combination of monomeric and polymeric ODS columns for the separation column. The switching time of the switching valve and the concentrations of TCPO and hydrogen peroxide in the chemiluminescence reagent solution were optimized. The detection limits ( $S/N=3$ ) of 1-, 2-NPs and 2-NFR by the proposed system were 1, 10 and 4 fmol, respectively. The proposed system determined trace levels of 1-, 2-NPs and 2-NFR in airborne particulates collected at Noto Peninsula. The atmospheric concentrations were not more than  $\text{pg m}^{-3}$  levels.

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## — 原 著 —

## 金沢市内における大気粉塵中多環芳香族炭化水素類および

## ニトロ多環芳香族炭化水素類の最近 12 年間の変遷

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in airborne particulates collected in urban Kanazawa, Japan, in last 12 yearsHirotaka Hama<sup>1\*</sup>, Takahiro Tokuda<sup>2</sup>, Akihiko Izaki<sup>2</sup>, Tomoko Ohno<sup>1</sup>, Yuri Watanabe<sup>1</sup>,  
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Airborne particulates were collected at a roadside site in Kanazawa, Japan, in the summer and winter, 1999, 2004/2005, 2007/2008 and 2010. The polycyclic aromatic hydrocarbons (PAHs) and nitropolycyclic aromatic hydrocarbons (NPAHs) were determined by HPLC with fluorescence and chemiluminescence detections, respectively. The total concentration of six PAHs (pyrene, benz[*a*]anthracene, chrysene, benzo[*b*]fluoranthene, benzo[*k*]fluoranthene and benzo[*a*]pyrene) decreased by the factor of 63.9% in the winter and 75.6% in the summer from 1999 to 2010. The total concentration of seven NPAHs (1,3-, 1,6-, 1,8-dinitropyrenes, 1-nitropyrene, 6-nitrochrysene, 7-nitrobenz[*a*]anthracene and 6-nitrobenzo[*a*]pyrene) significantly decreased by the factor of 88.0% in the winter and 89.2% in the summer during the same period. The concentration ratio of 1-nitropyrene to pyrene also significantly decreased in this period, suggesting a decrease in the emission amount. As the main reason for the significant decrease in the PAHs and NPAHs, the regulation of exhaust gas/particulates from automobiles was considered.

**Key words :** Polycyclic aromatic hydrocarbon, nitropolycyclic aromatic hydrocarbon, urban air, Kanazawa, automobile

## 1. はじめに

大気中に存在する様々な化学物質の中に、多環芳香族炭化水素 (polycyclic aromatic hydrocarbon; PAH) とニトロ多環芳香族炭化水素 (nitropolycyclic aromatic hydrocarbon; NPAH) がある。これらの中には強い発がん性、変異原性をもつものがある (国際がん研究機関 (IARC), 2010)。IARC でグループ 1 に分類されている benzo[*a*]pyrene (BaP) に関しては、これまでその活性代謝物が DNA に結合することにより、発がん性を示すと考えられていたが、最近では、そのフリーラジカル生成能による発がん性も疑われている (Kim et al., 1997)。また、1,3-, 1,6-, 1,8-dinitropyrenes (DNPs) や 1-nitropyrene (1-NP) は強い直接変異原性をもつ (Ames et al., 1975; Epstein et al., 1979)。したがって、これら PAH、NPAH の大気中濃度を測定し、監視することは極めて重要である。

PAH、NPAH は主に石炭、石油等の化石燃料の燃焼によって生じる。我が国の都市大気中 PAH、NPAH の主な発生源として自動車、工場、焼却炉、石油暖房器具などが考えられている。NPAH には一次生成するものと二次生成するものがあり、一次生成する NPAH としては 1,3-, 1,6-, 1,8-DNPs、1-NP、6-nitrochrysene (6-NC)、7-nitrobenz[*a*]anthracene (7-NBaA)、6-nitrobenzo[*a*]pyrene (6-NBaP) などがある。これらは、PAH が NO<sub>2</sub> の存在下で求電子反応を受けることで生成し (Nielson et al., 1984)、我が国の都市域における主な発生源はこれまでディーゼル車であることが明らかにされている (Hayakawa et al., 1994; Murahashi et al., 1995)。一方、二次生成する NPAH としては 2-nitropyrene (2-NP) や 2-nitrofluoranthene (2-NFR) が知られており、これらは OH ラジカルとの反応が引き金となって大気中で生成する (Pitts et al., 1985)。

大気中に存在するこれら PAH、NPAH は蒸気圧や気温によ

って、ガス相または粒子相に存在する。通常の気温であれば、2,3環のPAHはガス相、5環以上のPAHは粒子相に存在し、4環PAHは温度が下がるほど粒子相への分布割合が増す(Yamasaki et al., 1982)。一方、DNPs、1-NPは4環ではあるが、そのほとんどが粒子相に存在する(Araki et al., 1983)。

著者が所属する研究室では、金沢市の市街地幹線道路脇にある自動車排ガス測定局において大気粉塵のサンプリングを1999年、2004年度、2007年度および2010年の夏と冬に行い、PAHおよびNPAHの濃度測定を行ってきた。大都市地域では自動車NO<sub>x</sub>・PM法により窒素酸化物(NO<sub>x</sub>)や粒子状物質(PM)の濃度が下がり、大気質の改善がみられるようになってきた(環境省, 2011)。また、我が国では新車に対する規制が段階的に強化されてきた(国土交通省, 2011)。しかし、こうした対策が我が国の都市沿道大気中のPAH、NPAH濃度どのように影響を及ぼしたかは、十分な解析が行われていない。そこで、本研究では上記期間中に捕集した沿道大気粉塵中のPAH、NPAH濃度を測定し、その変遷と要因を明らかにした。

## 2. 実験

### 2.1 大気粉塵のサンプリング

大気粉塵のサンプリング地点は幹線道路(国道8号)沿いでその上を北陸自動車道が通る金沢市藤江自動車排ガス測定局で行った。捕集期間は1999年1月26日~30日、2月1日の毎日(n=6)、1999年7月26日~27日、7月29日~8月1日の毎日(n=6)、2004年8月2日~8日、8月11日~8月15日の毎日(n=12)、2005年2月7日~20日の毎日(n=14)、2007年7月24日~8月5日の隔日1日(n=7)、2008年1月21日~2月3日の隔日(n=7)、2010年2月16日~2月22日の毎日(n=7)、2010年8月2日、4~8日の毎日および8月10日(n=7)である。以下の記載では、1~2月の捕集日は冬、7~8月の捕集日は夏にグループ化した。大気粉塵はハイボリュームエアサンプラー(123VL型、紀本電子工業株式会社)を用い、1,000 L min<sup>-1</sup>の流速で石英繊維フィルター(2500QAT-UP、8×10 inch、PALLFLEX)上に24 hr捕集した。フィルターは毎日交換した。

### 2.2 試薬

米国環境保護庁610 PAH Mixおよび1,3-,1,6-DNPs、1-NP、2-fluoro-7-nitrofluorene (FNF)はシグマアルドリッチジャパン(大阪)から購入した。Pyrene (Pyr)及びBaPの重水素化体であるPyr-d<sub>10</sub>及びBaP-d<sub>12</sub>は和光純薬工業株式会社(大阪)から購入した。1,8-DNP、6-NC、7-NBaA、6-NBaPはChrion AS(トロンハイム、ノルウェー)から購入した。

### 2.3 捕集フィルターの処理

フィルターの前処理は著者らの研究グループの既報(Hayakawa et al., 2011)に従った。大気粉塵を捕集したフィルターは細切して三角フラスコにいれ、回収率補正ならびに

定量のために内標準物質としてPAH分析にはPyr-d<sub>10</sub>、BaP-d<sub>12</sub>、NPAH分析にはFNFを加えた。さらにベンゼン/エタノール(3:1, v/v) 40 mLを加えて超音波処理を行い、得られた抽出液を5%水酸化ナトリウム水溶液80 mL、20%硫酸80 mLでそれぞれ1回ずつ洗浄し、最後に超純水80 mLで2回洗浄した。有機溶媒相にジメチルスルホキシドを100 μL加えて減圧し、ベンゼン層を留去した。残渣にエタノールを900 μL加え、メンブランフィルター(HLC-DISK3、直径3 mm、孔径0.45 μm、関東化学、東京)でろ過して、HPLC分析用の検液とした。

### 2.4 PAHおよびNPAHの分析

PAHはHPLC-蛍光検出法により分析を行った。分析システムは島津製作所製の2台のポンプ(LC-10AD)、オートインジェクター(SIL-10A)、デガッサー(DGU-14A)、カラムオープン(CTO-10AS VP)、蛍光検出器(RF-10A XL)、システムコントローラー(SCL-10A)およびインテグレータ(CR-7Ae plus)で構成した。これに、ガードカラム(4.0 i.d. × 10 mm、5 μm、Inertsil ODS-P、ジーエルサイエンス)および分離カラム(4.6 i.d. × 250 mm、5 μm、Inertsil ODS-P、ジーエルサイエンス)を導入した。移動相はアセトニトリルと水の混液を用い、流速1 mL min<sup>-1</sup>で、濃度グラジェント方式で流した。蛍光検出器は各PAHの最適励起波長と蛍光波長に設定し、タイムプログラムを用いてPAHを測定した。その他の条件は著者らの研究グループの既報(Toriba et al., 2003)に従った。Pyr、benz[a]anthracene (BaA)、chrysene (Chr)、benzo[b]fluoranthene (BbF)、benzo[k]fluoranthene (BkF)、BaPの回収率は91~106%、繰り返し精度(RSD、n=5)は0.31~1.9%、検出限界値(S/N=3)は7.9~43 fmol/注入であった。

NPAHは、著者らの研究グループが開発したHPLC-化学発光検出法により分析を行った(Hayakawa et al., 1991; Tang et al., 2003)。分析システムはいずれも島津製作所製の5台のポンプ(LC-20AD)、オートインジェクター(SIL-20A)、デガッサー(DGU-20A<sub>s</sub>)、2台のカラムオープン(還元カラム用; CTO-10A、分離カラム用; CTO-20AC)、化学発光検出器(CLD-10A)、システムコントローラー(CBM-20A)、インテグレータ(LCsolution)で構成した。更にガードカラム1(4.6 i.d. × 30 mm、5 μm、Spheri-5 RP-18、PerkinElmer)、ガードカラム2(6.0 i.d. × 10 mm、5 μm、ODP-50G 6A Shodex Asahipak、昭和電工)、クリーンアップカラム(4.6 i.d. × 150 mm、5 μm、Cosmosil 5C18-MS-II、ナカライテスク)、Pt/Rh還元カラム(4.0 i.d. × 10 mm、ニトロアレーン還元カラム、島津製作所)、濃縮カラム(4.6 i.d. × 30 mm、5 μm、Spheri-5 RP-18、PerkinElmer)、分離カラム(4.6 i.d. × (250+150) mm、5 μm、Cosmosil 5C18-MS-II、ナカライテスク)を用いた。クリーンアップカラムとPt/Rh還元カラム用の移動相として、75%エタノール-酢酸緩衝液(pH 5.5)を流速0.3 mL min<sup>-1</sup>で流した。濃縮カラム用移動相として、30 mMアスコルビン酸溶液を流速1.8 mL min<sup>-1</sup>で流し、クリーンアップカラム溶出



Table 1 Variation of PAH and NPAH concentrations in Kanazawa (Fujie).

Compound	1999				2004/2005			
	summer(n = 6)		winter(n = 6)		summer(n = 12)		winter(n = 14)	
	mean	SD	mean	SD	mean	SD	mean	SD
TSP	33.6 ± 2.3		49.7 ± 30.6		31.4 ± 8.6		30.2 ± 13.2	
PAHs								
Pyr	1.22 ± 0.26		2.20 ± 0.74		0.804 ± 0.385		0.927 ± 0.630	
BaA	0.701 ± 0.154		1.77 ± 0.69		0.449 ± 0.218		0.753 ± 0.344	
Chr	0.957 ± 0.188		2.74 ± 0.81		0.733 ± 0.324		1.17 ± 0.47	
BbF	1.64 ± 0.28		2.72 ± 0.82		1.55 ± 0.94		1.01 ± 0.41	
BkF	0.590 ± 0.118		1.16 ± 0.36		0.833 ± 0.617		0.540 ± 0.226	
BaP	1.12 ± 0.29		1.65 ± 0.70		0.562 ± 0.143		0.974 ± 0.520	
Total	6.24 ± 0.97		12.2 ± 3.95		4.93 ± 1.60		5.37 ± 2.30	
NPAHs								
1,6-DNP	2.04 ± 1.05		3.20 ± 0.60		0.748 ± 0.559		0.669 ± 0.372	
1,8-DNP	2.41 ± 1.47		2.20 ± 0.68		3.19 ± 2.09		1.81 ± 2.37	
1,3-DNP	1.41 ± 0.57		4.53 ± 1.41		0.602 ± 0.311		0.552 ± 0.220	
1-NP	176 ± 44		424 ± 186		101 ± 52		101 ± 44	
6-NC	93.3 ± 15.4		55.3 ± 16.5		41.0 ± 20.5		29.3 ± 12.1	
7-NBaA	9.10 ± 1.89		20.2 ± 10.8		0.900 ± 0.994		6.57 ± 7.10	
6-NBaP	47.5 ± 15.5		43.7 ± 18.0		5.50 ± 2.30		12.1 ± 4.8	
Total	331 ± 61		553 ± 172		153 ± 76		152 ± 59	

Compound	2007/2008				2010			
	summer(n = 7)		winter(n = 7)		summer(n = 7)		winter(n = 7)	
	mean	SD	mean	SD	mean	SD	mean	SD
TSP	39.1 ± 13.9		24.0 ± 8.20		29.5 ± 5.1		27.6 ± 6.1	
PAHs								
Pyr	0.526 ± 0.122		1.66 ± 0.53		0.395 ± 0.093		1.51 ± 0.54	
BaA	0.255 ± 0.064		0.835 ± 0.125		0.167 ± 0.049		0.431 ± 0.152	
Chr	0.400 ± 0.095		1.51 ± 0.18		0.306 ± 0.084		1.02 ± 0.19	
BbF	0.417 ± 0.108		1.33 ± 0.20		0.309 ± 0.052		0.785 ± 0.173	
BkF	0.160 ± 0.036		0.594 ± 0.089		0.126 ± 0.024		0.305 ± 0.064	
BaP	0.243 ± 0.044		0.673 ± 0.089		0.220 ± 0.054		0.363 ± 0.114	
Total	2.00 ± 0.38		6.60 ± 0.76		1.52 ± 0.32		4.41 ± 1.13	
NPAHs								
1,6-DNP	0.200 ± 0.140		0.478 ± 0.128		0.815 ± 0.918		0.234 ± 0.102	
1,8-DNP	0.248 ± 0.175		0.256 ± 0.071		0.577 ± 0.362		0.586 ± 0.209	
1,3-DNP	0.283 ± 0.168		0.253 ± 0.063		0.482 ± 0.264		0.247 ± 0.066	
1-NP	41.5 ± 14.7		51.1 ± 9.1		15.3 ± 5.2		43.3 ± 15.1	
6-NC	16.7 ± 5.7		21.0 ± 3.4		14.9 ± 4.3		15.8 ± 8.4	
7-NBaA	3.04 ± 1.97		17.3 ± 11.2		1.99 ± 4.57		3.23 ± 4.99	
6-NBaP	2.48 ± 0.51		4.29 ± 1.04		1.83 ± 1.12		2.91 ± 1.27	
Total	64.5 ± 22.2		94.7 ± 15.8		35.8 ± 10.2		66.3 ± 25.0	

Units:  $\mu\text{g m}^{-3}$  (TSP),  $\text{pmol m}^{-3}$  (PAH),  $\text{fmol m}^{-3}$  (NPAH)

PAH Total = Pyr + BaA + Chr + BbF + BkF + BaP

NPAH Total = DNPs + 1-NP + 6-NC + 7-NBaA + 6-NBaP

液と混合した。分離カラム用移動相として、アセトニトリルと 10 mM イミダゾール緩衝液 (pH 7.6) をそれぞれ流速 0.5 mL  $\text{min}^{-1}$  で混合して流した。化学発光試薬として、アセトニトリルに別々に溶解した 0.04 mM bis(2,4,6-trichlorophenyl)oxalate と 30 mM  $\text{H}_2\text{O}_2$  をそれぞれ流速 0.5 mL  $\text{min}^{-1}$  で流して混合した。1,3-, 1,6-, 1,8-DNP、1-NP、6-NC、7-NBaP、6-NBaP の回収率は 93~105%、繰り返し精度 (RSD, n = 5) は 1.3~2.7%、

検出限界値 (S/N = 3) は 0.27~11 fmol/注入 であった。なお、濃度が定量限界値以下になった場合は、その 1/2 の値とした。

### 3. 結果

サンプリング地点における最近 12 年間の全浮遊粒子状物質 (total suspended particulate; TSP) 濃度の変遷を Fig. 1 に示す。夏は顕著な変化が見られなかったが、冬についてはエアーバ

一が大きいため有意ではないが、平均値だけみれば 44.4% (49.7  $\mu\text{g}/\text{m}^3$  から 27.6  $\mu\text{g}/\text{m}^3$ ) 減少した。

最近 12 年間の変化を追跡するために、共通の分析対象化合物として、PAH は 4 環の Pyr, BaA, Chr, 5 環の BbF, BkF および BaP, NPAH は 1,3-, 1,6-, 1,8-DNPs, 1-NP, 6-NC, 7-NBaA および 6-NBaP を選んだ。各 PAH, NPAH の種類別濃度の変遷を季節別に Table 1 に示す。PAH, NPAH 共に、概ね冬の方が夏より濃度が高かったが、この傾向はこれまでの金沢市の大気中 PAH, NPAH についてなされた報告 (Murahashi et al., 1995; Kakimoto et al., 2000; Hayakawa et al., 2002; Tang et al., 2002) と一致している。冬の方が高濃度となる要因の一つとして、地表面の大気が冬季に比較的安定しているために、拡散しにくいことが挙げられる。

PAH 濃度 (Fig. 2) は 2008 年冬に上昇がみられたが、両季節共に概ね減少傾向であった。冬はこの期間に 63.9% (12.2  $\text{pmol}/\text{m}^3$  から 4.41  $\text{pmol}/\text{m}^3$ )、夏は 75.6% (6.24  $\text{pmol}/\text{m}^3$  から 1.52  $\text{pmol}/\text{m}^3$ ) 減少した。各 PAH の種類別の割合を Fig. 3 に示したが、いずれの季節でも 4 環 PAH, 特に Pyr の割合が増加し、5 環 PAH の割合は減少傾向が見られた。

NPAH 濃度 (Fig. 4) は最近 12 年間に冬 88.0% (553  $\text{fmol}/\text{m}^3$  から 66.3  $\text{fmol}/\text{m}^3$ )、夏 89.2% (331  $\text{fmol}/\text{m}^3$  から 35.8  $\text{fmol}/\text{m}^3$ ) 減少し、TSP や PAH に比較して大幅な減少が見られた。NPAH の種類別の割合を Fig. 5 に示したが、サンプリングした年と季節によらず 1-NP が大きな割合を占めた。

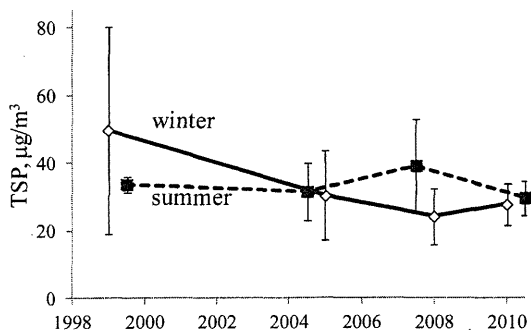


Fig. 1 Variations of TSP concentrations in Kanazawa (Fujie).

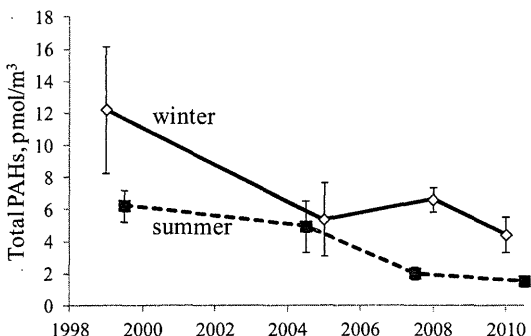


Fig. 2 Variations of Total PAH concentrations in Kanazawa (Fujie).  
Total PAHs = [Pyr] + [BaA] + [Chr] + [BbF] + [BkF] + [BaP]

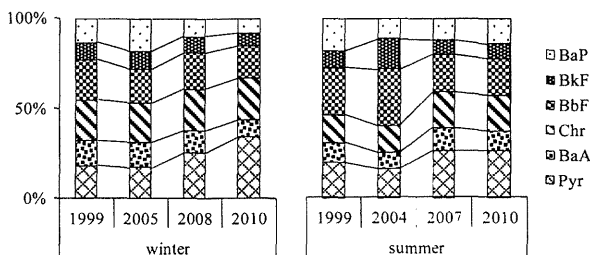


Fig. 3 The ratio of each PAH in Kanazawa (Fujie).

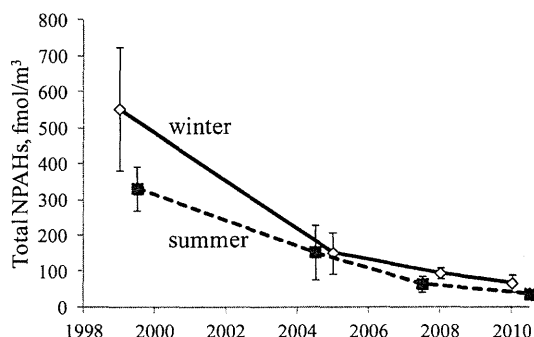


Fig. 4 Variations of Total NPAH concentrations in Kanazawa (Fujie).  
Total NPAHs = [DNPs] + [1-NP] + [6-NC] + [7-NBaA] + [6-NBaP]

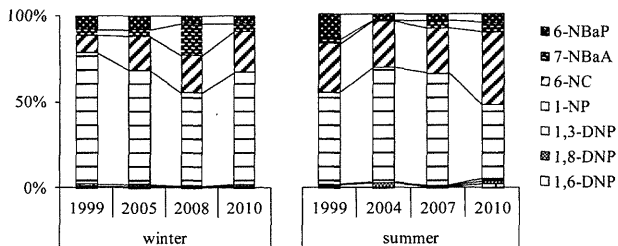


Fig. 5 The ratio of each NPAH in Kanazawa (Fujie).

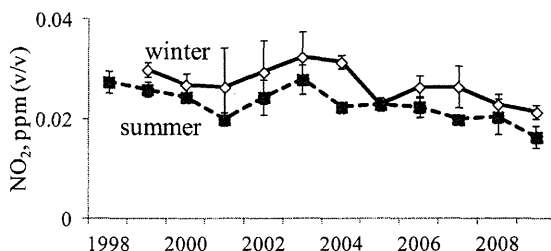


Fig. 6 Variations of NO<sub>2</sub> concentrations in Kanazawa (Fujie).

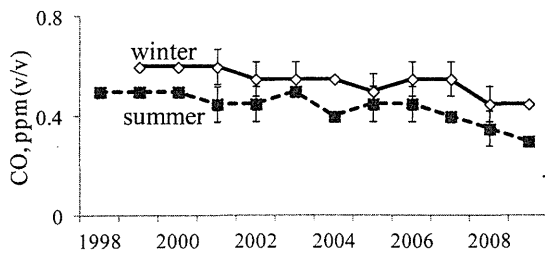


Fig. 7 Variations of CO concentrations in Kanazawa (Fujie).

藤江自動車排出ガス測定局における NO<sub>2</sub> 濃度、CO 濃度の推移をそれぞれ Fig. 6、Fig. 7 に示す。冬は 1~2 月の平均値、夏は 7~8 月の平均値を用いた。主要発生源がディーゼル車といわれる大気中 NO<sub>2</sub> は最近 11 年間の濃度推移を見ると、冬は 28.3%、夏は 36.5% 減少した。また、主要排出源が自動車といわれる大気中 CO は、同じ 11 年間に冬 25%、夏 40% 減少した。NO<sub>2</sub> と CO も夏より冬の濃度が高かった。以上の結果より、最近 12 年間に金沢市市街地沿道大気中の PAH、NPAH は、TSP、NO<sub>2</sub>、CO より顕著に濃度が減少したことが明らかになった。

4. 考察

上述した大気中 PAH、NPAH 濃度の顕著な減少の理由を明らかにするため、気象（気温）、交通量、燃料および自動車排出ガスの変化を順に考察した。

まず、PAH、NPAH のガス相と粒子相の分布比は気温の影響を受ける。しかし、サンプリングをした両季節の気温は 1999 年から 2010 年までの 12 年間にほぼ横ばいであった (Fig. 8 (気象庁 HP の公開データより作成、冬は 1~2 月の平均値、夏は 7~8 月の平均値))。したがって、気温が上昇したために PAH、NPAH の大半がガス相に移行し、粒子相中の PAH、NPAH 濃度の低下につながったとは考えられない。

かつて粉塵排出量はガソリン車よりディーゼル車の方が 30~100 倍多かったことから、1990 年代の我が国の都市大気中 1-NP の排出源は主にディーゼル車と報告されている (Murahashi et al., 1995)。また、燃焼温度に依存して PAH のニトロ化が促進するため、粉塵中の 1-NP と Pyr の濃度比 ([1-NP]/[Pyr]) は大きくなることが知られている。例えば、燃焼温度が高いディーゼル車 (2,500°C 以上) 排出粉塵の [1-NP]/[Pyr] 値は 0.36、燃焼温度が低い石炭ストーブ (約 1,100°C) 排出粉塵の値は 0.001 と報告されている (Tang et al., 2005)。そこで、Table 1 より算出した [1-NP]/[Pyr] 値の推移を Fig. 9 に示す。夏は 0.14 から 0.04、冬は 0.19 から 0.03 というずれの季節でも最近 12 年間に [1-NP]/[Pyr] 値が顕著に減少したことが明らかになった。夏の値が冬より概ね大きかった理由の一つとして、夏は気温が高いため比較的蒸気圧が高く、Pyr のガス相への移行が増し、粒子相中の Pyr 濃度が低下したことが考えられる。しかし、最近 12 年間の減少は、季節間差を

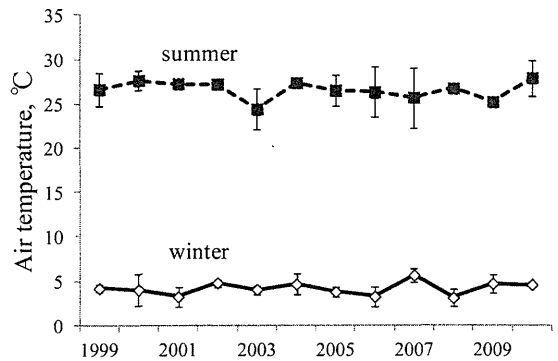


Fig. 8 Average air temperature of summer and winter in Kanazawa.

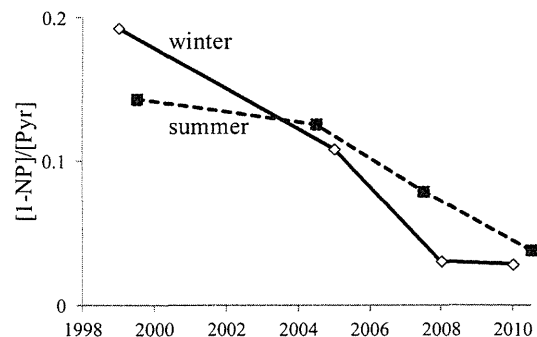


Fig. 9 Variation of [1-NP]/[Pyr] ratio in Kanazawa (Fujie).

大きく凌いでいる。サンプリング地点の金沢市藤江自排局は、近くに石炭を燃焼するような施設はない。しかも、最近 12 年間の Pyr 濃度は顕著に減少した (Fig. 2)。したがって、この期間の [1-NP]/[Pyr] 値の減少は、石炭燃焼が増加したためとは考えられない。

金沢市の自動車登録台数 (Fig. 10) (金沢市, 2011) は、データを手に入れた 2000 年から 2009 年までの 9 年間に 306,789 台から 323,296 台に 5.4% 増加したが、この増加の主体は軽自動車 (Light cars) である。一方、その多くがディーゼルエンジンを搭載していると思われる貨物用自動車 (Trucks)、乗合用自動車 (Buses) は微減している。また、サンプリング地点における交通量はデータを手に入れた 2004 年と 2010 年とでほとんど変化しなかった (夏: 2004 年 8 月 2~15 日の平均値 13637 台、2010 年 8 月 2~15 日の平均値 15023 台、冬: 2005 年 2 月 7~20 日の平均値 9263 台、冬: 2010 年 2 月 10~23 日の平均値 9172 台)。よって、交通量の減少が上述の PAH、NPAH 濃度の減少の原因とは考えられない。以上のことから、上述の [1-NP]/[Pyr] 値の顕著な低下は、自動車からの 1-NP 排出量自体の減少、即ち排出源としての自動車の寄与が減少したことを示している。

大気中の PAH、NPAH 濃度が共に大幅に減少した (Fig. 2 および Fig. 4) 理由として、第一に排ガス規制の強化があげ

られる。例えば、ディーゼル重量車について、1997年の長期規制の規制値は、 $\text{NO}_x$  は 5.80 g/kWh、PM は 0.49 g/kWh であったが、2003 年の新短期規制で  $\text{NO}_x$  は 3.38 g/kWh、PM は 0.18 g/kWh に下げられ、更に 2005 年の新長期規制で  $\text{NO}_x$  は 2.0 g/kWh、PM は 0.027 g/kWh に下げられた。そして、2009 年のポスト新長期規制で  $\text{NO}_x$  は 0.7 g/kWh、PM は 0.010 g/kWh にまで下げられた (Fig. 11) (国土交通省, 2011)。しかも、規制値の推移が、PAH と NPAH の大気中濃度の推移 (Fig. 2 および Fig. 4) と相関していることより、PM と  $\text{NO}_x$  の規制の寄与は大きいと推察される。東京都でも自動車排ガス規制により大気中の PAH と 1-NP の濃度が減少したとの報告 (Kojima et al., 2010) は、本研究結果と符合する。

その他の寄与として、エンジンの改良と燃料の質の改善も考えられる。ディーゼルエンジンには尿素 SCR システム (尿素水を噴射し、尿素水からアンモニアを生成させ、アンモニアにより  $\text{NO}_x$  を  $\text{N}_2$  に還元する)、ディーゼルエンジン用  $\text{NO}_x$  触媒 (通常運転時は  $\text{NO}_x$  を吸蔵剤に溜めこみ、リッチバーン

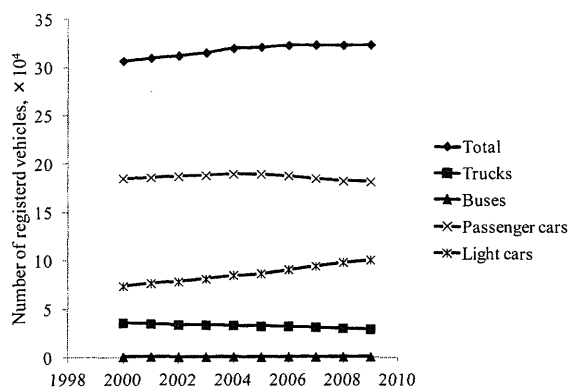


Fig. 10 Number of registered automobiles in Kanazawa.

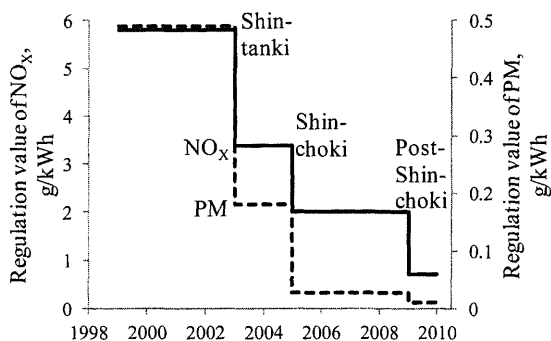


Fig. 11 Transition of Japanese regulation values of  $\text{NO}_x$  and PM against new diesel-engine automobiles.

の時に排出ガスに含まれる  $\text{CO}$ 、 $\text{HC}$ 、 $\text{H}_2$  により  $\text{NO}_x$  を  $\text{N}_2$  に還元する)、ディーゼル微粒子捕集フィルター (DPF) (ディーゼル排ガス中の PM を捕集する) が採用されてきた (日

本自動車工業会, 2011)。一方、ガソリンや軽油の品質は、「揮発油等の品質の確保等に関する法律」により規定されており、軽油中の硫黄の規制値は 2005 年までに 50 ppm (w/w) 以下に、2007 年までに 10 ppm (w/w) に下げられた。実際には、石油業界の自主的な取り組みにより、2005 年には硫黄含有量が 10 ppm (w/w) 以下の軽油が供給され始めた。軽油中の硫黄成分はディーゼル車の粒子状物質 (PM) の発生に関係している。即ち、硫黄成分から酸化によって生じた硫黄酸化物 ( $\text{SO}_x$ ) により、DPF の酸化触媒やディーゼル用  $\text{NO}_x$  触媒が覆われてしまうと触媒作用が低下する (新エネルギー・産業技術開発機構, 2011)。したがって、軽油中の硫黄成分の濃度を下げることによって、PM の発生が抑制され、その結果、大気粉塵中の PAH、NPAH も減少した可能性も併せて考慮する必要がある。

ここで、1-NP 濃度 (Fig. 4) の方が Pyr 濃度 (Fig. 2) より大きな減少率を示した理由は、次のように考察した。エンジン内で Pyr と  $\text{NO}_x$  が発生しているので、1-NP の生成速度  $v$  は反応速度定数を  $k$  とし、以下のように表されると考えた。

$$v = k[\text{Pyr}][\text{NO}_x] \quad (1)$$

式 (1) より、Pyr と  $\text{NO}_x$  両方の濃度が減少すると、相乗効果によって 1-NP 濃度は Pyr 濃度より大きく減少するものと考察した。

なお、2004 年 10 月～2005 年 4 月の中国の暖房期における、能登半島先端での BaP 濃度は平均  $0.03 \text{ ng/m}^3$  ( $0.01 \text{ pmol/m}^3$ ) であり (Yang et al., 2007)、その北西風下に位置する金沢市 (藤江) の 2005 年冬の BaP 濃度の約 1/10 である。したがって、市内発生 PAH に比較して長距離輸送の影響は極めて小さい。NPAH も同様に、中国の暖房期に含まれる 2007 年 1 月の能登半島の先端での 1-NP 濃度は  $840 \text{ fg/m}^3$  ( $3.40 \text{ fmol/m}^3$ ) (Hayakawa et al., 2011) と、金沢市 (藤江) の 2008 年冬の 1-NP 濃度の約 1/15 であり、長距離輸送の影響は極めて小さい。

## 5. まとめ

金沢市市街地幹線道路脇で 1999 年から 2010 年までの夏と冬大気粉塵を捕集し、PAH、NPAH を分析した結果、以下のことがわかった。

- PAH 濃度は、冬 63.9%、夏 75.6% 減少した。
- NPAH 濃度は、冬 88.0%、夏 89.2% 減少した。
- PAH と NPAH の濃度の減少率は、 $\text{NO}_2$ 、 $\text{CO}$  よりはるかに顕著であった。
- $[\text{1-NP}]/[\text{Pyr}]$  値は著しく減少した。その理由として排出源としての自動車の寄与が低下したことが考えられた。1-NP 濃度の大幅な減少は  $\text{NO}_x$  と Pyr の減少の相乗効果によると推察された。
- PAH と NPAH の濃度の減少の最大要因として、排ガス規制が考えられた。さらに、エンジンの改良、燃料質の改善も推察された。

## 謝 辞

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## 金沢市内における大気粉塵中多環芳香族炭化水素類および ニトロ多環芳香族炭化水素類の最近 12 年間の変遷

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石川県金沢市藤江の幹線道路脇において 12 年間 (1999~2010 年) の夏と冬の大気粉塵を捕集し、多環芳香族炭化水素 (PAH) 6 種類 (pyrene, benz[a]anthracene, chrysene, benzo[b]fluoranthene, benzo[k]fluoranthene, benzo[a]pyrene) およびニトロ多環芳香族炭化水素 (NPAH) 7 種類 (1,3-, 1,6-, 1,8-dinitropyrenes, 1-nitropyrene, 6-nitrochrysene, 7-nitrobenz[a]anthracene, 6-nitrobenzo[a]pyrene) をそれぞれ HPLC-蛍光検出法、HPLC-化学発光検出法で測定し、その変遷を明らかにした。この間に、PAH 濃度は冬 63.9%、夏 75.6%減少、NPAH 濃度は冬 88.0%、夏 89.2%減少した。また [1-NP]/[Pyr]値は冬と夏ともに著しく減少した。自動車排ガス規制の強化によって排出源としての自動車の寄与率が低下したことが、大気中 PAH、NPAH 濃度および [1-NP]/[Pyr]値が顕著に減少した主要因と考えられた。

## On-Line Concentration and Fluorescence Determination HPLC for Polycyclic Aromatic Hydrocarbons in Seawater Samples and Its Application to Japan Sea

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An on-line concentration and fluorescence determination HPLC for polycyclic aromatic hydrocarbons (PAHs) in seawater was proposed. An online concentration column packed with octadecyl polyvinyl alcohol polymer, a pump and a column switching valve were introduced in the conventional HPLC with a fluorescence detector. Only 1.0–100 mL seawater sample was introduced into the concentration column at 1.0 mL min<sup>-1</sup> without any other pretreatment except filtration. Then the trapped PAHs totally flew into the separation column and eluted separately to be detected fluorogenically. The proposed method had good linearity with correlation coefficients (*r*) ranged from 0.951 to 0.998, and limits of detection ranged from 0.002 to 0.50 ng L<sup>-1</sup> for 15 PAHs as 100 mL seawater was loaded. The sensitivity of the method was 10 to 100 times higher than those reported by other works. The proposed method was applied to the determination of PAHs in the seawater samples collected in the Japan Sea with satisfactory results and to check the present benzo[*a*]pyrene concentration at the beaches in Noto peninsula, Japan polluted with C-heavy oil spilled from the tanker in 1997.

**Key words** polycyclic aromatic hydrocarbon; HPLC; seawater; Japan Sea

Polycyclic aromatic hydrocarbons (PAHs) are ubiquitous environmental pollutants. Humans and animals are exposed to PAHs from environmental, dietary and occupational sources. Benzo[*a*]pyrene (BaP) is carcinogenic to humans, dibenz[*a,h*]anthracene is probably carcinogenic to humans and benz[*a*]anthracene (BaA), chrysene (Chr), benzo[*b*]fluoranthene (BbF), benzo[*k*]fluoranthene (BkF), indeno[1,2,3-*cd*]pyrene (IDP) are possibly carcinogenic to humans.<sup>1)</sup> Due to their potential or proven carcinogenic or mutagenic properties, some PAHs have been designated as priority pollutants for monitoring by the U.S. and European environmental agencies. It is for these reasons that the European Union has fixed very restrictive limits for these compounds in different kinds of superficial water.<sup>2)</sup>

Many kinds of PAHs are contained in natural oil and oil spilled from tankers and wells causes serious marine contamination with PAHs. The Japan Sea is fruitful of fishery resources. In January 1997, a large amount of C-heavy oil more than 6000 kL was spilled from the Russian tanker, Nakhodka, in the central part of the Japan Sea and the spilled oil was beached on the coastlines from Shimane to Akita, Japan. This accident gave the serious damage to the fishery in Japan. The contamination of PAHs was monitored for several years after the accident.<sup>3)</sup> Now, more than 10 years after the accident, it is believed that the Japan Sea has become clean without detailed monitoring of PAHs.

For determining PAHs in marine, specific analytical protocols have been described in detail in several reviews.<sup>4–6)</sup> Traditional pretreatment techniques for extraction of these organic compounds from aqueous samples are liquid–liquid extraction (LLE) and solid-phase extraction (SPE).<sup>7–10)</sup> However, these multistep techniques have disadvantages of labor-inten-

sive, heavy usage of toxic solvents and contamination. Solid-phase microextraction with different coating materials or polymeric fiber was employed for the determination of PAHs in tap water, river water or rainwater, and these techniques provided large extraction capacity and high sensitivity ranged ng L<sup>-1</sup> level when coupling with gas chromatography-mass spectrometry (GC-MS)/HPLC.<sup>11–13)</sup> However, the evaporation of the eluate reduced the recoveries of PAHs having 2 to 3 rings which have high vapor pressures.

Compared with the PAHs concentrations in rainwater, river water, sewage and even marine sediments, the concentrations of PAHs in seawater were much lower, so the reports concerning PAHs in seawater samples were scarce until now.<sup>8,9,14)</sup> When seawater samples were analyzed, it is necessary to collect big volume sample, e.g. several or several-hundred liter sample, in order to achieve the limits of detection.<sup>5,7,8)</sup> Moreover, the recoveries of PAHs became lower with decreasing concentrations.

In this work, in order to overcome the disadvantages of traditional methods described above, an on-line concentration and fluorescence determination HPLC was proposed for determining trace levels of PAHs in seawater samples. As effective applications of the proposed method, concentrations of PAHs were also determined in seawater samples collected in the southwest part of the Japan Sea and at beaches in Noto Peninsula which were heavily damaged by the oil spill in 1997.

### Experimental

**Materials** An EPA 610 PAHs mix containing naphthalene (Nap), acenaphthene (Ace), fluorene (Fle), phenanthrene (Phe), anthracene (Ant), fluoranthene (Flu), pyrene (Pyr), BaA, Chr, BbF, BkF, BaP, dienz[*a,h*]anthracene (DBA), benzo[*g,h,i*]perylene (BgPe) and IDP was purchased from Supelco

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(Bellefonte, PA, U.S.A.). Five deuterated PAHs (Nap- $d_8$ , Ace- $d_{10}$ , Phe- $d_{10}$ , Pyr- $d_{10}$  and BaP- $d_{12}$ ) were purchased from Wako Pure Chemical (Osaka, Japan) as internal standards. Both PAHs and deuterated PAHs were dissolved in acetonitrile (Kanto Chemical, Tokyo, Japan). All other chemicals used were of analytical reagent grade.

#### Collection and Pretreatment of Seawater Sample

Seawater samples were collected at five sites (S1–5), whose latitudes and longitudes were respectively 32.8° and 129.5° (S1), 33.8° and 129.7° (S2), 34.8° and 130.5° (S3), 35.5° and 131.5° (S4), 36.0° and 132.5° (S5), in the Japan Sea in August 2008. Seawater samples were collected from the bow of Nagasaki-maru, expedition ship, moving forward, and were collected by immersing pre-cleaned borosilicate amber glass bottles (2L) at *ca.* 0.5 m below the water surface (opening and closing it underwater). Each water sample was immediately filtered through a 0.45  $\mu$ m micropore membrane (GC-50, diameter 90 mm, Advantec, Tokyo, Japan) and 10% (v/v) ethanol was added to each bottle to prevent the adsorption of PAHs on the wall. The seawater samples were stored in the refrigerator (at 4°C) no more than 1 week before analysis and no other preservatives were needed. Seawater samples collected at two beaches in Noto Peninsula, Japan damaged by the oil spill in 1997 were also used for comparison.

Artificial seawater was prepared by dissolving 32 g of NaCl, 14 g of MgSO<sub>4</sub>·7H<sub>2</sub>O and 0.2 g NaHCO<sub>3</sub> in 1 L of Milli-Q water.

**On-line Concentration and Determination HPLC System for PAHs in Seawater** The proposed HPLC system was shown in Fig. 1. The system consisted of three Hitachi L-2130 pumps (Tokyo, Japan), a Hitachi degasser, a Hitachi L-2485 fluorescence detector and a Hitachi organizer. An Asahipak ODP-50G column (4.6 mm i.d.×10 mm, Shodex, Tokyo, Japan) was used as the concentrator column. An Inertsil ODS-P column (4.6 mm i.d.×250 mm, GL-Science Company, Tokyo, Japan) and an Inertsil ODS-P column (4.6 mm i.d.×33 mm) were used as the separator and guard columns, respectively. PAHs were concentrated on the concentrator column by loading seawater sample through the solid-line in Fig. 1.

The on-line concentration and elution were operated as follows with the Teflon tubing. After seawater sample (with 10% ethanol) was added with internal standards of Nap- $d_8$ , Ace- $d_{10}$ , Phe- $d_{10}$ , Pyr- $d_{10}$  and BaP- $d_{12}$ , an aliquot (1.0–100 mL) of the mixture was introduced into the HPLC system through the HPLC pump 1. The inlet tubing was rinsed with the sample

for 10 min before loading. The loading volume depended on the concentrations of PAHs in the samples. PAHs in the sample were adsorbed and concentrated on the concentrator column (ODP-50G). The column was washed with 15 mL of distilled water to remove the interfering compounds and salts. The volume of samples and washing solutions was controlled by the loading time when the optimized flow rate of pump 1 was 1.0 mL min<sup>-1</sup>. After the valve was switched (from the solid line to the dotted line in Fig. 1), PAHs were eluted from the concentrator column and separated on the separator column (ODS-P) with the HPLC mobile phase. Then, PAHs were determined fluorogenically under the following conditions according to our previous report<sup>15)</sup> with minor modification as follows. The mobile phase consisted of acetonitrile and Milli-Q water and the flow rate of mobile phase was kept constant at 1.0 mL min<sup>-1</sup>.

The gradient time program of pumps 2 and 3 was controlled by the Hatachi organizer. Initially, the content of acetonitrile in the mobile phase was 55% (v/v) for the first 20 min, and then was changed from 70 to 80% (20–35 min), after that, it was kept at 90% (35–45 min), then was increased to 100% (45–60 min) and kept for 20 min. The excitation and emission wavelengths were set at 280 and 340 nm (0–31.5 min) for Nap, Nap- $d_8$ , Ace, Ace- $d_{10}$ , Fle, Phe, Phe- $d_{10}$ , 250 and 400 nm (31.5–34 min) for Ant, 286 and 433 nm (34–36 min) for Flu, 331 and 392 nm (36–41 min) for Pyr and Pyr- $d_{10}$ , 264 and 407 nm (41–68.9 min) for BaA, Chr, BbF, BkF, BaP, BaP- $d_{12}$ , DBA, BgPe and 294 and 482 nm (68.9–80 min) for IDP, respectively.

The whole system was checked routinely with blank samples for preventing from contamination.

## Results and Discussion

**Optimization of On-line Concentration** As the solid-phase extraction sorbent for the on-line determination of PAHs in natural water, a kind of fluorocarbon polymer was used. In the system, only PAHs with high concentration, *e.g.* Nap, Ant, and Pyr, were determined.<sup>14)</sup> In the present work, an octadecyl polyvinyl alcohol polymer (ODP) column, which was made of porous vinyl alcohol copolymer modified with octadecyl groups on the surface, was used as the concentrator column for enrichment of PAHs having 2 to 6 rings in seawater samples. The polymeric ODP column was more resistant to strong acid and basic solutions than commonly used silica-based octadecyl silyl (ODS) columns. Moreover, the polymeric ODP column showed selective retention power for PAHs compounds due to the existence of  $\pi$ -electron interaction between PAHs molecule and ODP polymer.<sup>16)</sup> Therefore, we first compared the PAHs retention abilities on ODP and ODS columns. The recoveries of PAHs were higher on the polymeric ODP column than on the ODS column, indicating that the ODP column had stronger retention of PAHs and greater selectivity for PAHs. The elution profiles showed that PAHs were more stable on the ODP column than on the ODS column when the columns were loaded with PAHs and stored for several days before elution respectively. The higher stability on the ODP column might be because octadecyl polyvinyl alcohol polymers was more resistant to water than silica. For these reasons, an ODP column was selected for the concentrator column. The new ODP concentrator column was conditioned with 20 mL methanol and distilled water, respectively, at the flow rate of 0.5 mL min<sup>-1</sup>.

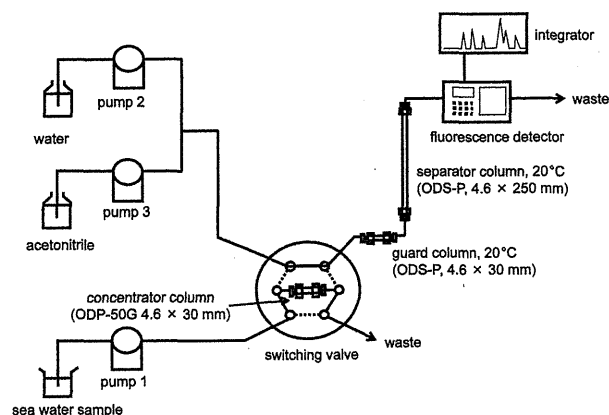


Fig. 1. Schematic Diagram of the Proposed System



The recoveries of PAHs depend on the flow rate of seawater, although a short time concentration is better. The peak heights were measured at flow rates from 0.5 to 2.0 mL min<sup>-1</sup>, and the maximum peak heights were observed at flow rates of 0.5 and 1.0 mL min<sup>-1</sup> for most PAHs. At flow rates over 1.0 mL min<sup>-1</sup>, the peak heights decreased with the increase in the flow rate (Fig. 2). So, the flow rate was set at 1.0 mL min<sup>-1</sup> for the following experiments.

The recoveries of PAHs were lower without the addition of ethanol. This might be attributed to the absorption of PAHs on the bottle walls due to the high hydrophobic property.<sup>17,18)</sup> Here, methanol and ethanol were added to the water samples respectively. We found that 10% ethanol was just as effective as 10% methanol to decrease the adsorption of PAHs and that the recoveries of PAHs were constant at the concentrations over 10%. Considering that ethanol was nontoxic and safe to bring it on the ships, 10% ethanol was immediately added to

the collected seawater samples after filtration. The seawater sample containing 10% ethanol was loaded on the column at 1.0 mL min<sup>-1</sup>. The column was washed with 15 mL distilled water after trapping the PAHs in order to remove the adsorbed interfering compounds and prevent salts out from the column.

The breakthrough volume was tested to evaluate the maximum sample volume which can be applied with a theoretical 100% recovery. The different volume of artificial seawater samples containing a constant concentration of analyte were treated as described in 'On-line Concentration and Determination HPLC System for PAHs in Seawater.' When the breakthrough volume of analyte began, this relationship started to deviate from linearity.<sup>19)</sup> There was no deviation from linearity observed when the tested volume was changed from 100 to 1000 mL. The relationships between the peak height ratios of PAHs and their respective internal standards against the sample volume were linear. This result suggested that the concentrator column (ODP) had enough capacity for concentrating PAHs in seawater samples and the breakthrough volume of the column was larger than 1000 mL of the polluted seawater such as samples collected in harbors.

**Performance of the Proposed Method** In order to evaluate the practical applicability of the proposed system, performance parameters such as linearity, precision, limits of detection (LODs) and limits of quantization (LOQs) were measured under optimum analytical conditions using 100 mL artificial seawater samples spiked with PAHs. Results were given in Table 1. Concentrations of PAHs in the artificial seawater samples were as follows: Nap and Ace, 20 ng L<sup>-1</sup>; Fle, Flu, BbF, DBA and BgPe, 4 ng L<sup>-1</sup>; IDP, 3.2 ng L<sup>-1</sup>; Phe, Ant, Pyr, BaA, Chr, BkF and BaP, 2.0 ng L<sup>-1</sup>. All the PAHs showed good linearity with correlation coefficients (*r*) ranging from 0.951 to 0.998 (Table 1). Recoveries ranged from 74% (IDP) to 110% (Ant) with relative standard deviation (R.S.D.) of 1.5–9.4%. R.S.D.s were between 0.4–5.0% for repeatability (*n*=3) and 1.8–9.8% for reproducibility (*n*=5).

Limits of detection (LODs) (concentrations giving a signal-to-noise ratio of 3) ranged from 0.002 (Ant) to 0.50 ng L<sup>-1</sup> (IDP) as the loading sample volume was 100 mL. LODs by the proposed method were smaller than those by GC-MS/MS

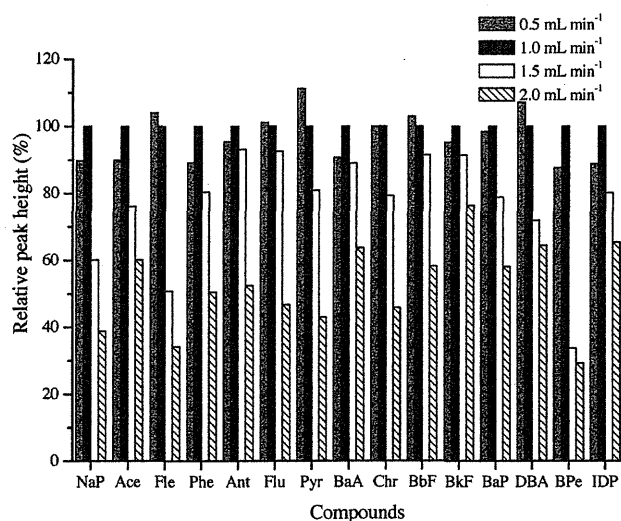


Fig. 2. Effect of Flow Rate of Pump 1 on the Relative Peak Height of PAHs

The samples were the spiked artificial seawater samples and the spiked concentration was shown in the text. The loading volume was 100 mL. The peak height at flow rate of 1.0 mL/min was defined as 100%.

Table 1. Linearity, Recovery, Repeatability and Reproducibility of the Proposed Method<sup>a)</sup>

PAHs	Linearity ( <i>r</i> )	LOD (ng L <sup>-1</sup> )	LOQ (ng L <sup>-1</sup> )	Recovery ± R.S.D. (% , <i>n</i> =5)	Repeatability (R.S.D., % , <i>n</i> =3)	Reproducibility (R.S.D., % , <i>n</i> =5)
Nap	0.951	0.042	0.14	98 ± 1.5	0.9	5.6
Ace	0.970	0.013	0.042	95 ± 1.5	1.6	7.7
Fle	0.984	0.020	0.067	101 ± 2.4	0.4	8.4
Phe	0.976	0.011	0.036	93 ± 4.2	4.7	5.8
Ant	0.998	0.002	0.007	110 ± 6.8	2.4	5.3
Flu	0.988	0.030	0.10	88 ± 9.4	4.9	9.8
Pyr	0.994	0.012	0.040	95 ± 9.3	2.5	7.3
BaA	0.990	0.012	0.040	92 ± 6.5	1.1	3.8
Chr	0.990	0.018	0.061	88 ± 5.3	1.2	1.8
BbF	0.996	0.060	0.20	84 ± 5.2	4.1	9.6
BkF	0.988	0.013	0.044	89 ± 5.6	2.6	5.1
BaP	0.988	0.012	0.039	80 ± 7.4	1.3	8.9
DBA	0.990	0.20	0.67	75 ± 8.2	5.0	7.1
BgPe	0.986	0.22	0.73	79 ± 7.3	2.3	9.1
IDP	0.986	0.50	1.7	74 ± 7.7	1.8	8.9

a) Loading sample volume, 100 mL.

method coupled with solid phase microextraction and HPLC-fluorescence detection method coupled with nanoextraction recently reported.<sup>8-12,17,20</sup> Especially LODs of Ace, Ant, Pyr, BaA, Chr, BkF and BaP by the proposed method were 1/10 to 1/100 of the reported values.

On the other hand, artificial seawater samples spiked with 100 times higher concentrations of PAHs were prepared and 1.0 mL of the solution was loaded to the system. The slopes of the calibration curves and LOQs of PAHs were almost the same as those obtained above, suggesting that the proposed method is useful for both highly polluted and clean seawater samples.

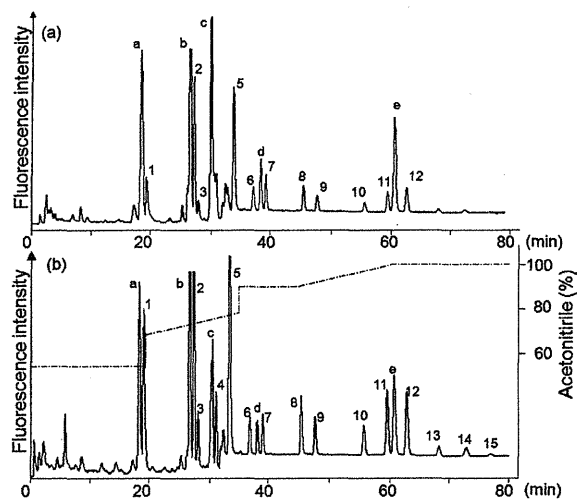


Fig. 3. Typical Chromatograms of (a) Real Japan Sea Water Sample and (b) Artificial Seawater Sample Spiked with PAHs

The sampling site of (a), S1. The spiked PAH concentrations, seen text; the dotted line in (b) indicated the acetonitrile concentration (%) in the mobile phase. Peaks: 1, Nap; 2, Ace; 3, Fle; 4, Phe; 5, Ant; 6, Flu; 7, Pyr; 8, BaA; 9, Chr; 10, BbF; 11, BkF; 12, BaP; 13, DBA; 14, BgPe; 15, IDP; a, Nap-*d*<sub>6</sub>; b, Ace-*d*<sub>10</sub>; c, Phe-*d*<sub>10</sub>; d, Pyr-*d*<sub>10</sub>; e, BaP-*d*<sub>12</sub>.

**PAHs Concentrations in Japan Sea Samples** The proposed method was applied to seawater samples collected from the Japan Sea to investigate the stage. Figure 3 showed representative chromatograms of the Japan Sea water and a spiked artificial seawater sample obtained by the proposed method under the optimized conditions. The loading volume was 100 mL. Table 2 showed the concentrations of PAHs at the five sampling sites. Average concentrations ranged from 0.1 (Ant) to 22.9 (Nap) ngL<sup>-1</sup> in the southwest part of the Japan Sea. Nap, a 2-ring PAH, was the predominant compound. BaA, BbF and BkF, whose concentrations were lower than LODs the reported in traditional methods, were quantified by the proposed method. DBA, BgPe and IDP showed trace peaks in Fig. 3, although they were lower than the LODs. The recoveries of PAHs spiked to the Japan Sea samples showed similar result as that of the spiked artificial seawater samples. These results suggested that the proposed method could determine PAHs at sub ppt (ngL<sup>-1</sup>) in marine water.

**Recovery of Beaches Damaged by Oil Spilled from Nakhodka from the View Point of BaP** Finally the BaP concentration was determined in seawater samples collected at two beaches (Kaiso and Hagahashi) in Noto Peninsula, which were heavily damaged by the beached oil from the Nakhodka in January 1997, in Table 3. The BaP concentrations at Kaiso (8.1 ngL<sup>-1</sup>) and Nagahashi (7.4 ngL<sup>-1</sup>) were very high one month after the accident and decreased with time. Then the concentrations at 3 years after the accident (April, 2000) became to the normal level of the Japan Sea (0.2–0.3 ngL<sup>-1</sup>).

## Conclusion

An on-line HPLC system for concentrating and determining PAHs in small volume seawater samples was proposed with the advantages of automatic, simple and on-line sampling pre-treatment. The polymeric ODP column concentrated PAHs in the samples with excellent matrix removal, and on-line elution and determination through switching valve guaranteed the

Table 2. Concentrations (ngL<sup>-1</sup>) of PAHs Collected at Different Sampling Sites in the Japan Sea

Method	Proposed work						MASE <sup>a)</sup> -GCMS <sup>21)</sup>	LLE-GCMS <sup>8)</sup>	SPE-HPLC <sup>9)</sup>
	Japan Sea						Bilbao, Spain	Alexandria Coast, Egypt	Saronikos Gulf, Greece
Sampling site	S1 <sup>b)</sup>	S2	S3	S4	S5	Ave. ± S.D.			
NaP	23.9	26.0	23.9	23.7	16.8	22.9 ± 3.5	166	n.d. <sup>c)</sup>	160
Ace	1.5	1.7	1.5	1.2	1.3	1.4 ± 0.2	110	n.d.	41
Fle	1.6	1.6	1.6	1.2	1.3	1.5 ± 0.2	37	2.1	42
Phe	i.p. <sup>d)</sup>	i.p.	i.p.	i.p.	i.p.	—	118	14.8	54
Ant	0.1	0.1	0.1	0.1	0.1	0.1 ± 0	76	3.8	30
Flu	1.5	1.6	1.5	1.6	1.4	1.5 ± 0.1	21	6.7	34
Pyr	0.8	1.0	0.8	0.7	0.9	0.8 ± 0.1	30	5.6	18
BaA	0.1	0.2	0.1	0.1	0.3	0.2 ± 0.1	n.d.	n.d.	—
Chr	0.2	0.2	0.2	0.2	0.4	0.3 ± 0.1	n.d.	5.1	—
BbF	0.6	0.6	0.6	0.5	0.5	0.6 ± 0.1	9	n.d.	55
BkF	0.3	0.4	0.3	0.2	0.2	0.3 ± 0.1	10.1	n.d.	—
BaP	0.2	0.3	0.2	0.2	0.2	0.2 ± 0.1	9.7	9.0	25
DBA	n.q. <sup>e)</sup>	n.q.	n.q.	n.q.	n.q.	—	n.d.	n.d.	—
BgPe	n.q.	n.q.	n.q.	n.q.	n.q.	—	n.d.	n.d.	—
IDP	n.q.	n.q.	n.q.	n.q.	n.q.	—	n.d.	n.d.	—
Total	30.8	33.7	30.8	29.7	23.4	29.7 ± 3.8	679	47.0	459

a) MASE, membrane-associated solvent extraction; b) sampling site (locations of each sampling site seen text); c) not detected; d) not quantified because of the interfering peaks; e) detected but not quantified because lower than LOD.

Table 3. BaP Concentrations (ngL<sup>-1</sup>) in Seawater Samples Collected at Beaches in Noto Peninsula Damaged by Nakhodka Spilled Oil in 1997 and South Part of the Japan Sea

Sampling date	Noto peninsula		Japan Sea (ave. of S1-5)
	Kaiso	Nagahashi	
Feb., 1997	8.1 <sup>a)</sup>	7.4 <sup>a)</sup>	— <sup>b)</sup>
Jan., 1998	1.6 <sup>a)</sup>	—	—
April, 2000	0.3 <sup>a)</sup>	0.2 <sup>a)</sup>	—
April, 2008	0.2	0.2	0.2

a) Cited from ref. 3. b) Sample was not collected.

higher sensitivity than currently available systems. Thus the proposed method which required a small volume sample was successfully applied for the determination of PAHs in not only polluted seawater but also clean background seawater. This is the first report to determine the normal levels of PAHs. This method also found that the BaP concentration in seawater at beaches in Noto Peninsula which were damaged by the oil spill in 1997 had become to the normal level.

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多環芳香族炭化水素類の生体影響に対し輸送及び生体内代謝がどのように関与しているのか

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## How Do Transport and Metabolism Affect on the Biological Effects of Polycyclic Aromatic Hydrocarbons?

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Polycyclic aromatic hydrocarbons (PAHs), some of which are carcinogenic/mutagenic, are generated by combustion of fossil fuels and also released through tanker or oilfield accident to cause a large scale environmental pollution. PAHs concentration in China is especially high in East Asia because of many kinds of generation sources such as coal heating systems, vehicles and factories without exhaust gas/particulate treatment systems. So, the atmospheric pollution caused by PAHs in China has been seriously concerned from the view point of health effects. Like yellow sand and sulfur oxide, PAHs exhausted in China are also transported to Japan. Additionally, strongly mutagenic nitrated PAHs (NPAHs), estrogenic/antiestrogenic PAH hydroxides (PAHOHs) and reactive oxygen species-producing PAH quinones (PAHQs) are formed from PAHs by the chemical reaction during the transport. Furthermore these PAHOHs and PAHQs are produced by the metabolism in animal body. In the biological activities caused by the above PAH derivatives, the structure-activity relationship was observed. In this review, our recent results on the generation of PAH derivatives by atmospheric transport and metabolism are reported. Also, the existing condition of PAHs as atmospheric pollutants is considered.

**Key words**—polycyclic aromatic hydrocarbon derivative; atmospheric transport; metabolism

### 1. はじめに

多環芳香族炭化水素 (PAH) 類は、石炭や石油などの化石燃料の燃焼に伴い生成され、タンカーや油田事故でも大規模な環境汚染を引き起こす環境汚染物質の1つである。PAH類はヒトや野生生物への影響が懸念されると同時に、偏西風等によって長距離輸送されることから越境汚染を引き起こす可能性も危惧されている。さらには、PAHは大気中を輸送される際には、様々な化学反応によって誘導体

へと姿を変え、例えばPAHのニトロ化体 (NPAH) が強い変異原性を示すように、多岐に渡る毒性を発現する可能性も示唆される。

本シンポジウムでは、PAHの大気輸送及び生体内代謝により生成されるPAH誘導体を中心に、これらが示す毒性と生体影響との係わりについて、筆者らの取り組みを紹介するとともに、PAH類の大気汚染物質としての現状について考えてみたい。

### 2. 環日本海域の主要都市におけるPAH/NPAH濃度の実態

筆者らは、環日本海域に位置する各都市の汚染状況を把握すべく、日本 (札幌, 金沢, 東京, 北九州), 韓国 (釜山), 中国 (瀋陽, 撫順, 鉄嶺, 北京), ロシア (ウラジオストク) における、大気粉塵中のPAHとNPAHの濃度測定を実施した。分

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