

## A diffusive sampling device for simultaneous determination of ozone and carbonyls

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### ABSTRACT

A new diffusive sampling method for the simultaneous determination of ozone and carbonyls in air has been developed. In this method, silica gel impregnated with a mixture of *trans*-1,2-bis(2-pyridyl)ethylene (2BPE) and 2,4-dinitrophenylhydrazine (DNPH) is used as the absorbent; further, a porous sintered polyethylene tube (PSP-diffusion filter), which acts as a diffusive membrane, and a small polypropylene syringe (PP-reservoir) for elution of the analytes from the absorbent are used. The carbonyls present in air react with DNPH in the absorbent to form hydrazone derivatives. Concurrently, ozone in the air reacts with 2BPE to form pyridine-2-aldehyde, which immediately reacts with DNPH to form a pyridine-2-aldehyde hydrazone derivative. All the hydrazones derived from airborne carbonyls, including pyridine-2-aldehyde (formed from ozone), are completely separated and analyzed by high-performance liquid chromatography. The sampling rates of ozone ( $44.6 \text{ mL min}^{-1}$ ) and formaldehyde ( $72.0 \text{ mL min}^{-1}$ ) are determined by comparison with the rates obtained in an active sampling method. The sampling rates of other carbonyl compounds are calculated from the respective molecular weights according to a rule based on Graham's law. The calculated sampling rates agree with the experimental values. The DSD-BPE/DNPH method is advantageous because it is simple and allows for the simultaneous analysis of ozone and carbonyls.

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### 1. Introduction

Carbonyl compounds such as formaldehyde and acetaldehyde have received a great deal of attention in environmental chemistry studies because they are hazardous substances and have a significant impact on the environment. These compounds are ubiquitous pollutants formed as a result of the oxidation of hydrocarbons by tropospheric ozone [1,2] and by the reaction between ozone and terpenoids in indoor air [3–5]. Long-term exposure to relatively high levels of formaldehyde is known to increase the risk for asthma [6], leukemia [7], and cancer [8,9] to humans. Additionally, ozone, which is also hazardous, causes lung inflammation [10], and hence, exposure to ozone is associated with various respiratory symptoms, including dyspnea, upper airway irritation, coughing, and chest tightness [11]. Tropospheric ozone is a major environmental pollutant produced by various routes, including photochemical transformation of nitrogen oxides, carbon monoxide, and volatile organic compounds in vehicle exhaust. Thus, ozone and carbonyl compounds are related substances in atmospheric chemistry, therefore, the mon-

itoring of the atmospheric levels of these substances is very important.

Diffusive samplers are small and lightweight and do not require a power source. Hence, a diffusive sampler is preferred over an active sampler for analyzing ambient air and indoor air and for monitoring personal exposure to airborne contaminants. Literature surveys indicate that a number of personal diffusive samplers for detecting carbonyl compounds are available; these samplers comprise 2,4-dinitrophenylhydrazine (DNPH)-coated filters [12–15], *N*-methyl-4-hydrazino-7-nitrobenzofurazan coated filters [16], and dansylhydrazine-coated silica [17]. In addition, in our previous study, we developed DSD (diffusive sampling device)-voc [18], DSD-carbonyl [19], and DSD-DNPH [20] diffusive samplers that comprise a porous extended polytetrafluoroethylene tube and a porous sintered polyethylene tube as a diffusion permeable media. These samplers offer many advantages over traditional samplers, including rapid sampling rate, operation simplicity, and omnidirectionality; moreover, these samplers can be connected to a pump and used for active sampling applications. The sampling rate when using DSD-DNPH is calculated according to a rule based on Graham's law, according to which the rate of diffusion of a gas is inversely proportional to the square root of the density of the gas; therefore, the sampling rate of the target compound can be calculated without using any standard gas. For ozone analysis,

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diffusive sampling methods in which various sampling reagents such as potassium iodide [21], 1,2-di-(4-pyridyl)ethylene [22,23], indigo carmine [24–26], and nitrite ions [25–27] are used, have been developed. Thus, numerous diffusive sampling methods are available for the analysis of carbonyl compounds and ozone. However, a diffusive sampler for the simultaneous analysis of carbonyl compounds and ozone has not been developed until now; although, it is well known that these substances are strongly interrelated.

Herein, we report an active sampling method involving the use of a using a 2-bed cartridge system (2BPE/DNPH-cartridge [28] and 4BPE/DNPH-cartridge [29]) for the simultaneous analysis of ozone and carbonyls in air. Each bed consists of reagent-impregnated silica particles. The first bed contains *trans*-1,2-bis-(2-pyridyl)ethylene (2BPE), while the second contains DNPH. Ozone present in the air sample is trapped in the first bed by the 2BPE-coated silica particles, and pyridine-2-aldehyde is formed. Airborne carbonyls pass uninterrupted through the 2BPE bed and are trapped in the second bed by the DNPH-coated silica particles, resulting in the formation of carbonyl 2,4-DNPhydrazones. When a solvent is eluted through the BPE/DNPH-cartridge, excess DNPH is flushed into the 2BPE bed, where it reacts with pyridine-2-aldehyde to form the corresponding hydrazone derivative. All the hydrazones derived from airborne carbonyls and pyridine-2-aldehyde (derived from ozone) are completely separated and analyzed by high-performance liquid chromatography (HPLC). In this study, we extended the BPE/DNPH-cartridge method to the diffusive sampling device (DSD-BPE/DNPH) for the simultaneous analysis of ozone and carbonyls.

## 2. Experimental

### 2.1. Apparatus and reagents

The HPLC system (Shimadzu, Kyoto, Japan) included two LC-20AD pumps, an SIL-20AC autosampler, and an SPD M20A photodiode-array detector. An analytical column with a stainless steel tube (Supelco Inc., Bellefonte, PA, USA; dimensions: 150 mm (L) × 4.6 mm (i.d.)) packed with Ascentis Express C18, 2.7 μm particles were used. The mobile phase mixture was an acetonitrile/water (55:45 (v/v)) mixture containing 5 mmol L<sup>-1</sup> ammonium acetate. The column temperature was 40 °C, and the injection volume was 10 μL. The environmental test chamber, supplied by Ohnishi Netsugaku Co., Ltd., Tokyo, Japan, was used for the sampler exposure tests. The test chamber had a volume of 34.8 m<sup>3</sup> (4.2 m × 3.6 m × 2.3 m) and equipped with an adjustable constant temperature and humidity controller. Air flow-rate in the test chamber is 1–2 m s<sup>-1</sup>. Ozone gas was generated using an Ozone Generator (model 1410, Dylec, Inc., Japan). Two air pumps (SP-100 Dual GL Sciences Inc., Saitama, Japan) and a wet gas meter (WS D-1A; Shinagawa Co., Tokyo, Japan) were used for air sample collection. The humidity and temperature of standard ozone gas were recorded using a TR-72U data logger (T&D Corporation, Japan).

Water used for HPLC and sample preparation was deionized and purified using a Milli-Q Water System equipped with a UV lamp (Millipore, Bedford, MA, USA). 2,4-Dinitrophenylhydrazine hydrochloride (>98%) and *trans*-1,2-bis(2-pyridyl)ethylene (>97%) were purchased from Tokyo Kasei Co., Ltd. (Tokyo, Japan). Acetonitrile (HPLC grade, >99.9%), 2-pyridinecarboxaldehyde (pyridine-2-aldehyde, 99%), phosphoric acid (85% solution in water), hydrochloric acid (37%), and ammonium acetate (99.999%) were purchased from Sigma-Aldrich Inc. (St. Louis, MO, USA). The BPE/DNPH-cartridge and DSD-DNPH were obtained from Supelco Inc. Silica gel (spherical, 60/80 mesh, 120 Å mean pore size) was obtained from AGC Si-Tech. Co., Ltd. (Fukuoka, Japan). Pyridine-2-aldehyde 2,4-DNPhydrazone was synthesized according to previously reported methods [29].

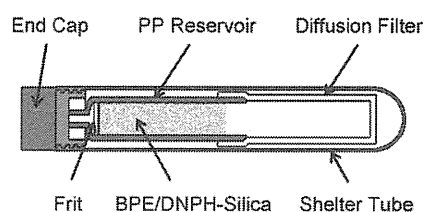


Fig. 1. Schematic representation of the DSD-BPE/DNPH sampler.

### 2.2. DSD-BPE/DNPH diffusive sampling device for collection of ozone and carbonyls

BPE/DNPH-coated silica particles. Silica gel (100 g) was washed with water (3 × 500 mL) and acetonitrile (2 × 500 mL) and then transferred to a distilling flask. DNPH HCl (1 g) was added to 200 mL water, and the solution was stirred for 10 min. After filtration through a Millipore filter (0.45 μm pore size), the precipitate was dissolved in 200 mL water and stirred for 10 min. This was repeated 3 times; lastly, the solution was filtered and added to 200 mL acetonitrile in a distilling flask with washed silica. 7 g of 2BPE and 1.2 mL of phosphoric acid were dissolved in 50 mL of acetonitrile and added to a distilling flask. Washed silica was added to the DNPH-2BPE mixture solution and dried by rotary evaporation at 40 °C.

The DSD-BPE/DNPH device comprised three sections: an exposure component made of a porous sintered polyethylene (diffusion filter), an analysis component comprising a polypropylene tubing (PP-reservoir), and an absorbent component made of BPE/DNPH-coated silica gel (Fig. 1). The DSD-BPE/DNPH device contained 250 mg of BPE/DNPH-coated silica particles. Samplers were packed in an aluminum-laminated bag and stored in a refrigerator (organic-solvent-free environment) at 4 °C.

Fig. 2 shows the scheme for the simultaneous determination of ozone and carbonyls by the DSD-BPE/DNPH method. Ozone present in the air reacts with 2BPE in the sampler to form pyridine-2-aldehyde and is thus trapped in the DSD-BPE/DNPH device. Next, pyridine-2-aldehyde reacts with DNPH in the sampler to form pyridine-2-aldehyde DNPhydrazone. Concurrently, carbonyl compounds in the air are trapped in the DSD-BPE/DNPH device via a reaction with DNPH in the sampler to form carbonyl DNPhydrazones.

In this reaction, water is required to decompose the ozonide to 2PA. Air humidity has much less influence on the reaction of 2-BPE with ozone. Above 18% relative humidity, the reaction efficiency of 2-BPE with ozone is almost complete [28].

### 2.3. Sampling and analysis of the DSD-BPE/DNPH

The sampler was removed from a heat-sealed aluminum plastic-laminated sachet. Next, the shelter tube was removed, and the absorbent was transferred from the PP-reservoir to the diffusion filter by orienting the DSD-DNPH device to an upright, vertical position. Sample exposure began at this point. After a fixed period, sampling was stopped by inverting the DSD-DNPH device to return the absorbent from the diffusion filter to the PP-reservoir, and the shelter tube was replaced. The DSD-BPE/DNPH device was then repacked in an aluminum-laminated bag and stored in a refrigerator (organic-solvent-free environment) at 4 °C.

For HPLC analysis, the PP-reservoir of the DSD-BPE/DNPH device was removed from the diffusion filter and connected to a clean 5 mL syringe. DNPH derivatives were eluted from the PP-reservoir absorbent by passing the solution through 25% dimethyl sulfoxide in acetonitrile solution containing 0.085% (v/v) phosphoric acid via the syringe to a graduated test tube over; this was done over a 1-min

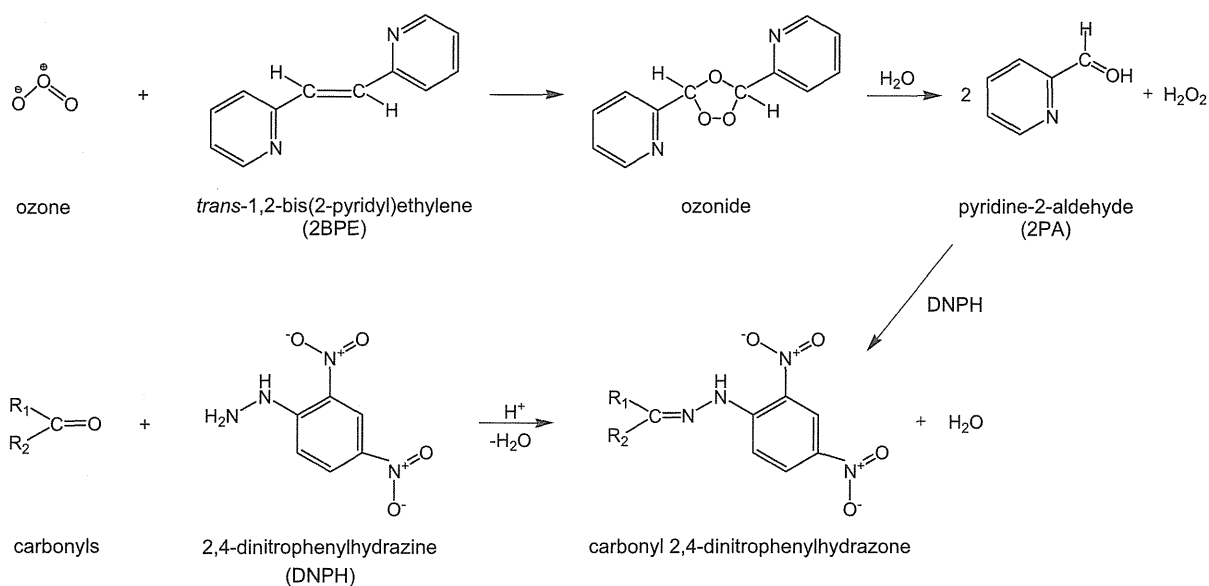


Fig. 2. Scheme for the simultaneous determination of ozone and carbonyls.

period, and 5 mL of eluate was collected. After 30 min of elution, the eluate was analyzed by HPLC.

### 3. Results and discussion

#### 3.1. HPLC analysis

The spectral profile of the 2PA-2,4-DNPhydrazone derivative revealed that the wavelength of maximum absorption was 378 nm and that the absorption coefficient was large ( $3.4 \times 10^4 \text{ L mol}^{-1} \text{ cm}^{-1}$ ) [29]; hence, this derivative could be detected in the presence of other aldehyde derivatives such as formaldehyde and acetaldehyde at a single wavelength of 360 nm. The analysis conditions for 2PA and C1–C3 carbonyl-DNPH derivatives were determined by taking into account the specifications of the HPLC conditions. Fig. 3 shows the chromatogram of a standard mixture containing pyridine-2-aldehyde (2PA), formaldehyde (FA), acetaldehyde (AA), and acetone (AC) 2,4-DNPhydrzones ( $100 \mu\text{mol L}^{-1}$ ).

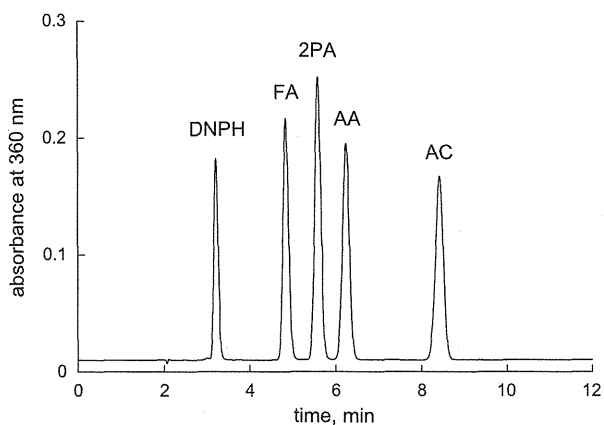


Fig. 3. Chromatogram of pyridine-2-aldehyde and other carbonyl 2,4-DNPhydrzones.

The limit of detection (LOD) and limit of quantitation (LOQ) of DSD-BPE/DNPH-HPLC analysis were calculated using the linear regression theory [30]. A standard mixture ( $100 \mu\text{L}$ ) containing 2PA, FA, AA, and AC 2,4-DNPhydrzones ( $100 \mu\text{mol L}^{-1}$ ) was introduced into the DSD-BPE/DNPH device and analyzed using the analytical conditions described above. LOD and LOQ were calculated as being three times and ten times the standard deviation obtained from the data of 10 replicate measurements, respectively (Table 1). The LOD values of DSD-BPE/DNPH-HPLC method were higher than that of DSD-DNPH-HPLC method [20]. The reason for this discrepancy was due to the fact that the concentration of DNPH in DSD-BPE/DNPH is two times higher than that of DSD-DNPH. HPLC analysis reproducibility was estimated from data of 10 samplers spiked with 5 nmol of 2,4-DNPhydrzones, including 2PA. The relative standard deviations (RSD) for 2PA, formaldehyde and acetaldehyde were 1.2%. RSD for acetone was 1.3%.

#### 3.2. Influence of 2BPE and phosphoric acid contents of DNPH-silica on the reaction with ozone

An ozone generator was operated in the environmental test chamber set at a temperature of 25 °C and a relative humidity of 60%. The ozone concentration reached a constant value of  $72 \mu\text{g m}^{-3}$  after 24 h. BPE/DNPH silica particles containing various concentrations of 2BPE, 1% (w/w) DNPH, and 0.85% (v/w) phosphoric acid were packed into the DSD-BPE/DNPH samplers and placed in the environmental test chamber for 24 h. Concurrently, active sampling was performed using the 2BPE/DNPH-cartridge (flow rate:  $100 \text{ mL min}^{-1}$ ). After air sampling, carbonyl compounds including 2PA were analyzed by HPLC. Fig. 4 shows the change in amount of 2PA with the 2BPE content of 2BPE/DNPH-silica.

Table 1  
LOD and LOQ of DSD-BPE/DNPH-HPLC method.

Compound	LOD, $\mu\text{mol L}^{-1}$	LOQ, $\mu\text{mol L}^{-1}$
2PA (ozone)	0.056	0.19
Formaldehyde	0.039	0.13
Acetaldehyde	0.054	0.18
Acetone	0.12	0.39

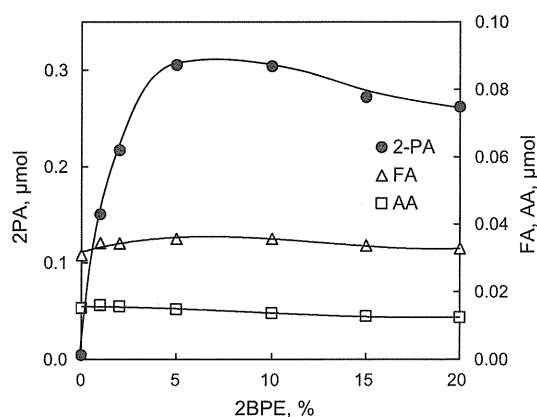


Fig. 4. Change in the amount of 2PA formed with the 2BPE content of 2BPE/DNPH-silica.

As can be seen in Fig. 4, 2PA formation increases with additional amounts of 2BPE until 5% (w/w) and slightly decreases over 10% (w/w) addition. When the 2BPE content exceeds 10% (v/w), precipitation was observed. The preferable 2BPE content for DSD-BPE/DNPH was determined to be 7% (w/w), as 2PA formation was constant in the range 5–10% (w/w).

Carbonyl compounds, including 2PA, react with DNPH to form stable hydrazones. For this reaction, a catalytic amount of acid is required. The ozone generator was operated in an environmental test chamber whose temperature and relative humidity were set to 25 °C and 60%, respectively. The ozone concentration reached a constant value of 72  $\mu\text{g m}^{-3}$  after 24 h. BPE/DNPH silica particles containing 0.01–1% (v/w) phosphoric acid, 7% (w/w) 2BPE, and 1% (w/w) DNPH were packed into the DSD-BPE/DNPH samplers and placed in the environmental test chamber for 24 h. Concurrently, active sampling was performed using the 2BPE/DNPH-cartridge, at the rate of 100  $\text{mL min}^{-1}$ . Fig. 5 shows the amount of 2PA formed as a function of the phosphoric acid content of 2BPE/DNPH-silica. 2PA formation reaches a maximum with additional amounts of 0.1% (v/w) of phosphoric acid. When the phosphoric acid content exceeds 0.1% (v/w), pyridine-2-aldehyde phosphate precipitates.

### 3.3. Simultaneous ozone and carbonyl collection

Seven DSD-BPE/DNPH samplers were placed in the environmental test chamber, which was set to conditions described above. The ozone generator was operated in the environmental test cham-

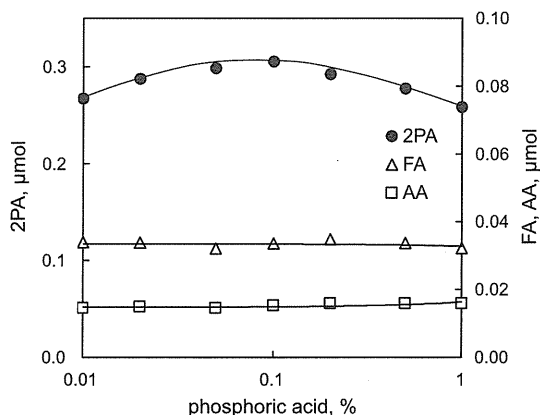


Fig. 5. Change in the amount of 2PA with the phosphoric acid content of 2BPE/DNPH-silica.

ber set to 25 °C and 60% relative humidity. The concentrations of ozone, formaldehyde, and acetaldehyde in the environmental test chamber were 72, 8.3, 8.6  $\mu\text{g m}^{-3}$ , respectively. Samplers were recovered separately every 24 h over a period of 1 week. Concurrently, DSD-DNPH samplers were measured for reference. Diurnal concentrations of ozone were calibrated with the BPE/DNPH active sampler. The active samplers were performed for 24 h at the flow rate of 100  $\text{mL min}^{-1}$ . Fig. 6 shows the amounts of 2PA (ozone), formaldehyde, and acetaldehyde collected with DSD-BPE/DNPH and DSD-DNPH from 1 to 7 days.

For ozone concentrations measured by the DSD-BPE/DNPH, a linear relationship (slope: 0.158  $\mu\text{mol d}^{-1}$ ; linear regression coefficient: 0.999) exists between the sampling duration and the ozone concentration for the first 7 days. Cumulative data obtained by using BPE/DNPH active sampling (ACT-BPE/DNPH) with a flow rate of 100  $\text{mL min}^{-1}$  and one-day sampling period showed the slope of 0.354  $\mu\text{mol d}^{-1}$ . Ozone experimental sampling rate with DSD-BPE/DNPH can be calculated from the slope ratio of the DSD-BPE/DNPH to the ACT-BPE/DNPH in Fig. 6 using the following equation:

$$\text{Ozone sampling rate by DSD-BPE/DNPH} = \frac{0.158}{0.354} \times 100 = 44.6 \text{ mL min}^{-1}$$

Additionally, the theoretical sampling rate of DSD-BPE/DNPH can be calculated from Graham's law of diffusion [20]. According to this law, the diffusion coefficient ( $D_{\text{gr}}$ ) is inversely proportional to the square root of the density ( $Z$ ) or molecular weight ( $M$ ) of the gas:

$$D_{\text{gr}} \propto \frac{1}{\sqrt{Z}} \propto \frac{1}{\sqrt{M}} \quad (1)$$

When the diffusion coefficient ( $D_f$ ) of formaldehyde is given, the diffusion coefficients of various other carbonyl compounds can be calculated from Eq. (2):

$$D_{\text{gr}} = D_f \sqrt{\frac{M_f}{M_d}} \quad (2)$$

where  $M_f$  is the molecular weight of formaldehyde, and  $M_d$  is the molecular weight of the desired compound. The sampling rates of the carbonyl compounds can be calculated from Eq. (3):

$$R = \frac{D_d}{D_f} R_f \quad (3)$$

where  $R$  is the sampling rate of the desired compound,  $D_d$  is the diffusion coefficient of the desired compound, and  $R_f$  (71.9  $\text{mL min}^{-1}$ ) is the sampling rate of formaldehyde by DSD-DNPH [20]. The theoretical sampling rate of the DSD-BPE/DNPH can be calculated from Eq. (3) because the diffusion filter is the same as DSD-DNPH. Dashed lines in Fig. 6 represent theoretical values calculated using Graham's law. For ozone, the theoretical sampling rate with DSD-BPE/DNPH is 56.9  $\text{mL min}^{-1}$  and the experimental sampling rate is approximately 80% of the theoretical value (44.6  $\text{mL min}^{-1}$ ). This suggests that ozone decomposes while passing through the diffusion filter of DSD-BPE/DNPH. However, the ozone concentration can be obtained by using the experimental sampling rate because a linear relationship (regression coefficient: 0.999) is observed between the sampling duration and the ozone concentration.

For formaldehyde measured by the DSD-BPE/DNPH, an approximately linear relationship (slope: 0.0280  $\mu\text{mol d}^{-1}$ ) is observed between the sampling duration and the ozone concentration until 5 days of sampling. Amounts collected with DSD-DNPH agreed with DSD-BPE/DNPH until 5 days; however, when sampling duration

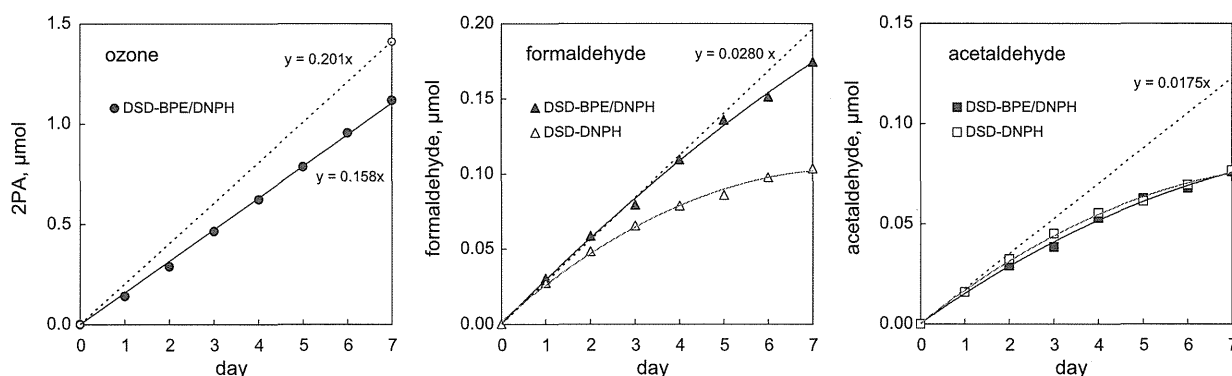


Fig. 6. Relationship between exposure time and collected amount by using DSD-BPE/DNPH and DSD-DNPH diffusive samplers. Air sampling was performed continuously for several days. Dashed lines represent theoretical values calculated from Graham's law of effusion.

was more than 6 days, the agreement gradually decreased. Experimental DSD-BPE/DNPH sampling rate can be calculated in the same way of ozone, as follows:

$$\text{Sampling rate of formaldehyde by DSD-BPE/DNPH} = \frac{0.0280}{0.0389} \\ \times 100 = 72.0 \text{ mL min}^{-1}$$

In a previous report, the DSD-DNPH sampling rate was determined to be  $71.9 \text{ mL min}^{-1}$  at a sampling duration of 24 h [20]; this value was very close to the sampling rate of DSD-BPE/DNPH. However, over 2 days, the DSD-DNPH sampling rate decreased with the sampling term. This suggested that ozone decomposes the formaldehyde hydrazone derivative [31,32] collected by DSD-DNPH and that 2BPE in DSD-BPE/DNPH acts as an ozone scrubber.

For acetaldehyde measured by the DSD-BPE/DNPH diffusive sampler (DSD-BPE/DNPH), an approximately linear relationship (slope:  $0.0175 \mu\text{mol d}^{-1}$ ) exists between the sampling duration and the ozone concentration until 2 days of sampling. Amounts collected with DSD-DNPH agreed with DSD-BPE/DNPH values until 2 days of sampling; however, when sampling duration was more than 3 days, this agreement gradually decreased. The DSD-BPE/DNPH experimental sampling rate for acetaldehyde can be calculated again as follows:

$$\text{Sampling rate of acetaldehyde by DSD-BPE/DNPH} = \frac{0.0175}{0.0295} \\ \times 100 = 59.3 \text{ mL min}^{-1}$$

In a previous report, the DSD-DNPH sampling rate was determined to be  $59.4 \text{ mL min}^{-1}$  at a sampling duration of 24 h [20]; this value was very close to the DSD-BPE/DNPH sampling rate. However, over 3 days, the DSD-DNPH and DSD-BPE/DNPH sampling rates significantly decreased with an increase in the sampling term. This suggested that ozone decomposes the acetaldehyde hydrazone derivative [31,32] collected by DSD-DNPH and DSD-BPE/DNPH. The high molecular weight carbonyls react slowly with DNPH and seemed to decompose with high concentration ozone. DSD-BPE/DNPH method is possible to measure formaldehyde and acetaldehyde under the condition of relatively high concentration of ozone ( $72 \mu\text{g m}^{-3}$ ) for 24 h.

### 3.4. High concentration and long-term ozone sampling

An ozone generator was operated at  $136 \mu\text{g m}^{-3}$  in the environmental test chamber, which was set to  $25^\circ\text{C}$  and 60% relative

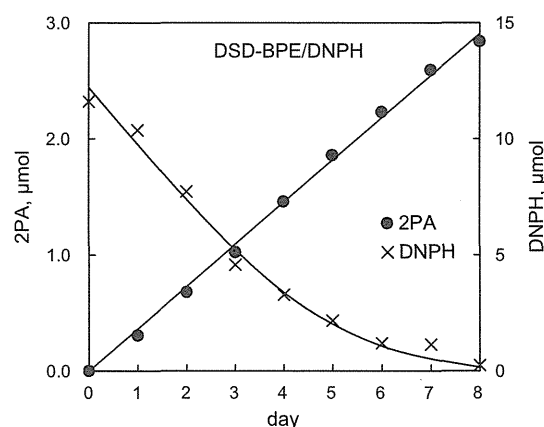


Fig. 7. Change in the amount of 2PA formed with sampling duration.

humidity. Ten DSD-BPE/DNPH samplers were placed in the environmental test chamber and recovered separately every 24 h over a period of 10 days. After air sampling, all the samplers were analyzed by HPLC. Fig. 7 shows 2PA (ozone) amounts collected with DSD-BPE/DNPH over a sampling duration of 1–8 days.

All DNPH in the DSD-BPE/DNPH was eliminated by reacting with ozone at 8 days. Until DNPH was eliminated, 2PA formation was proportional to sampling duration. During long-term sampling, 2PA, formed from ozone and 2BPE, is likely to be oxidized by excess ozone, forming pyridine-2-carboxylic acid (picolinic acid). However, this phenomenon does not occur in the DSD-BPE/DNPH method, because 2PA reacts immediately with coexistent DNPH to form the hydrazone derivative.

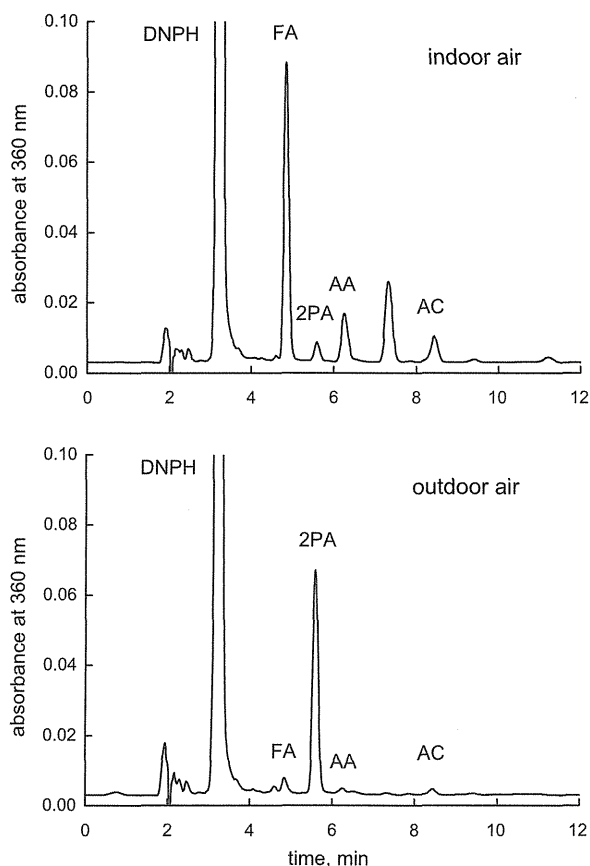
### 3.5. Measurement of indoor and outdoor air

Indoor and outdoor air was collected over the period October 19–26, 2010, using DSD-BPE/DNPH. In case of outdoor measurement, the DSD-BPE/DNPH samplers were placed on the rooftop of Chiba City's Air Monitoring Station in Japan. In case of indoor measurement, the DSD-BPE/DNPH samplers were placed in the living room of a house near by Chiba City's Air Monitoring Station. Collections were performed for 24 h; the ozone auto-analyzer at the Air Monitoring Station records every 1-h mean value obtained from every 1-min data. Fig. 8 shows carbonyl profiles of indoor and outdoor air measured during the period October 21–22, 2010. All the peaks were well separated and baseline-resolved.

**Table 2**

Ozone and carbonyl concentrations measured in indoor air (I) and outdoor air (O) collected by DSD-BPE/DNPH samplers. Concentration units are in  $\mu\text{g m}^{-3}$ . Values in parentheses indicate data measured by the auto-analyzer at the Air Monitoring Station.

Date (2010)	Weather conditions			Ozone		Formaldehyde		Acetaldehyde		Acetone	
	WS ( $\text{m}^{-1}$ )	Temp ( $^{\circ}\text{C}$ )	RH (%)	I	O	I	O	I	O	I	O
Oct, 19–20	(2.7)	(18)	(82)	3.2	42 (43)	72	3.5	25	2.6	22	3.4
Oct, 20–21	(2.2)	(18)	(93)	2.4	14 (17)	82	3.8	27	2.5	24	3.2
Oct, 21–22	(3.7)	(18)	(80)	4.4	61 (59)	61	2.8	21	2.1	19	3.3
Oct, 22–23	(2.7)	(16)	(70)	3.4	57 (57)	55	2.4	22	2.2	20	3.3
Oct, 23–24	(1.6)	(15)	(81)	3.8	43 (40)	54	3.4	21	2.2	21	3.5
Oct, 24–25	(1.4)	(16)	(94)	2.4	17 (19)	63	3.7	24	3.9	24	2.5
Oct, 25–26	(2.3)	(17)	(94)	2.0	11 (12)	86	2.5	26	2.5	18	2.2



**Fig. 8.** Comparative carbonyl 2,4-DNPhydrazone profiles of indoor air (upper chromatogram) and outdoor air (lower chromatogram).

Measured ozone and carbonyl concentrations are listed in Table 2.

Ozone concentrations in outdoor air measured by the DSD-BPE/DNPH method were very similar to those obtained by the auto-analyzer at the Air Monitoring Station, and are approximately 10 times higher than in indoor air. A negative correlation between ozone and formaldehyde concentrations was found in indoor air, with a correlation coefficient of  $-0.736$ . Formaldehyde in indoor air may be decomposed by ozone.

#### 4. Conclusions

We developed a new diffusive sampler (DSD-BPE/DNPH) for the simultaneous determination of ozone and carbonyl in air, by using 2BPE and DNPH. The carbonyls in air react with DNPH in the absorbent to form hydrazone derivatives. Concurrently,

ozone in the air reacts with 2BPE to form 2PA, which reacts immediately with DNPH to form a 2PA-hydrazone derivative. All the hydrazones derived from airborne carbonyls and 2PA (formed from ozone) are completely separated and analyzed by HPLC. The DSD-BPE/DNPH sampling rates for the carbonyls agree well with those for commercially available DSD-DNPH. The DSD-BPE/DNPH sampling rate for ozone is determined to be  $44.6 \text{ mL min}^{-1}$  by comparison with that obtained in an active sampling method. The DSD-BPE/DNPH method is advantageous because it is simple and allows for the simultaneous analysis of ozone and carbonyls.

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## Review

Derivatization of carbonyl compounds with 2,4-dinitrophenylhydrazine and their subsequent determination by high-performance liquid chromatography<sup>☆</sup>Shigehisa Uchiyama<sup>\*</sup>, Yohei Inaba, Naoki Kunugita

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## ABSTRACT

Derivatization of carbonyl compounds with 2,4-dinitrophenylhydrazine (DNPH) is one of the most widely used analytical methods. In this article, we highlight recent advances using DNPH provided by our studies over past seven years. DNPH reacts with carbonyls to form corresponding stable 2,4-DNPhydrazone derivatives (DNPhyrazones). This method may result in analytical error because DNPhyrazones have both *E*- and *Z*-stereoisomers caused by the C=N double bond. Purified aldehyde-2,4-DNPhydrazone demonstrated only the *E*-isomer, but under UV irradiation and the addition of acid, both *E*- and *Z*-isomers were seen. In order to resolve the isometric problem, a method for transforming the C=N double bond of carbonyl-2,4-DNPhydrazone into a C–N single bond, by reductive amination using 2-picoline borane, has been developed. The amination reactions of C1–C10 aldehyde DNPhyrazones are completely converted into the reduced forms and can be analyzed with high-performance liquid chromatography. As a new application using DNPH derivatization, the simultaneous measurement of carbonyls with carboxylic acids or ozone is described in this review.

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## 1. Introduction

Carbonyl compounds such as aldehydes and ketones have received much attention as hazardous substances in studies of environmental and biological chemistry. Long-term exposure to relatively high levels of formaldehyde is known to increase the risk to human [1–4]. In 2004, the International Agency for Research on Cancer (IARC) reclassified formaldehyde as a human carcinogen that causes nasopharyngeal cancer and also concluded that there is a “strong but not sufficient evidence for

a causal association between leukemia and occupational exposure to formaldehyde” [5]. It is a great public health problem whether there is an association between formaldehyde exposure and leukemia. IARC classification of formaldehyde has started controversial discussions. Recently, some new epidemiological reports including meta-analysis were published [1,3,6–10]. The two studies [7,8] found an elevated mortality rate from myeloid leukemia in individuals occupationally exposed to formaldehyde. In addition, formaldehyde affects indoor air quality, and is known to trigger acute adverse health effects such as skin, eye, nose, and throat irritation. It has been reported that formaldehyde is significantly associated with a higher risk of the “Sick Building Syndrome (SBS)” [11–13]. Acetaldehyde, an analogue of formaldehyde, is listed as possibly carcinogenic to humans (Group 2B) by IARC. Ethanol in alcoholic drinks is mainly oxidized in the liver by alcohol dehydro-

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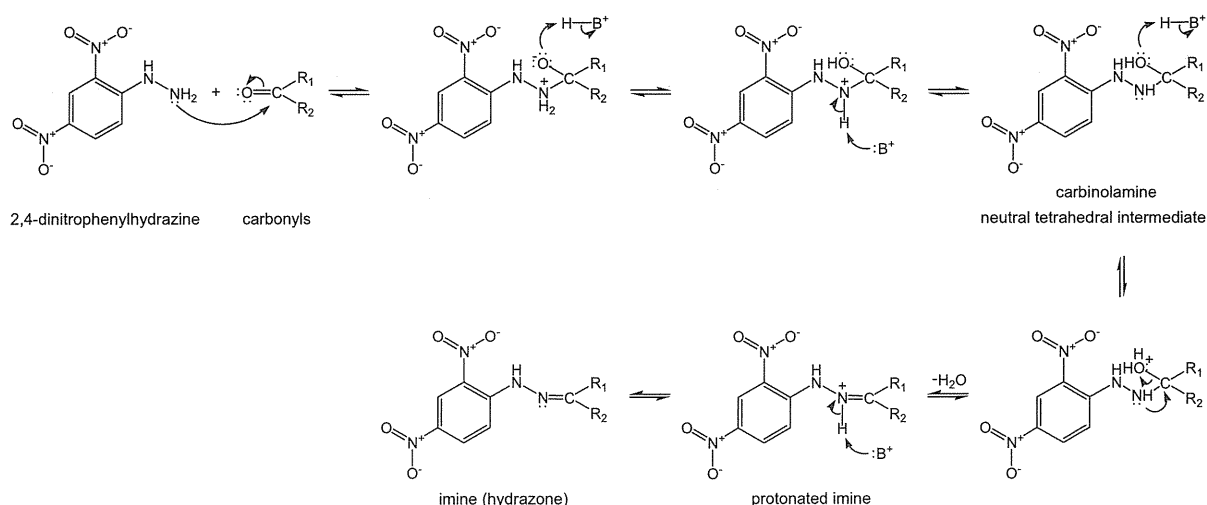


Fig. 1. Scheme of the derivatization reaction of DNPH with carbonyls.

genases to acetaldehyde. Acetaldehyde is subsequently oxidized to acetic acid by aldehyde dehydrogenase 2 (ALDH2). Approximately 40% of Japanese have single nucleotide polymorphisms (SNPs) of the ALDH2 gene. The ALDH2 \*2 allele produces an inactive protein subunit, which is unable to metabolize acetaldehyde. Exposure to high levels of acetaldehyde may be responsible to increase the risk of head and neck cancer and esophageal cancer [14–16]. Estimation of aldehydes is also the most common approach for the study of lipid peroxidation [17]. Malonaldehyde can be very useful as a presumptive marker for the development of oxidative stress in tissues and plasmas [18]. Determination of hexanal as an indicator of the lipidic oxidation state in some food samples has been presented [17].

The specific reaction of carbonyl compounds with 2,4-dinitrophenylhydrazine (DNPH) forming the corresponding 2,4-DNPhhydrazones is one of the most important qualitative and quantitative methods in organic analysis. This method has been used to measure aldehydes and ketones in plasma [18–20], urine [21–23] and other biological samples [24–26], as well as environmental air [27] and water samples [28]. It was first published by Allen [29] and Brady [30]. Carbonyl compounds react with DNPH to form hydrazones as Fig. 1 [31]. In the first step of the mechanism for hydrazone formation, the amine attacks the carbonyl carbon. Gain of a proton by the alkoxide ion and loss of a proton by the ammonium ion form a neutral tetrahedral intermediate. The neutral tetrahedral intermediate, called a carbinolamine, is in equilibrium with two protonated forms. Protonation can take place on either the nitrogen or the oxygen atom. Elimination of water from the oxygen-protonated intermediate forms a protonated hydrazone that loses a proton to yield the hydrazone. Hydrazone formation is reversible. In acidic aqueous solutions, the hydrazone derivatives are hydrolyzed back to the carbonyl compound and DNPH, and then the reaction attains equilibrium. The main advantage of the DNPH derivatization method is the ability to analyze various aldehydes and ketones simultaneously in a complex mixture. Sampling can be performed using acidic solutions of DNPH in impingers [32] or with acidic solid sorbents coated with DNPH in a cartridge. A number of cartridge devices containing solid sorbents coated with DNPH have recently been provided for sampling aldehydes. The solid sorbents include XAD-2 [33,34], silica gel [35,36], glass beads [37], octadecylsilane bonded silica gel [38], Florisil [39], and glass fiber filters [40]. In aqueous samples, an acidic solution of DNPH is used to form the corresponding hydrazones followed by high per-

formance liquid chromatography (HPLC) separation and ultraviolet (UV) detection at 360 nm (depending on the absorption maximum wavelength of the hydrazones) [28,41–43] or mass spectrometry (MS) [44–46]. Due to the importance of the method, it has been introduced as a standard procedure by several national standardization bodies. Recent research has resulted in the identification of chemical interferences caused by the presence of ozone [47–49] or nitrogen dioxide [50].

This review looks at the fundamental principles of and new applications for the derivatization of carbonyl compounds with DNPH; mainly through our studies over past seven years.

## 2. Isomerization of carbonyl 2,4-DNPhhydrazones

Usually, the DNPH derivatizations are performed under acidic conditions and 2,4-DNPhhydrazone derivatives are separated by means of HPLC followed by detection using UV spectrophotometry at 360 nm (depending on the absorption maximum wavelength of the hydrazones). However, this DNPH derivatization method may cause an analytical problem as 2,4-DNPhhydrazones have both *E*- and *Z*-stereoisomers due to the C=N double bond. Fig. 2 shows the structure of *E*- and *Z*-stereoisomers of acetaldehyde DNPhhydrazone. The formation of isomeric 2,4-DNPhhydrazones from unsymmetrical carbonyl compounds in the liquid phase has long been known [51–53]. Behforouz et al. [54] and Tayyari et al. [55] reported that a trace of acid catalyzed the *E*-*Z* isomerization, which was detected via melting point anomalies. However, until recently, this evidence seemed irrelevant with regard to possible analytical problems it would cause in the determination of aldehydes and ketones. Purified alkanal-2,4-DNPhhydrazones demonstrated only the *E*-isomer. However under UV irradiation and the addition of acid, both *E*- and *Z*-isomers were seen [56]. The spectral patterns of *Z*-isomers were different from those of *E*-isomers and the absorption maximum wavelengths were shifted towards shorter wavelengths by 5–8 nm. Fig. 3 shows the variation of *Z*- to *E*-isomer ratios of acetaldehyde and propanal DNPhhydrazone derivatives with various phosphoric acid concentrations. An equilibrium *Z/E* isomer ratio was observed in 0.02–1% (v/v) phosphoric acid solutions. Propanal- and other aldehyde-2,4-DNPhhydrazone derivatives showed similar behavior. The isomer ratios of alkanal-2,4-DNPhhydrazones are listed in Table 1. In the case of acetaldehyde- and propanal-2,4-DNPhhydrazones, the equilibrium *Z/E* isomer ratios were 0.32 and 0.14, respectively. However, when irradiated with ultraviolet light



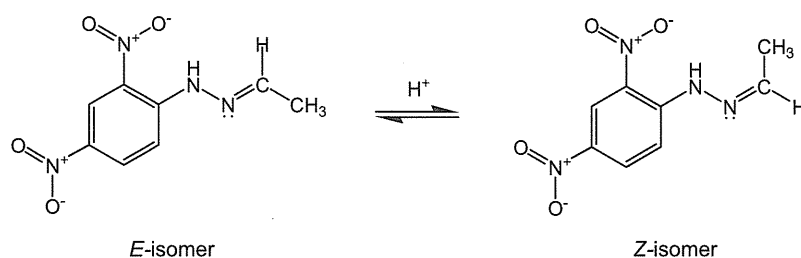


Fig. 2. Chemical structures of *E*- and *Z*-stereoisomers of acetaldehyde DNPhhydrazone.

at 364 nm, the isomer ratios were increased beyond this constant ratio and reached 0.55 and 0.33, respectively. Zero-order rates for decreases of aldehyde derivatives were observed under UV irradiation (364 nm). However, the decreases in concentration were not observed in phosphoric acid solutions.

Similar to alkanals, purified alkenal-2,4-DNPhhydrazone derivatives comprise only the *E*-isomer. However, partial isomerization to the *Z*-isomer occurs upon the addition of acid to attain an equilibrium isomer ratio [57]. The UV–visible spectral properties of the isomers differ; the *Z*-isomer exhibits a 6–10 nm lower absorption maximum wavelength compared to the *E*-isomer. Alkenal-2,4-DNPhhydrazones having a C=C double bond at the 2- or 3-position of the alkenal exhibited similar absorption maximum wavelengths with an equilibrium isomer ratio (0.035) that was much lower than those of other alkenals. The isomer ratio of alkenal-2,4-DNPhhydrazones is listed in Table 1. The C=C double bond at the 3-position migrates to a position of conjugation with the C=N double bond during hydrazone synthesis to form

a stabilized molecular structure. Alkenal-2,4-DNPhhydrazones having a double bond at the 4-position or greater exhibited similar absorption maximum wavelengths and equilibrium isomer ratio (0.14) to alkenal-2,4-DNPhhydrazones. The quantitative analysis of carbonyl compounds using DNPH is usually conducted in the presence of an acid catalyst. Consequently, the solution of the direct extract prepared for HPLC or GC analysis contains both *E*- and *Z*-isomers.

In the case of ketones, purified ketone-2,4-DNPhhydrazones were present only as the *E*-isomer [58]. When acid was added, both *E*- and *Z*-isomers were seen. The isomer ratios of ketone-2,4-DNPhhydrazones are listed in Table 1. In the case of 2-butanone-, 2-pentanone- and 2-hexanone-2,4-DNPhhydrazone, the equilibrium *Z/E* isomer ratios were 0.20, 0.21 and 0.22, respectively. In addition, when trace water was added to the hydrazone derivatives in acetonitrile solution, the concentrations of ketone derivatives were seen to decrease and the concentration of free DNPH was seen to increase. The decomposition rate of 2-butanone-2,4-DNPhhydrazone was dependent on the concentration of acid-catalyst and reached an equilibrium state – carbonyl, DNPH, hydrazone-derivative and H<sub>2</sub>O – within 10 h at 0.1 mol/L phosphoric acid solution. The equilibrium constants of ketone-2,4-DNPhhydrazones,  $[\text{carbonyl}][\text{DNPH}]/[\text{hydrazone}][\text{H}_2\text{O}]$ , were relatively large and ranged from  $0.74 \times 10^{-4}$  to  $5.9 \times 10^{-4}$ . Hydrazone derivatives formed from 2-ketones such as 2-pentanone,

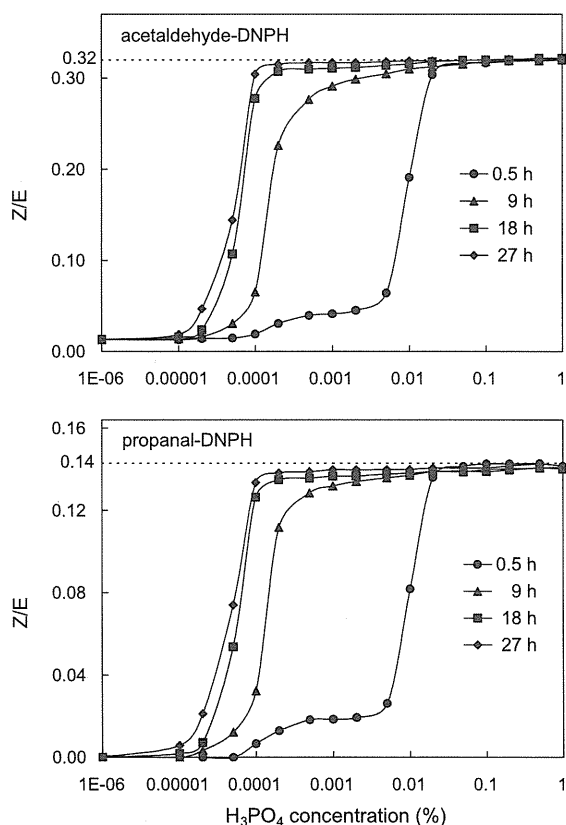


Fig. 3. The changes in the isomer ratios of acetaldehyde and propanal DNPhhydrazone with phosphoric acid. Reproduced with permission from Fig. 5 in Ref. [56].

Table 1

The isomer ratio and maximum absorption wavelengths of (*E*-) and (*Z*-) isomers of DNPhhydrazone derivatives at 50/50 (v/v) acetonitrile/water.

Carbonyls	Isomer ratio <i>Z/E</i>	$\lambda_{\text{max}}$ (nm) <i>Z</i> -isomer	$\lambda_{\text{max}}$ (nm) <i>E</i> -isomer
<b>Alkanals</b>			
Formaldehyde	n.a.	356	
Acetaldehyde	0.32	360	365
Propanal	0.14	358	366
Butanal	0.15	358	365
Pentanal	0.15	358	365
Hexanal	0.16	358	365
Heptanal	0.15	358	365
Octanal	0.15	358	364
Nonanal	0.15	358	364
Decanal	0.16	358	364
<b>Alkenals</b>			
2-Propenal	0.018	367	374
<i>trans</i> -2-Butenal	0.035	373	383
<i>trans</i> -2-Pentenal	0.035	373	383
<i>trans</i> -2-Hexenal	0.035	373	383
<i>trans</i> -2-Heptenal	0.035	373	383
<i>trans</i> -2-Octenal	0.035	373	383
<i>trans</i> -2-Nonenal	0.036	373	383
<i>trans</i> -2-Decenal	0.036	373	383
<b>Ketones</b>			
2-Propanone	n.a.	369	
2-Butanone	0.20	367	369
2-Pentanone	0.21	367	368
2-Hexanone	0.22	367	370

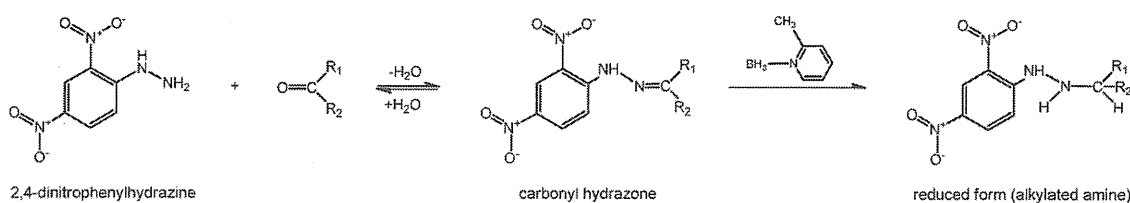


Fig. 4. Scheme of the reductive amination of carbonyl 2,4-DNPhyrazones with 2-picoline borane.

2-hexanone and 4-methyl-2-pentanone showed lower equilibrium constants than corresponding 3-ketones. Consequently, only a minimum concentration of catalytic acid must be added. The better method for the determination of ketone-2,4-DNPhyrazones by HPLC or GC is to add phosphoric acid to both the standard reference solution and samples, forming a 0.001 mol/L acid solution, and analyzing after 27 h.

### 3. Reductive amination of aldehyde 2,4-DNPhyrazones

As mentioned above, the traditional method for the measurement of carbonyl compounds, using DNPH to form the corresponding 2,4-DNPhydrazone derivatives, is subject to analytical errors because DNPhyrazones form both *E*- and *Z*-stereoisomers as a result of the C=N double bond. In order to resolve the isometric problem, it is necessary to transform the C=N double bond to a C-N single bond through use of a reducing agent. Various kinds of reducing agents, such as sodium cyanohydrinborate ( $\text{NaBH}_3\text{CN}$ ) [59,60], sodium triacetoxyborohydride ( $\text{Na}(\text{OAc})_3\text{BH}$ ) [61–65], pyridine-borane ( $\text{pyr-BH}_3$ ) [66–68], titanium(IV) isopropoxide/sodium borohydride ( $\text{Ti}(\text{Oi-Pr})_4/\text{NaBH}_4$ ) [69–72], borohydride exchange resin [73], zinc borohydride/silica gel ( $\text{Zn}(\text{BH}_4)_2/\text{SiO}_2$ ) [74], and phenylsilane/dibutyltin dichloride ( $\text{PhSiH}_4/\text{Bu}_2\text{SnCl}_2$ ) [75] have been developed for this conversion. The choice of the reducing agent is very critical to the success of the reaction, since the reducing agent must reduce imines selectively. Pyridine-borane has been widely used as a reductive amination reagent for aldehydes and ketones [68]. However, this reagent is quite unstable to heat and attempted distillation of the liquid residue at reduced pressures sometimes results in violent decompositions [76–78]. Thus, extreme care must be used if this reagent is handled in large quantities. Sato et al. [79] have developed an expeditious, easy-to-handle and environmentally friendly approach to the synthesis of a variety of amines through a three-component one-pot reaction of carbonyl compounds, amines, and 2-picoline borane. The later is a thermally stable transparent solid that be stored on a shelf for months without appreciable loss of the reduction capability. The use of 2-picoline borane eliminates the problems encountered with the use of other less stable reducing agents such as pyridine borane.

Recently, we developed a method for transforming the C=N double bond into a C-N single bond, using reductive amination of DNPhydrazone derivatives with 2-picoline borane [80]. Reductive amination of aldehyde DNPhyrazones is achieved by adding 2-picoline borane to the acetonitrile solution used to elute the DNPH-cartridge. Fig. 4 shows a scheme of the reductive amination of carbonyl 2,4-DNPhyrazones with 2-picoline borane. Aldehyde DNPhyrazones (C1–C10) are completely converted into their reduced forms within 40 min in the presence of 1 mmol/L 2-picoline borane and 20 mmol phosphoric acid. Fig. 5 shows the chromatograms at the state of coexistent aldehyde DNPhyrazones and their reduced forms. Before the addition of 2-picoline borane, only *E*- and *Z*-DNPhydrazone isomers are detected (upper panel). After the addition of 2-picoline borane, peaks of the reduced

forms began to appear between the *Z*- and *E*-isomer peaks of the corresponding DNPhydrazone. Twenty minutes after the addition of 2-picoline borane solution, reductive amination proceeds to 46–50% (middle panel). Sixty minutes later (80 min total), all DNPhydrazone derivatives, including *Z*- and *E*-isomers, are completely converted to their respective reduced forms (lower panel). These reduced forms are very stable and do not change when stored for two weeks at room temperature. The absorption maximum wavelengths of the reduced forms from C1 to C10 aldehyde DNPhyrazones were 351–352 nm, which shifted 6–7 nm towards shorter wavelengths when compared to the corresponding

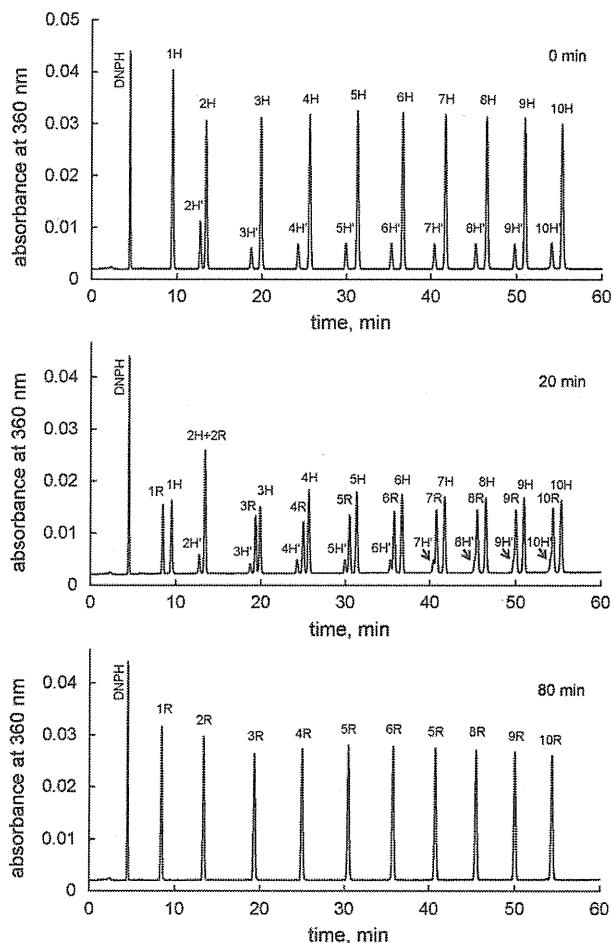


Fig. 5. Chromatographic profiles of DNPhyrazones and their reduced forms changing with reaction time. Number of peak name indicates carbon number of precursor aldehyde (1: formaldehyde, 2: acetaldehyde, 3: propanal, 4: butanal, 5: pentanal, 6: hexanal, 7: heptanal, 8: octanal, 9: nonanal, and 10: decanal). "H" indicates DNPhydrazone derivative and "R" indicates reduced form of DNPhydrazone derivative. Prime sign indicates *Z*-isomer of DNPhydrazone derivative. Reproduced with permission from Fig. 3 in Ref. [80].

DNPhydrazones. The molar absorption coefficients were  $1.5 \times 10^4$  (C1)– $2.2 \times 10^4$  L/mol/cm (C10). Complete separation between C1 and C10 aldehyde DNPhydrazones and the corresponding reduced forms can be achieved by operating the HPLC in gradient mode using an Ascentis RP-Amide column (150 mm  $\times$  4.6 mm i.d.). The RSDs of DNPhydrazone (*Z + E*) peak areas ranged from 0.40 to 0.66 and those of the corresponding reduced forms ranged from 0.26 to 0.41. This demonstrates that the reductive amination method gave improved HPLC analytical precision because of the absence of stereoisomers.

#### 4. Derivatization of phthalaldehydes

Glutaraldehyde is a powerful biocide that was first introduced in 1963. Until relatively recently it has been the only widely available disinfectant for the reprocessing of flexible endoscopes and other heat-sensitive equipment. Orthophthalaldehyde (OPA) was introduced in 1999 as a safer alternative to glutaraldehyde, even though there was little evidence available to support such claims. OPA is a potential dermal and respiratory sensitizer and irritates the skin and respiratory tract [81]. Various analysis methods for difunctional glutaraldehyde have been developed. For the most part, they are based on solid substrate sampling and involve the use of derivatizing agents [82–86]. When derivatized with DNPH, OPA was collected using a silica gel cartridge impregnated with acidified 2,4-dinitrophenylhydrazine (DNPH-cartridge) and derivatives were analyzed by HPLC. The derivatization was examined by comparing the process with three phthalaldehyde isomers (ortho-, iso- and tere-) [87]. Fig. 6 shows chromatograms of OPA-DNPhydrazone, isophthalaldehyde (IPA) - DNPhydrazone and terephthalaldehyde (TPA) - DNPhydrazone synthesized with a fourfold molar excess of DNPH and with a fourfold molar excess of aldehyde. Chromatograms resulting from the use of excess aldehyde or excess DNPH are designated with the suffix “-A” or “-D” respectively. Only one peak is observed in OPA-DNPhydrazone, and two peaks are observed in IPA-DNPhydrazone and TPA-DNPhydrazone. In the early eluting peaks, peak areas of IPA-A and TPA-A are much larger than those of corresponding IPA-D and TPA-D. In the late eluting peaks, peak areas of IPA-D and TPA-D are much larger than those of corresponding IPA-A and TPA-A. Dialdehydes such as phthalaldehydes may give two types of derivatives, namely mono- and bis-DNPhydrazone derivatives. The early eluting peaks are mono-DNPhydrazone derivatives and late eluting peaks are bis-DNPhydrazone derivatives. In the case of iso- and terephthalaldehyde, derivatives synthesized with excess aldehyde consisted mainly of mono-derivatives and derivatives synthesized with excess DNPH consist mainly of the bis-derivative. In the case of OPA, only the bis-derivative was detected and the mono-derivative was never observed under any conditions. OPA is completely retained by the DNPH-cartridge. The derivatization reaction was incomplete and unreacted OPA was flushed from the cartridge during the subsequent solvent extraction process. Unreacted OPA and DNPH react in the extraction solvent solution. Immediately after solvent extraction, both mono- and bis-DNPhydrazone derivatives of OPA are present in the solution. Over time, the mono-derivative decreased and the bis-derivative increased in concentration until only the bis-derivative remained; allowing accurate determination of the OPA concentration. The transformation of mono-derivative to bis-derivative was faster in polar aprotic solvents such as acetonitrile, dimethyl sulfoxide and ethyl acetate. Transformation is found to occur most quickly in acetonitrile solvent and is completed within 4 h. It is suggested that the reaction of OPA and DNPH proceeded in polar aprotic solvents and mono-derivative was completely transformed to bis-derivative according to the reaction of Fig. 7. It is possible to measure OPA as

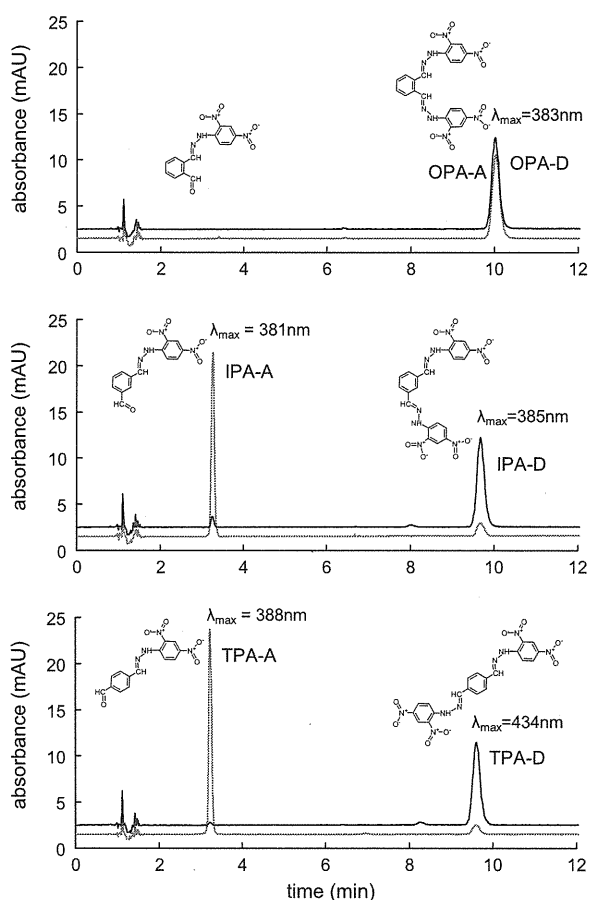


Fig. 6. HPLC chromatograms of OPA-DNPhydrazone (upper), IPA-DNPhydrazone (middle) and TPA-DNPhydrazone (lower) at maximum wavelengths by photo diode array detector. Light-colored chromatograms indicate the derivatives synthesized with excess of aldehyde and dark-colored chromatograms indicate the derivatives synthesized with excess of DNPH. The concentration was 2 mg/L. Reproduced with permission from Fig. 1 in Ref. [87].

the bis-derivative using a DNPH impregnated silica cartridge and HPLC analysis.

#### 5. Application of DNPH derivatization to new analytical methods

##### 5.1. Simultaneous determination of carboxylic acids and carbonyls

It has been recognized that DNPH only reacts with the carbonyl functional groups in aldehydes and ketones and not with those in compounds such as carboxylic acids, esters and amides. However in our experiments, we have found that carboxylic acids such as formic acid and acetic acid react with DNPH to form the corresponding carboxylic-2,4-dinitrophenylhydrazides under specific conditions [88]. A DNPH-cartridge saturated with formic acid vapor becomes gradually discolored and completely changes to light yellow in 6 h at 25 °C. The HPLC chromatogram of the eluant from this DNPH-cartridge indicates complete consumption of DNPH accompanied with formation of formic-2,4-dinitrophenylhydrazide (formic-DNPhydrazone). Fig. 8 shows the peak area changes with time of DNPH and formic-DNPhydrazone at wavelength 360 nm. Acetic acid, propionic acid and butyric acid exhibit similar behavior with longer reaction time in order of

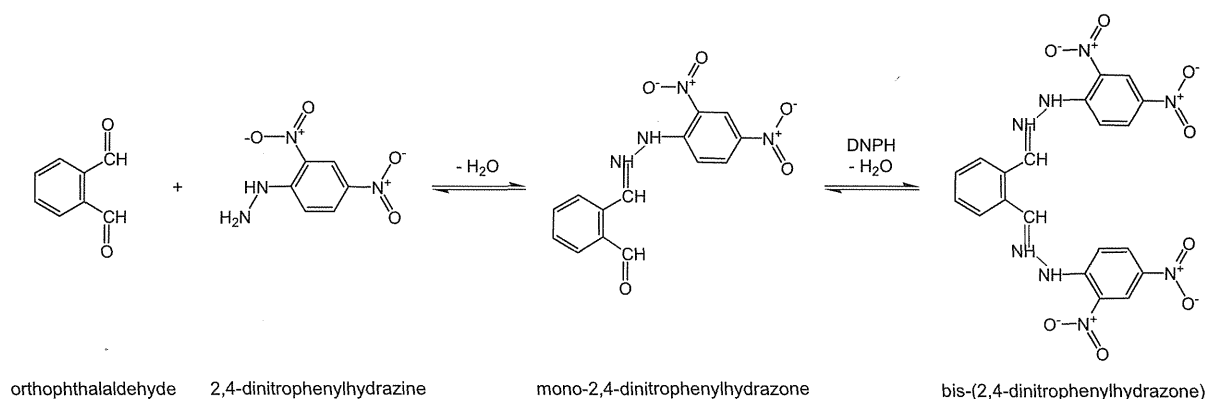


Fig. 7. Scheme of the derivatization reaction of DNPH with orthophthalaldehyde.

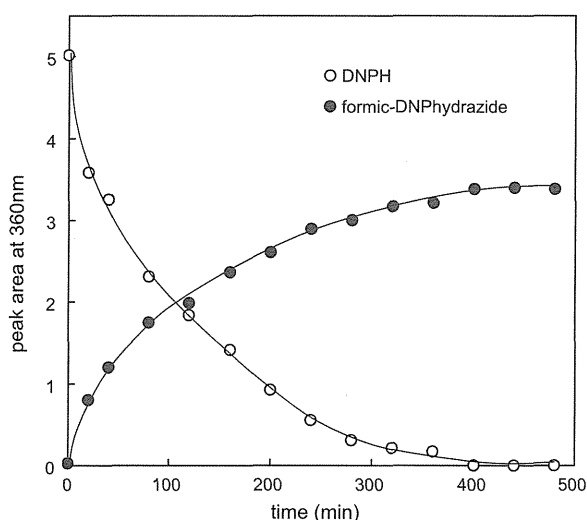


Fig. 8. The reaction of adsorbed formic acid and DNPH with time. ( $\lambda = 360$  nm). Reproduced with permission from Fig. 1 in Ref. [88].

increasing carbon number. Fig. 9 shows the derivatization reaction of DNPH with carboxylic acid. It is suggested that carboxylic acids react with DNPH to initially form corresponding hydrazone derivatives, which then isomerize to hydrazides by keto-enol tautomerization. These hydrazide derivatives have excellent thermal stability with melting points higher than those of the corresponding hydrazones by 32–50 °C. They exhibit maximum absorption wavelengths of 331–334 nm and molar absorption coefficients of  $1.4 \times 10^4$  L/mol/cm. In reversed-phase HPLC analysis, the separations of hydrazide and hydrazones derivatives may be incomplete. The retention times of DNPhydrazone peaks vary with mobile phase

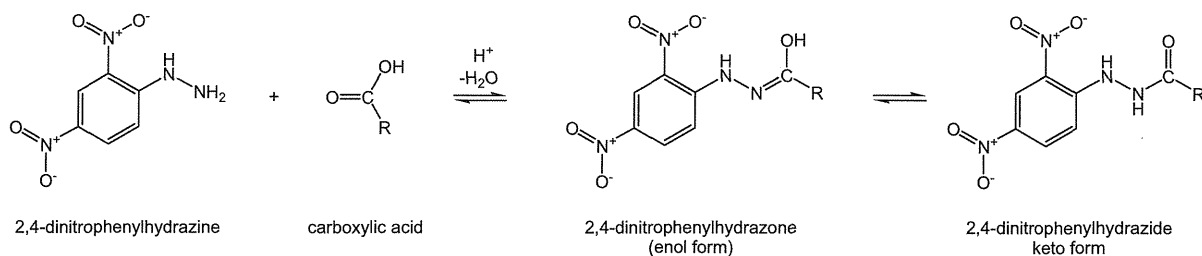
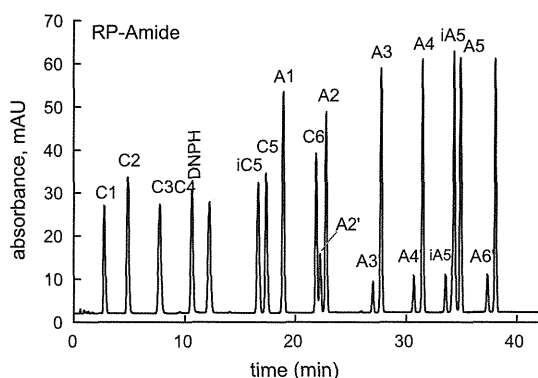


Fig. 9. Scheme of the derivatization reaction of DNPH with carboxylic acid.

pH. The addition of base to the mobile phase shortens the retention times of C1–C6 DNPhydrazone peaks and shifts the UV/vis spectrum profiles to longer wavelengths. Under the conditions of 0, 0.1, and 1.0 mmol/L dibasic potassium phosphate, the spectra of formic DNPhydrazone are unimodal with a maximum wavelength of 339 nm, bimodal with a maximum wavelength of 339 and 423 nm, and unimodal with a maximum wavelength of 423 nm, respectively. The DNPhydrazone derivatives of carboxylic acids exist in equilibrium with their enol tautomer and exhibit an isosbestic point at 370 nm. Complete separation of C1–C6 carboxylic acids and aldehydes was achieved on an RP-Amide column with the use of ACN–H<sub>2</sub>O (40:60) containing dibasic potassium phosphate (0.1 mmol/L) as the mobile phase and UV detection at 370 nm. Fig. 10 shows chromatogram of C1–C6 hydrazide and hydrazone derivatives using an RP-Amide C16 column. The derivatization reaction to hydrazide progressed essentially to completion for the DNPH-cartridges containing 0.2–1% (v/w) phosphoric acid. The best condition for the simultaneous measurement of carboxylic acids and aldehydes is 1% (v/w) phosphoric acid because acidic conditions are needed for the measurement of aldehydes. Cartridges packed with DNPH-coated silica particles (DNPH-cartridge) are used for sampling formic acid and aldehydes. Formic acid is physically adsorbed on the silica particles as the first step of the sampling mechanism. A gradual reaction with DNPH follows. Formic acid reacts very slowly with DNPH at room temperature (20 °C), but reacts completely at 80 °C over 4 h.

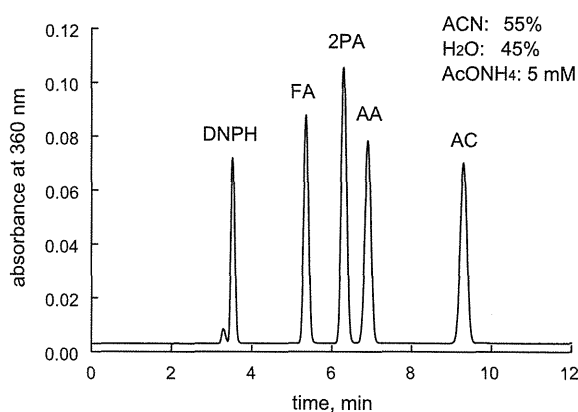
## 5.2. Simultaneous determination of ozone and carbonyls

A new method for the simultaneous determination of ozone and carbonyls in air using a two-bed cartridge system has been developed [89,90]. Each bed consists of reagent-impregnated silica particles. The first bed contains *trans*-1,2-bis-(2-pyridyl)ethylene (2BPE) while the second contains 2,4-dinitrophenylhydrazine (DNPH). Fig. 11 shows the reaction pathways for the simulta-



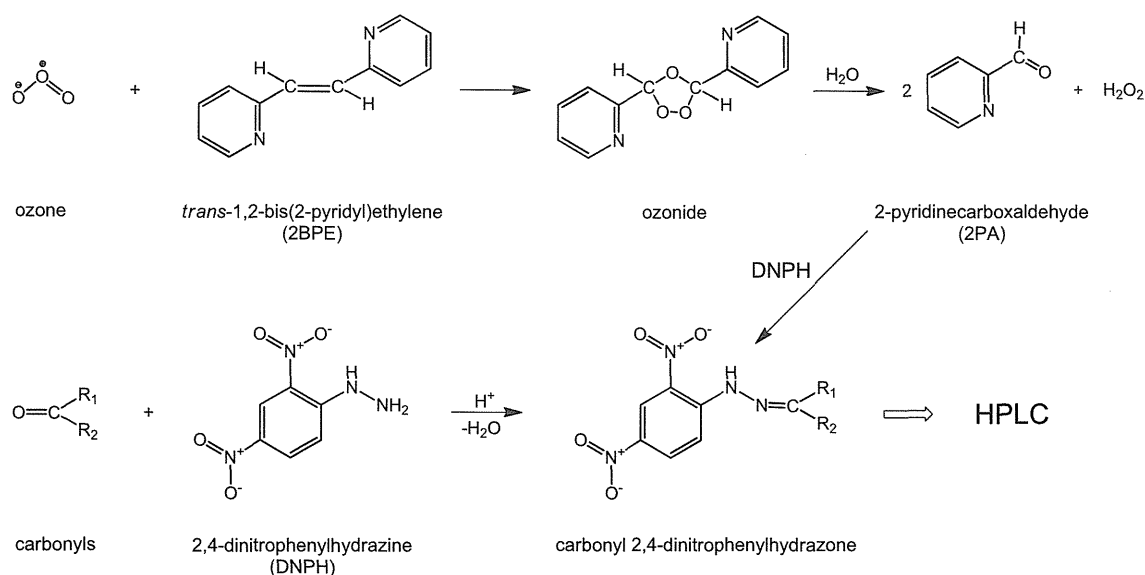
**Fig. 10.** Chromatographic profiles of C1–C6 carboxylic-DNPhydrazides and aldehyde-2,4-DNPhydrazones on an Ascentis RP-Amide column (100  $\mu\text{mol/L}$ ) at maximum absorption wavelengths between 300 nm and 500 nm. A prime sign indicates the Z-isomer C1, formic acid DNPhydrazide; C2, acetic acid DNPhydrazide; C3, propionic acid DNPhydrazide; C4, butyric acid DNPhydrazide; i-C5, i-pentanoic acid DNPhydrazide; n-C5, n-pentanoic acid DNPhydrazide; C6, hexanoic acid DNPhydrazide, A1, formaldehyde DNPhydrazide; A2, acetaldehyde DNPhydrazide; A3, propionaldehyde DNPhydrazide; A4, butyraldehyde DNPhydrazide; i-A5, i-pentanal DNPhydrazide; n-A5, n-pentanal, DNPhydrazide; A6, hexanal DNPhydrazide.

neous determination of ozone and carbonyls. Air samples are drawn through the cartridge first through the 2BPE bed and then through the DNPH. Ozone in the air sample is trapped in the first bed by the 2BPE-coated silica particles to produce pyridine-2-aldehyde. Airborne carbonyls pass unimpeded through the 2BPE and are trapped in the second bed by the DNPH-coated silica particles. They produce carbonyl DNPhydrazones. Fig. 12 shows the chromatographic profiles of 2PA (derived from ozone) and carbonyl DNPhydrazone derivatives. DNPH and carbonyl 2,4-DNPhydrazones are not influenced by ozone because of effective trapping by the 2BPE. Extraction is performed in the direction reverse to air sampling. When solvent is eluted through the 2BPE/DNPH-cartridge, excess DNPH is washed into the 2BPE bed where it reacts with pyridine-2-aldehyde and forms the corresponding hydrazone derivative. The use of a 2BPE/DNPH-cartridge has made possible the simultaneous determination of ozone



**Fig. 12.** Chromatogram of pyridine-2-aldehyde and other carbonyl 2,4-DNPhydrazones. Reproduced with permission from Fig. 2 in Ref. [90].

and carbonyls. A separate ozone scrubber is not necessary with the 2BPE/DNPH cartridge because the 2BPE portion of the sampler serves this function. Initially, *trans*-1,2-bis-(4-pyridyl)ethylene (4BPE) was used for the BPE/DNPH-cartridge [89]. However, the method suffered from long reaction times in the eluate, low solubility of the DNPH derivative and a strong dependence on atmospheric moisture. These problems could be overcome using *trans*-1,2-bis-(2-pyridyl)ethylene (2BPE) in place of 4BPE [90]. The efficiency of the reaction of ozone with 2BPE to form pyridine-2-aldehyde (2PA) is higher than the corresponding reaction with 4-BPE. Under the optimized elution conditions, the reaction times of 2PA and 4PA with DNPH are within 15 min and 120 min, respectively. During elution from the sampling cartridge, 2PA formed from 2-BPE and ozone is easier to dissolve in the elution solvent. A stronger influence of humidity was observed in ozone recovery by the 4-BPE/DNPH method. 2BPE exhibits a maximum reaction efficiency of 84% at 32% R.H., while 49% R.H. is required for 4BPE to attain a maximum reaction efficiency of 82%. Humidity has much less influence on the reaction of 2-BPE with ozone. Above 18% R.H., the reaction efficiency of 2-BPE with ozone is in the range 80–84%. Thus, 2-BPE is the more useful reagent for ozone analysis. The measured concentrations of ozone and carbonyls by the improved 2-BPE/DNPH



**Fig. 11.** Scheme of the simultaneous determination of ozone and carbonyls.

method corresponded with the values obtained using an ozone auto analyzer and a DNPH cartridge coupled with a KI-ozone scrubbing cartridge.

## 6. Conclusions

The specific reaction of carbonyl compounds with DNPH forming the corresponding DNPhydrazones is one of the most important qualitative and quantitative methods in analytical chemistry. In this review, basic research such as isomerizations of DNPhydrazones and reductive amination of aldehyde 2,4-DNPhydrazones were described. Moreover, applications of new analytical methods, such as the analyses of carboxylic acids and ozone, were introduced. We expect that the traditional DNPH derivatization method will be more useful to analyze carbonyls or other compounds.

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# 健康建築の構図

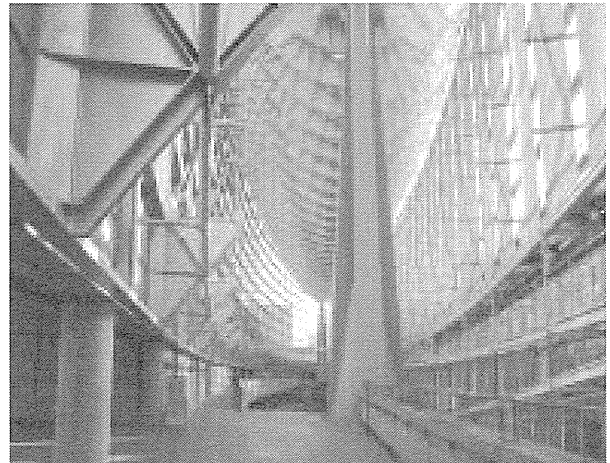
国立保健医療科学院  
統括研究官 大澤 元毅

## 1. はじめに

建築や都市は、住人に必要な資源を供給処理したり、外敵や外乱を防いで、利便性とやすらぎをもたらすシステムである。しかし、先進国では成熟して当たり前になりすぎ、残念なことだが災害時などで機能を失なってみないとそのありがたさも分かりにくくなってしまった。その一方、近年は新しい状況(例えばエネルギー制約、新技術・新材料導入や耐震設計)、新しいパラダイム(環境志向や健康指向)がその調和を乱す事態が増えてきた。そこでは、安全や安心に対する期待への裏返しも重なって、信頼が裏切られた時の不安や不信が、かえって大きくふくらみかねない皮肉な状況さえみられている。

日進月歩、多様化する都市と建築には、衛生と安全にかかわる社会の信頼に応え続け、その確保と増進を図るために、最新情報に基づく予測・予防と、効果的な監視・管理を行う努力が、今まで以上に求められていると言えるだろう。

この連載では、健康を守るために建築は何ができるか、何をすべきかを考えるために役立つ話題と情報を「建築と健康」をキーワードにお伝えしていきたい。



## 2. 建築物における健康とは

### —新公衆衛生とのかかわり—

堅い話で恐縮だが、最初は「健康とは何か」から始めようと思う。

図-1は、健康を守るべき人体のまわりを、衣服、室内空間、建築躯体、近隣(都市)環境が重層的にとりまいている状況を表している。良く知られているように人体自体も刺激や環境変化に対して、ホメオスタシス(恒常性)という、状態を一定に保とうとする性質・機能を持っていて、それを維持するために暑ければ発汗、寒ければ発熱、異物が侵入すれば排除・免疫などの様々なフィードバックが発動される。

そしてその負担がある限度を超えると生体のストレスに、さらに限度を超えると様々な体調不良や病気になるという図式だ。守る側としては、そのような極端な状

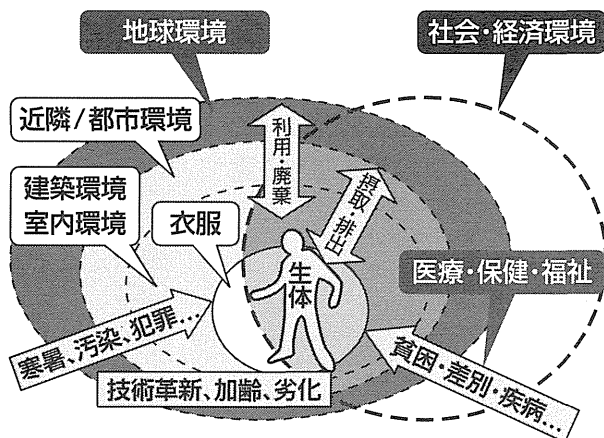


図-1 人と環境のかかわり

態におちいらないよう、体の表面には適切な衣服、さらにそのまわりには環境調整された空間などを用意して「肉体的健康」の維持に努めるのが基本戦略である。ところが、このカッコつきの「肉体的健康」は、保健衛生がめざす広義の「健康」の必要条件ではあっても十分条件ではない。ご承知の通り、WHO(世界保健機関)憲章の前文(1948年)には、健康とは「身体的・精神的・社会的に完全に良好な状態であり、たんに病気あるいは虚弱でないことではない」と謳われている。肉体的な側面ばかりでなく、社会的・経済的環境にかかわるストレスも様々な形で人の生活を脅かし、間接的に健康な状態を損なうことを考えれば、社会経済環境(図中右側円)の整備と、物理的環境(図中左側円)の整備は、健康実現プロセスの両輪と言える。さらに言い換えれば、過度に外部のエネルギーや資源に依存したり、外部環境に負荷をかける建築は社会や経済環境の変動に脆弱で社会経済的に不健

全なものであろう。なお、この二つの側面は働きかける対象と方法の違いから、保健衛生分野では、人・集団を対象に医療や保健・福祉の体制やシステムを介して健康の改善を図るアプローチを「対人保健」、それを取りまく物的環境から改善を図るアプローチを「対物保健」と分類している。そして近代の公衆衛生は、健康の維持増進にかかわる環境整備の役割に着目してその増進を重視し、これら様々な社会資源や手段を総動員して実現をはかる戦略「新公衆衛生(New Public Health)」へと傾斜してきた。

建築における環境づくりが「新公衆衛生」の主要な構成要素の担い手であることは言うまでもない。

例えば、地域住民の健康と衛生を支える保健所は、保健指導・保健サービスにかかわる対人保健と、食品・獣医や薬事など生活衛生にかかわる対物保健を担当しており、建築物衛生には建築物衛生法(建築物の衛生確保に関する法律)に規定される「対物保健」業務の一環としてかかわっている。

### 3. 建築環境衛生の現代的な捉え方

図-2は定番の環境要素別の健康影響要因である。

建築が利用者・居住者・所有者に提供する様々なサービスのうち、健康や利便性・快適性を害するおそれのある主な要因とそ



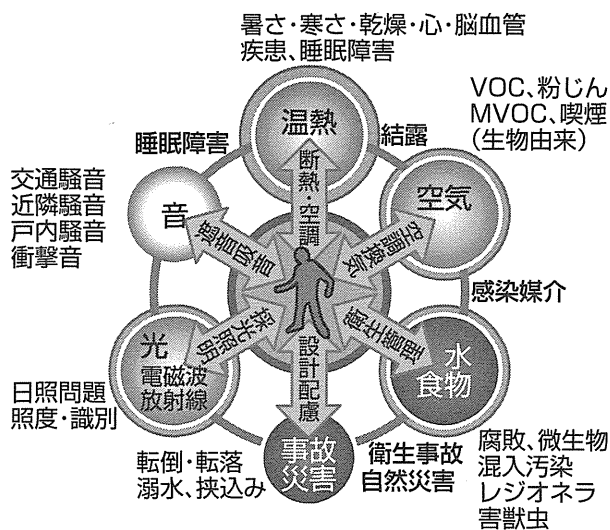


図-2 環境要素と健康影響要因

の形成・制御にかかわる手段を書き出した。

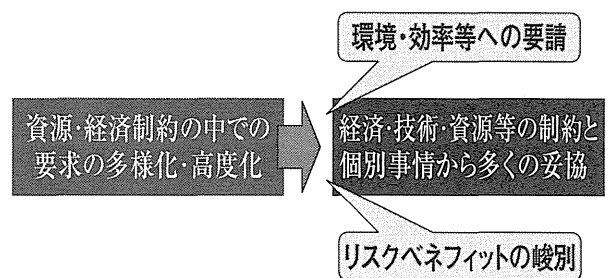
最上部から右回りに「温熱」「空気」「水・食物」「事故・災害」「光・電磁波・放射線」「音」の6要素を挙げたが、便宜上、給排水や害獣・虫の部分は「水」に区分してある。「温熱」には空調設備と建物側の断熱・気密・遮熱・通風性等が暑さ・寒さを、「空気」には空調換気設備と建物側の気密・通風・汚染放散・吸脱着性等が空気汚染状況を形成するというイメージを示している。なお、図中の要素や現象はあくまでも例示であり、健康・衛生と直接的なかわりの薄い防犯性やデザイン性、経済性、情報機能などには触れていない。また、建築物衛生法では、音・光・事故についても扱っていないのがわが国の実情である。

この図で強調したいのは、健康等に関して配慮すべき要因が多岐にわたり、それを制御・緩和する手段が、建物構造の企画・

設計から、空調など建築設備の設計・施工、それら全ての維持管理・保全までと多彩な点である。長年の蓄積で定型的な設計・施工技術が熟成されてきたことは事実だが、ひとつとして同じ条件で設計・施工・運用されない一品生産品である建築物において、これら要因をバランスよく実現・維持することは容易ではない。

#### 4. 健康にかかわる状況変化と建築側の対応

健康を支える建築が何をすべきかを考えるため、状況を「資源・経済制約」「人の要求変化」「技術革新」三つの視点から見直してみよう。



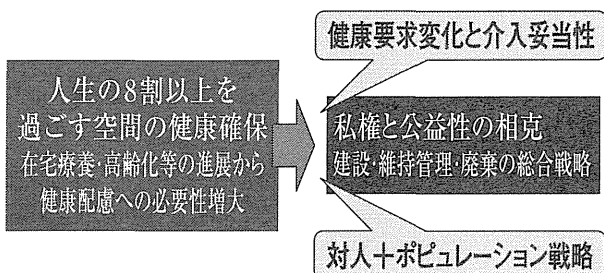
今日の資源・経済制約が招いた要求変化の最たるものは、気候変動対策に関する温暖化ガス削減(具体的には省エネルギー化)であるが、耐震・防火などの安全性確保、耐久性の改善などが続く。もちろん、どれも昔から建築での要求として一般的なものだが、昨今の地球環境問題や防災意識の高まりのなかでクローズアップされてきた。

しかし、これから何度も繰り返すことになるが、有限な資金・材料・技術と時間の

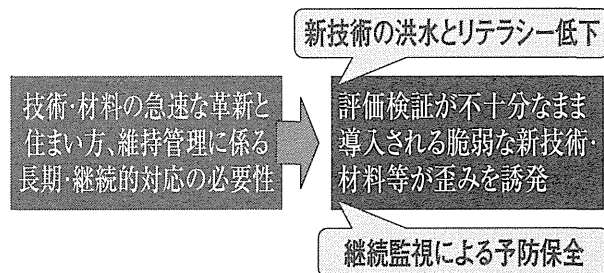
制約のなかで、何を優先させ、どこに配分するか(何をあきらめるか)のマネジメントが現実の最大の課題である。保健衛生分野でも「リスク」の評価・配分とその共有は大きなテーマだが、日々の設計・施工・管理の中で行われる予算や工程の管理においても、技術的な裏づけも不十分ななかでこの重い課題を背負っていかなくてはならない。もうひとつ、建築におけるリスクの扱い方について補足しておこう。

以上に述べてきたように求められる要求と性能は多岐に及んでおり、改善を意図した努力が思いもよらない形や、他の性能を損なう形で現れることがしばしばある。

例えば、耐震性を良くしようと合板などを多用したために気密性が上がりすぎて空気汚染物質が溜まってしまったり、空気汚染を防止しようとホルムアルデヒドを放散しない材料を用いたために、防黴性(カビの生えにくさ)が低下してしまったりという事態は日常茶飯事である。現時点で、個別の検討段階で姿を見せないこれらのリスクを完全に予防する手立てはなく、現場での管理を通じてその前兆や訴えを現場で速やかに捉え、対処する努力が今後、さらに重要になってくる。



現代人の生活は、睡眠を減らしてでも自分の時間を確保する傾向にあり、8割以上の時間を室内で過ごすことが明らかになっている。また、在宅医療・介護の増加なども考慮すると、健常でない方或いは高齢な方にも対応できる高品質な環境づくりが求められることが予想される。伝統的な公衆衛生対策は、大規模な建築物や不特定多数を扱うポピュレーション戦略を基本とするが、二つ目に挙げるこの動きは個別の対人対応を加味することとなるため、建築技術・設備技術と協力して新しい枠組みが求められるのではないだろうか。



冒頭でも指摘したように、室内環境は構造躯体、設備機器、居住/管理者の努力の総力戦で維持されているが、その設計や運用の基準自体が時代とともに揺れている。

性能や管理に目標や基準が定められるのは基本的な部分に限られるし、時代の変化に追いつかない場合もこれから増えてくるだろう。そしてそれに追い討ちをかけているのが3番目に挙げる様々な技術革新の登場である。ブラックボックス化した成分組成、制御技術や設定は、法の網をくぐったり、思いもかけない挙動を示す場合もまま

ある。医療・薬品の治験に相当するシステムもない中では、歴史的試練を経ていない技術や材料の影響を様々な方法で監視して行くほかない。

## 5. 建築衛生と環境

図-1を見返して欲しい。ここでは建築単体が左側の多重円の中で、資源利用と排出を行いながら内部環境を維持するイメージだが、これは時間的・空間的な全体像を十分に表していない。現実の建築は、過去に蓄積された資源を消費しながら、環境負荷となる廃棄物や温暖化ガスを閉じた地球系のなかに排出し、未来の社会に負の遺産を残していると言うのが現代的認識である。そしてその最も身近なしっぺ返しが、温暖化を含む気候変動やヒートアイランドであることが明らかになってきた。

人体や室内環境が守るべき対象で、そのまわりがそれを脅かす外敵や外乱だと言う一元的な認識はもう古い。本稿の趣旨から

外れるので詳しくは述べないが、わが国のエネルギー消費の約三分の一は建築物においてである。環境問題の系譜の中で、建築とその集合体である都市は、ある時には被害者、ある時は加害者という二つの顔を持っており、その調和を模索していかなくてはならないと言えるだろう。

## 6. おわりに—そしてこれから

建築と健康とのかかわりについて、その幅広さと時代の流れのなかで揺れ動き、対処が難しいことを書き綴った。背景と理念を主とし、個別の要因を語らなかったため、具体性を欠くところのご指摘もあると思うが、序論的な部分なのでこれからにご期待いただきたい。

以後、図-1に示した温湿度や空気汚染、水管理、高齢者対応、災害との関連などに触れながら、話を進めていく予定である。

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昭和53年建設省建築研究所に奉職以来、居住環境の安全・健康・快適性と効率にかかわる調査・評価・開発に従事。その間、住宅省エネ法や結露対策指針、シックハウス対策にかかる建築基準法改正の支援などを手掛け、平成20年より厚生労働省保健医療科学院で、建築物衛生施策の支援などに携わっている。博士（工学）。

