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

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-DNPhydrazone 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-DNPhydrazones 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-DNPhydrazones 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-DNPhydrazones having a double bond at the 4-position or greater exhibited similar absorption maximum wavelengths and equilibrium isomer ratio (0.14) to alkanal-2,4-DNPhydrazones. 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-DNPhydrazones 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-DNPhydrazones are listed in Table 1. In the case of 2-butanone-, 2-pentanone- and 2-hexanone-2,4-DNPhydrazone, 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-DNPhydrazone was dependent on the concentration of acid-catalyst and reached an equilibrium state – carbonyl, DNPH, hydrazone-derivative and H_2O – within 10 h at 0.1 mol/L phosphoric acid solution. The equilibrium constants of ketone-2,4-DNPhydrazones, [carbonyl][DNPH]/[hydrazone][H_2O], were relatively large and ranged from 0.74×10^{-4} to 5.9×10^{-4}. Hydrazone derivatives formed from 2-ketones such as 2-pentanone,
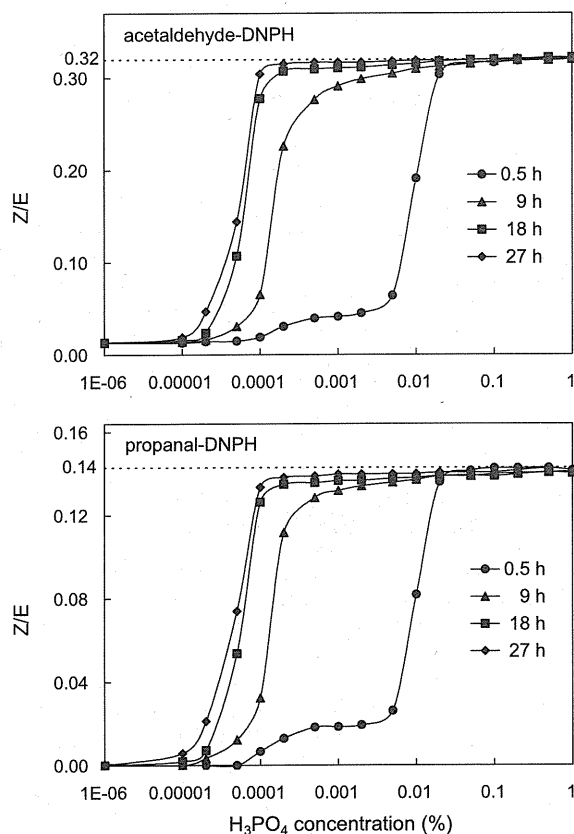

Fig. 3. The changes in the isomer ratios of acetaldehyde and propanal DNPhydrazone with phosphoric acid. Reproduced with permission from Fig. 5 in Ref. [56].

Table 1

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

| Carbonyls	Isomer ratio Z/E	λ_{max} (nm) Z-isomer	λ_{max} (nm) E-isomer
Alkanals			
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
Alkenals			
2-Propenal	0.018	367	374
trans-2-Butenal	0.035	373	383
trans-2-Pentenal	0.035	373	383
trans-2-Hexenal	0.035	373	383
trans-2-Heptenal	0.035	373	383
trans-2-Octenal	0.035	373	383
trans-2-Nonenal	0.036	373	383
trans-2-Decenal	0.036	373	383
Ketones			
2-Propanone	n.a.	369	
2-Butanone	0.20	367	369
2-Pentanone	0.21	367	368
2-Hexanone	0.22	367	370
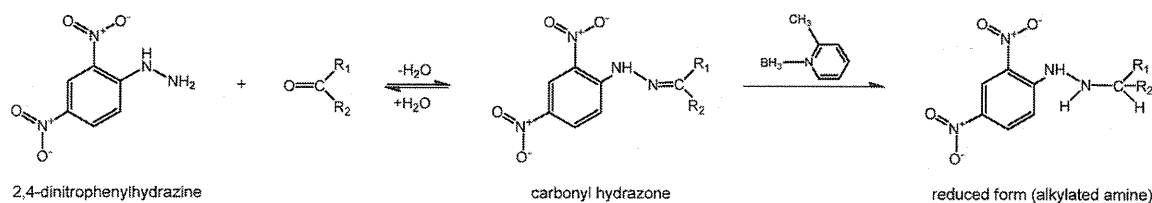
Fig. 4. Scheme of the reductive amination of carbonyl 2,4-DNPhydrazones with 2-picoline borane.

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-DNPhydrazones 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-DNPhydrazones

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 DNPhydrazones 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 cyanohydridoborate ($NaBH_3CN$) [59,60], sodium triacetoxyborohydride ($Na(OAc)_3BH$) [61–65], pyridine-borane (pyr-BH_3) [66–68], titanium(IV) isopropoxide/sodium borohydride ($Ti(Oi-Pr)_4/NaBH_4$) [69–72], borohydride exchange resin [73], zinc borohydride/silica gel ($Zn(BH_4)_2/SiO_2$) [74], and phenylsilane/dibutyltin dichloride ($PhSiH_4/Bu_2SnCl_2$) [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 DNPhydrazones 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-DNPhydrazones with 2-picoline borane. Aldehyde DNPhydrazones (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 DNPhydrazones 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 DNPhydrazones were 351–352 nm, which shifted 6–7 nm towards shorter wavelengths when compared to the corresponding
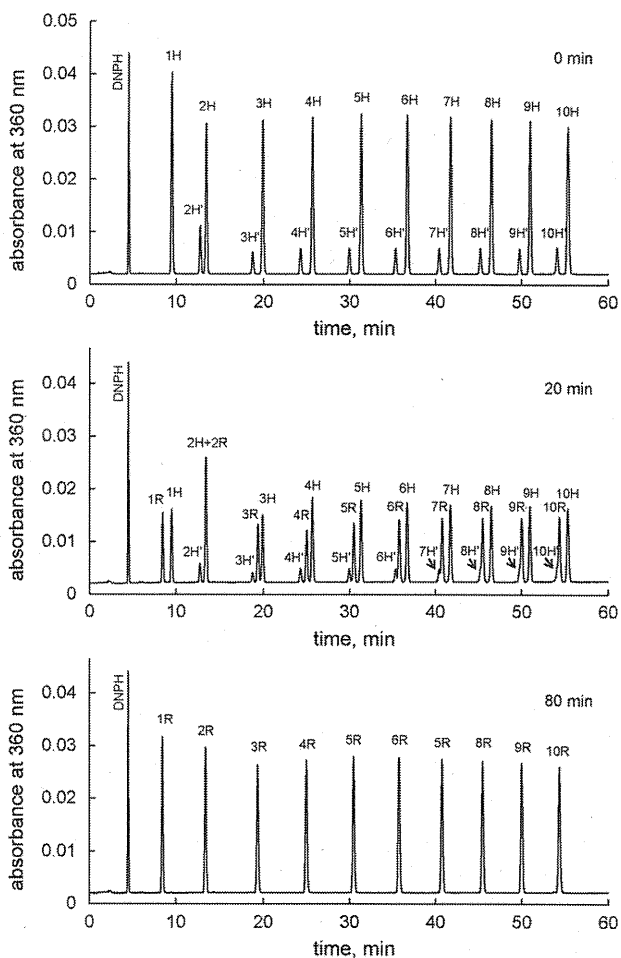

Fig. 5. Chromatographic profiles of DNPhydrazones and their reduced forms changing with reaction time. Number of peak name indicates carbon number of precursor aldehyde (1: formaldehyde, 2: acetaldehyde, 3: propanal, 4: butanal, 5: pentanal, 6: hexanal, 7: heptanal, 8: octanal, 9: nonanal, and 10: decanal), "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 1.5×10^4 (C1)–2.2×10^4 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 × 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
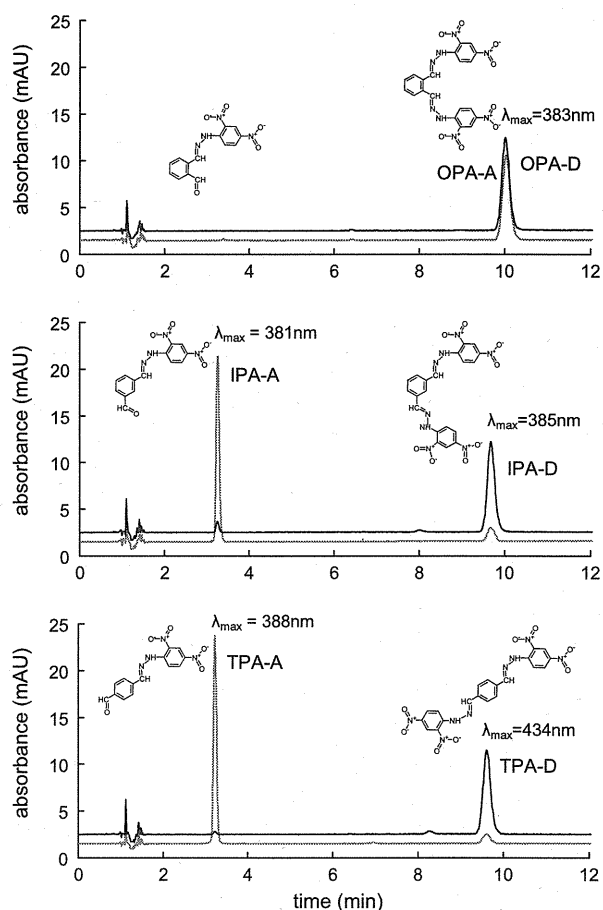

Fig. 6. HPLC chromatograms of OPA-DNPhydrazone (upper), IPA-DNPhydrazone (middle) and TPA-DNPhydrazone (lower) at maximum wavelengths by photo diode array detector. Light-colored chromatograms indicate the derivatives synthesized with excess of aldehyde and dark-colored chromatograms indicate the derivatives 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-DNPhydrazide). Fig. 8 shows the peak area changes with time of DNPH and formic-DNPhydrazide at wavelength 360 nm. Acetic acid, propionic acid and butyric acid exhibit similar behavior with longer reaction time in order of

orthophthalaldehyde   2,4-dinitrophenylhydrazine   mono-2,4-dinitrophenylhydrazone   bis-(2,4-dinitrophenylhydrazone)
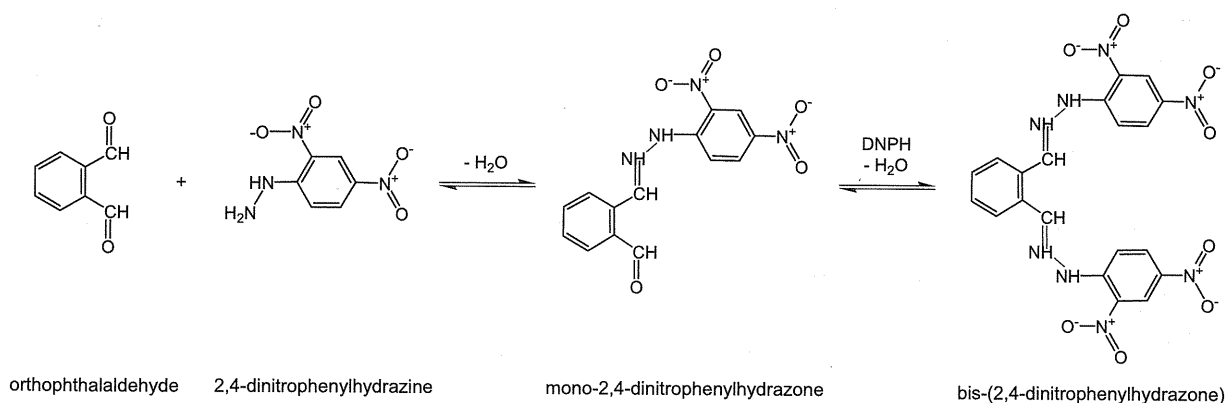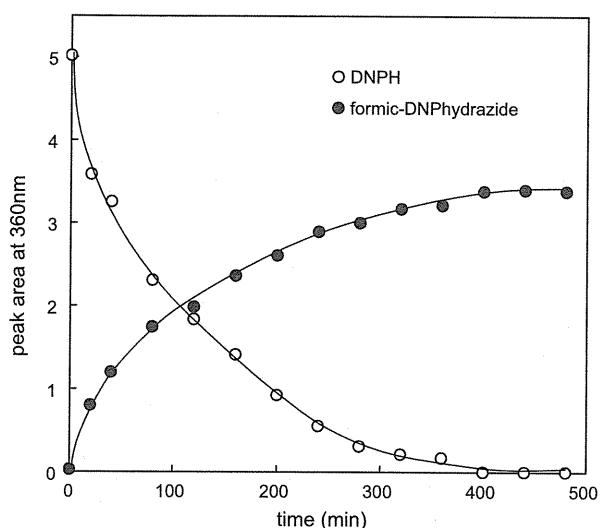
Fig. 7. Scheme of the derivatization reaction of DNPH with orthophthalaldehyde.

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 1.4×10^4 L/mol/cm. In reversed-phase HPLC analysis, the separations of hydrazide and hydrazones derivatives may be incomplete. The retention times of DNPhydrazide peaks vary with mobile phase
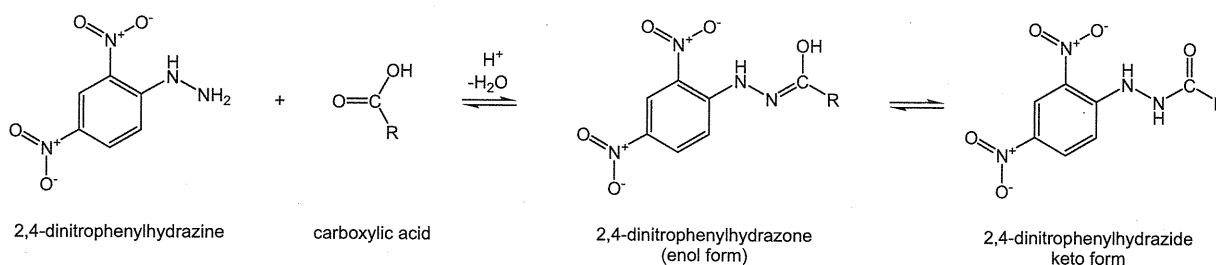
pH. The addition of base to the mobile phase shortens the retention times of C1–C6 DNPhydrazide peaks and shifts the UV/vis spectrum profiles to longer wavelengths. Under the conditions of 0, 0.1, and 1.0 mmol/L dibasic potassium phosphate, the spectra of formic DNPhydrazide are unimodal with a maximum 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 DNPhydrazide 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_2O (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 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-
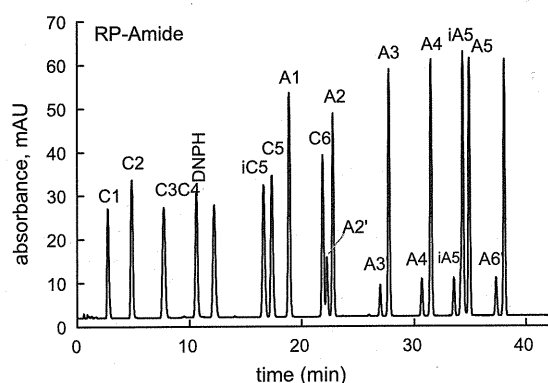

2,4-dinitrophenylhydrazine   carboxylic acid   2,4-dinitrophenylhydrazone   2,4-dinitrophenylhydrazide
                                                                      (enol form)   keto form

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

Fig. 10. Chromatographic profiles of C1–C6 carboxylic-DNPhydrazides and aldehyde-2,4-DNPhydrazones on an Ascentis RP-Amide column (100 μmol/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 DNPhydrazone; A2, acetaldehyde DNPhydrazone; A3, propionaldehyde DNPhydrazone; A4, butyraldehyde DNPhydrazone; i-A5, i-pentanal DNPhydrazone; n-A5, n-pentanal, DNPhydrazone; A6, hexanal DNPhydrazone.

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 thorough 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
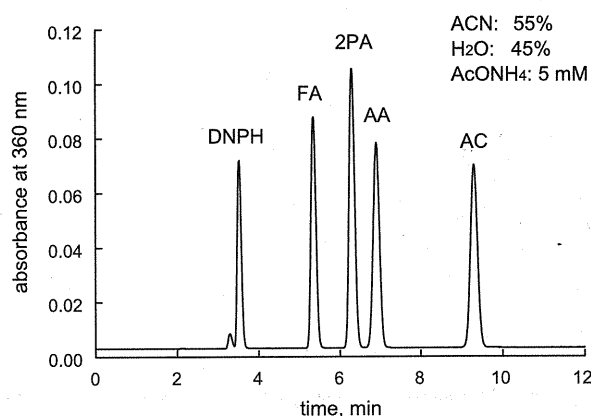

Fig. 12. Chromatogram of pyridine-2-aldehyde and other carbonyl 2,4-DNPhydrazones. Reproduced with permission from Fig. 2 in Ref. [90].

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
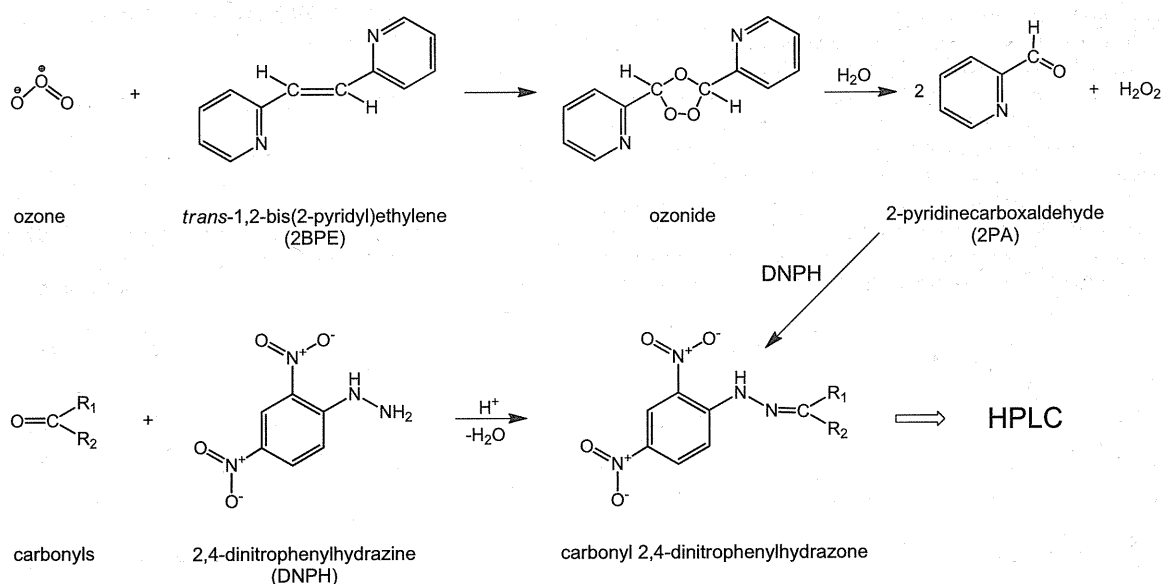

Fig. 11. Scheme of the simultaneous determination of ozone and carbonyls.

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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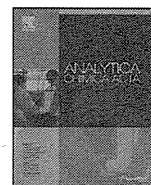
A diffusive sampling device for simultaneous determination of ozone and carbonyls

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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 mL min^{-1}) and formaldehyde (72.0 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.

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 thorough 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 BEP/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^{-1} 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^3 (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^{-1}. 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].
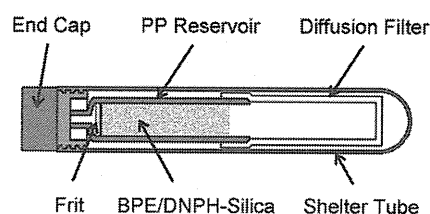

Fig. 1. Schematic representation of the DSD-BPE/DNPH sampler.

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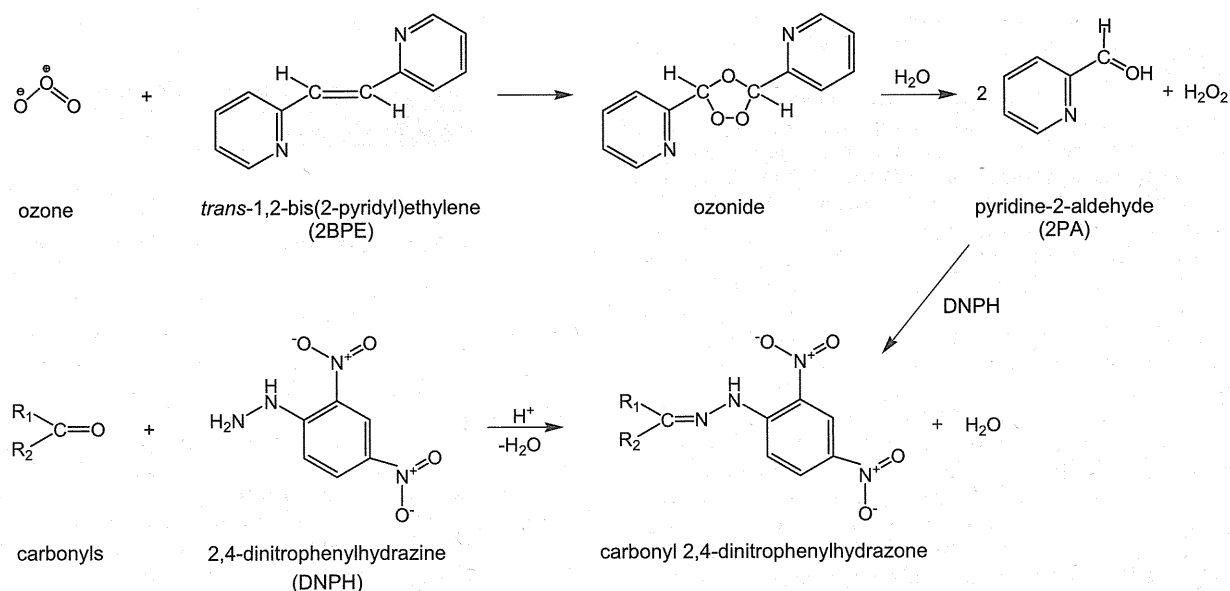
Fig. 2. Scheme for the simultaneous determination of ozone and carbonyls.

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 $(3.4 \times 10^4\,L\,mol^{-1}\,cm^{-1})$ [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-DNPhydrazones $(100\,\mu mol\,L^{-1})$.
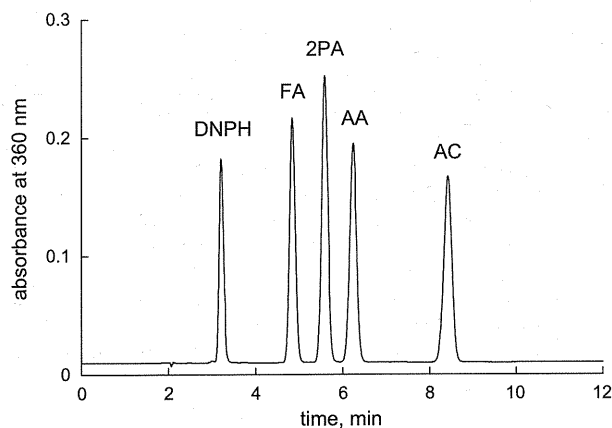

Fig. 3. Chromatogram of pyridine-2-aldehyde and other carbonyl 2,4-DNPhydrazones.

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 μL) containing 2PA, FA, AA, and AC 2,4-DNPhydrazones $(100\,\mu mol\,L^{-1})$ 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-DNPhydrazones, 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 $72\,\mu g\,m^{-3}$ 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: $100\,mL\,min^{-1}$). 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.

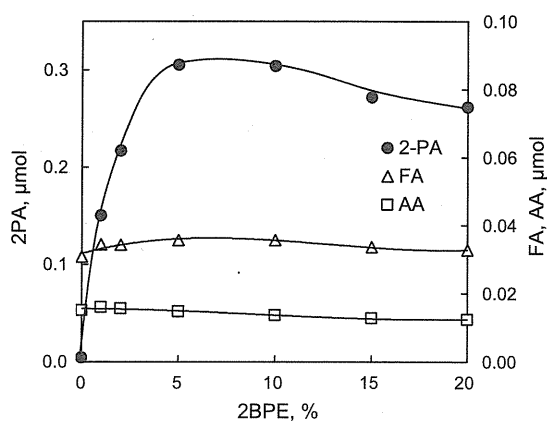| Compound	LOD, $\mu mol\,L^{-1}$	LOQ, $\mu mol\,L^{-1}$
2PA (ozone)	0.056	0.19
Formaldehyde	0.039	0.13
Acetaldehyde	0.054	0.18
Acetone	0.12	0.39

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 $\mu g\,m^{-3}$ 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 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-
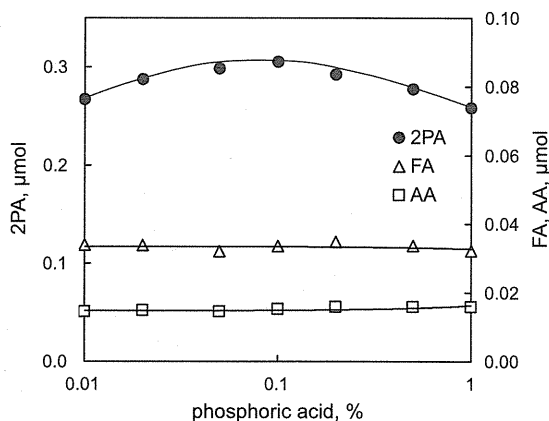

Fig. 5. Change in the amount of 2PA with the phosphoric acid content of 2BPE/DNPH–silica.

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 $\mu g\,m^{-3}$, 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 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 $\mu mol\,d^{-1}$; 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 mL min^{-1} and one-day sampling period showed the slope of 0.354 $\mu mol\,d^{-1}$. 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_{gr}) is inversely proportional to the square root of the density (Z) or molecular weight (M) of the gas:

$$D_{gr} \propto \frac{1}{\sqrt{Z}} \propto \frac{1}{\sqrt{M}} \tag{1}$$

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

$$D_{gr} = D_f \sqrt{\frac{M_f}{M_d}}, \tag{2}$$

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):

$$R = \frac{D_d}{D_f} R_f, \tag{3}$$

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 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 mL min^{-1} and the experimental sampling rate is approximately 80% of the theoretical value (44.6 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 $\mu mol\,d^{-1}$) 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
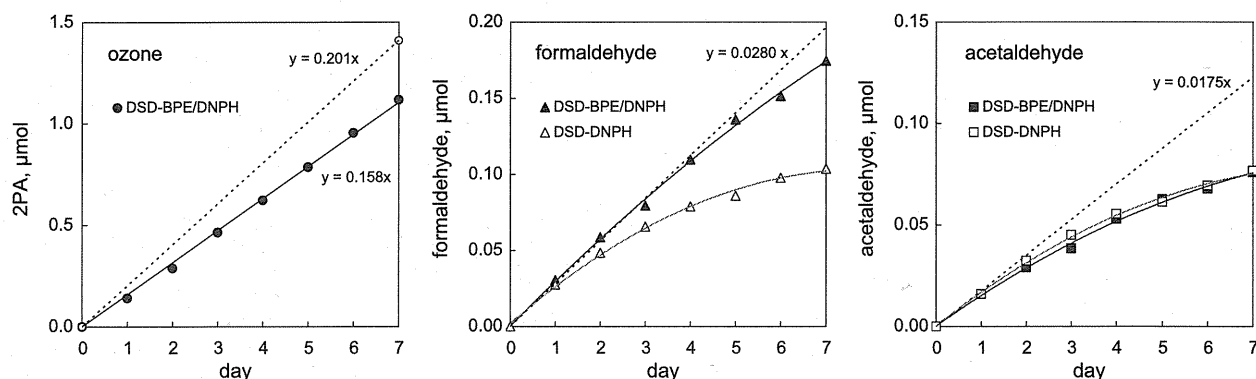
Fig. 6. Relationship between exposure time and collected amount by using DSD-BPE/DNPH and DSD-DNPH diffusive samplers. Air sampling was performed continuously for several days. Dashed lines represent theoretical values calculated from Graham's law of effusion.

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:

$$\text{Sampling rate of formaldehyde by DSD-BPE/DNPH} = \frac{0.0280}{0.0389}$$

$$\times 100 = 72.0 \, \text{mL} \, \text{min}^{-1}$$

In a previous report, the DSD-DNPH sampling rate was determined to be $71.9 \, \text{mL} \, \text{min}^{-1}$ 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: $0.0175 \, \mu\text{mol} \, \text{d}^{-1}$) 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:

$$\text{Sampling rate of acetaldehyde by DSD-BPE/DNPH} = \frac{0.0175}{0.0295}$$

$$\times 100 = 59.3 \, \text{mL} \, \text{min}^{-1}$$

In a previous report, the DSD-DNPH sampling rate was determined to be $59.4 \, \text{mL} \, \text{min}^{-1}$ 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 ($72 \, \mu\text{g} \, \text{m}^{-3}$) for 24 h.

3.4. High concentration and long-term ozone sampling

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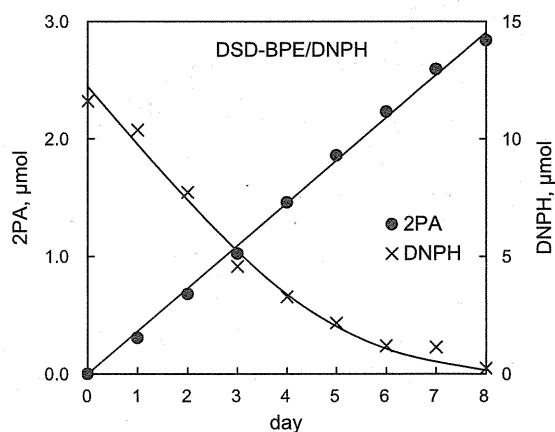

Fig. 7. Change in the amount of 2PA formed with sampling duration.

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 dose 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 $\mu g\,m^{-3}$. Values in parentheses indicate data measured by the auto-analyzer at the Air Monitoring Station.

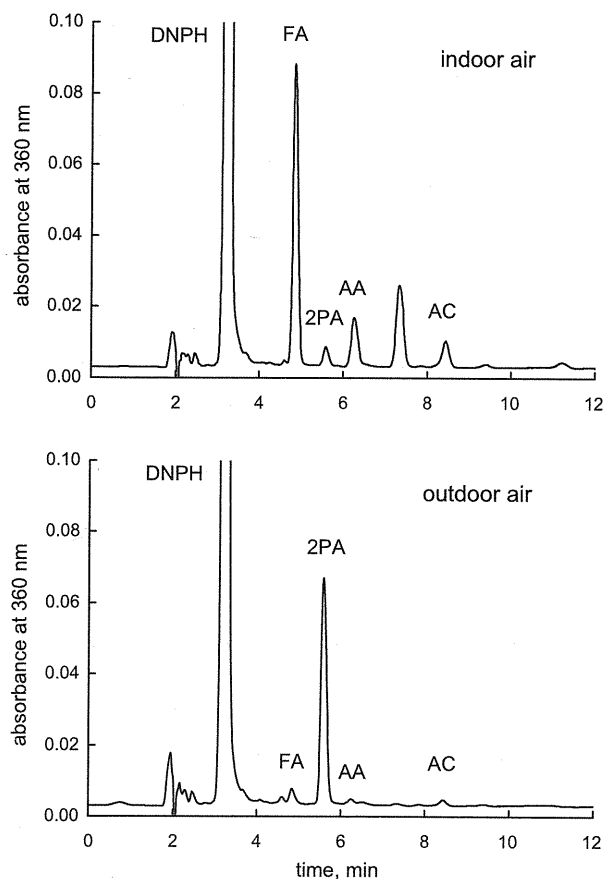| Date (2010)	Weather conditions			Ozone		Formaldehyde		Acetaldehyde		Acetone	
	WS (m⁻¹)	Temp (°C)	RH (%)	I	O	I	O	I	O	I	O
Oct, 19–20	(2.7)	(18)	(82)	3.2	42 (43)	72	3.5	25	2.6	22	3.4
Oct, 20–21	(2.2)	(18)	(93)	2.4	14 (17)	82	3.8	27	2.5	24	3.2
Oct, 21–22	(3.7)	(18)	(80)	4.4	61 (59)	61	2.8	21	2.1	19	3.3
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 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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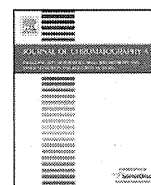
Short communication

Ozone removal in the collection of carbonyl compounds in air

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ABSTRACT

The most widely used method for measuring carbonyl compounds in air is 2,4-dinitrophenylhydrazine (DNPH) derivatization followed by high-performance liquid chromatography (HPLC). However, substantial negative interference caused by the presence of ozone in air has been reported. To avoid the influences of ozone, a potassium iodide scrubber (KI-scrubber) is commonly used. However, when air sampling using a DNPH-cartridge and a KI-scrubber is performed under conditions of high humidity, moist potassium iodide in the KI-scrubber traps carbonyls before they reach the DNPH-cartridge. Moreover, wet KI reacts with I_2 to form KI_3 and this oxidative reagent moves to the DNPH-cartridge and destroys the DNPH and DNPhydrazone derivatives. In order to alleviate these problems, new ozone scrubbers (BPE-scrubber, HQ-scrubber) have been developed. BPE-scrubber and HQ-scrubber consist of silica gel particles impregnated with trans-1,2-bis-(2-pyridyl) ethylene (BPE) and hydroquinone (HQ), respectively. BPE reacts with ozone to form pyridine aldehyde and HQ reacts with ozone to form benzoquinone. The amounts of reducing agent in silica gel (130 mg) for ozone scrubber are 1% (w/w) for BPE-cartridge; 0.2% (w/w) for HQ-scrubber. These scrubbers can be used in air containing 140 μg/m³ of ozone for 24 h at a flow rate of 200 mL/min. When the relative humidity exceeded 80%, KI in the KI-scrubber was gradually moistened and changed to yellow in color. Peak abundance of formaldehyde, acetaldehyde and acetone DNPhydrazones was diminished to 25%, 15%, and 2%, respectively, compared with the BPE-scrubber or HQ-scrubber. When using a BPE-scrubber or HQ-scrubber, decomposition of DNPH and DNPhydrazones was not observed at a wide range of relative humidities (3–97%).

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1. Introduction

Formaldehyde, acetaldehyde and other carbonyl compounds are ubiquitous pollutants that are formed through oxidation of hydrocarbons by ozone in the troposphere [1–3] and by the reaction between ozone and terpenoid in indoor air [4–6]. Long-term exposure to relatively high levels of carbonyl compounds such as formaldehyde and acetaldehyde is known to increase the risk of asthma [7] and cancer [8]. Accurate aldehyde measurements are therefore important both for determining the formation mechanism of aldehydes and for evaluating the implications for human health.

The most widely used method for qualitative and quantitative analyses of carbonyl compounds is 2,4-dinitrophenylhydrazine (DNPH) derivatization followed by high-performance liquid chromatography (HPLC). Sampling can be performed using acidic solutions of DNPH in impingers or with acidic DNPH-coated solid sorbents in a cartridge. At the present time, a number of cartridge devices packed with DNPH-coated silica gel particles are commercially available for sampling aldehydes in air. Due to the importance of the method, it has been introduced as a standard procedure by several national and international standardization bodies.

While the derivatization reaction at first glance appears straightforward, substantial negative interference caused by the presence of ozone in the air sample has been reported [9–11]. Ozone decomposes DNPH and DNPhydrazone derivatives to form 2,4-dinitrophenol, 2,4-dinitroaniline and 1,3-dinitrobenzene [12]. Additionally, Rodier and Birks reported that sampling atmospheres containing isoprene and ozone lead to the formation of artifact carbonyl peaks in a system using DNPH or dansylhydrazine-coated C18 cartridges [13]. The peaks were purportedly due to a reaction of isoprene with ozone on the cartridge surface, which led to positive artifacts for a number of compounds including formaldehyde.

To avoid the influences of ozone, a potassium iodide scrubber (KI-scrubber) can be used to destroy ozone before sampling the carbonyl compounds. In this case the air sample is first drawn over a surface on which solid KI is adsorbed. Ozone reacts with KI to form iodine and potassium hydroxide (Fig. 1). At the present time, KI-scrubbers are commercially available from many suppliers and are widely used. However, KI-scrubbers have two disadvantages. When air sampling using a DNPH-cartridge with a KI-scrubber is performed at high humidity, moist potassium iodide in the

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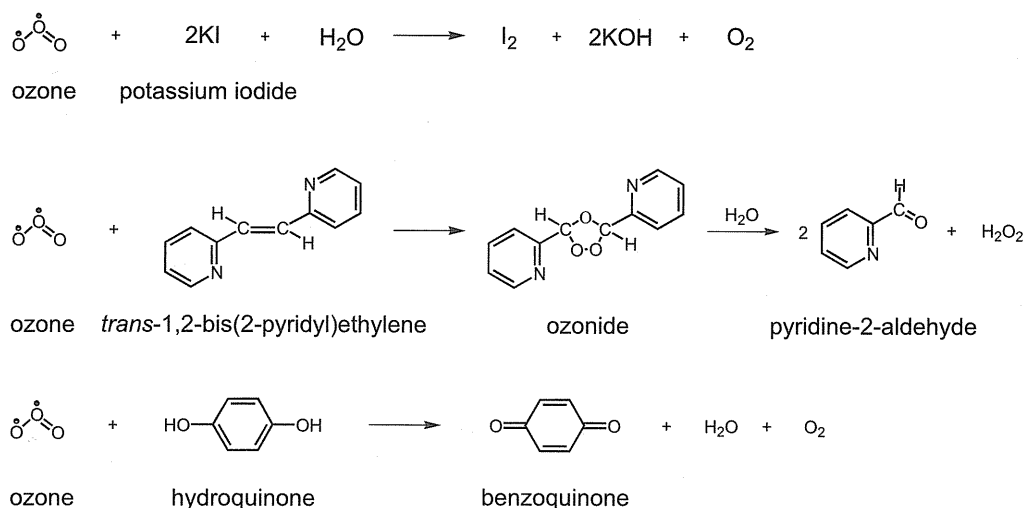
Fig. 1. Reactions of ozone with reducing agents.

KI-scrubber traps carbonyl compounds before they can reach the DNPH-cartridge. Moreover, wet KI reacts with I_2 to form KI_3 and this oxidative reagent moves to the DNPH-cartridge and destroys the DNPH and DNPhydrazone derivatives.

We have previously developed a method for the simultaneous determination of ozone and carbonyls in air using a two-bed cartridge system [14,15]. Each bed consists of reagent-impregnated silica particles. The first contains trans-1,2-bis-(pyridyl) ethylene (BPE), while the second contains 2,4-dinitrophenylhydrazine (DNPH). Air samples are drawn through the cartridge first through the BPE and then through the DNPH. Ozone in the air sample is trapped in the first bed by the BPE-coated silica particles and produce pyridine aldehyde (Fig. 1). In this method, BPE acts as an ozone scrubber. We have also developed a method for the determination of acrolein and other carbonyls in cigarette smoke using a dual cartridge system [16]. Each cartridge consists of reagent-impregnated silica particles. The first contains hydroquinone (HQ) for the inhibition of acrolein polymerization, while the second contains DNPH for the derivatization of carbonyls. HQ is a radical and ozone-trapping reagent and is used to inhibit acrolein radical polymerization and to remove ozone. Ozone reacts with HQ to form benzoquinone (Fig. 1). Thus, both BPE and HQ can function as ozone scrubbers. In this study, the effectiveness of KI-scrubber, BPE-scrubber and HQ-scrubber as ozone removers was investigated.

2. Experimental

2.1. Apparatus and reagents

The HPLC system (Shimadzu, Kyoto, Japan) used included two LC-20AD pumps, an SIL-20AC autosampler and an SPD M20A photodiode array detector. The analytical column was an Ascentis Express RP-Amide, 2.7 μm particle size, 150 mm × 4.6 mm i.d. column (Supelco Inc, Bellefonte, PA, USA). Solution A of the mobile phase mixture was acetonitrile/water (45/55, v/v) containing 5 mmol/L ammonium acetate and solution B was acetonitrile/water (75/25, v/v). HPLC elution was carried out with 100% A for 5 min, followed by a linear gradient from 100% A to 100% B in 50 min and then held for 10 min. The flow rate of the mobile phase was 0.7 mL/min. The column temperature was 40 °C, the autosampler temperature was 25 °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 m³ (4.2 m × 3.6 m × 2.3 m) and was equipped with an adjustable constant temperature and humidity controller. Ozone gas was generated using an Ozone Generator (TGO-1, Funatech Ltd., Tokyo, Japan). Air pumps (MP-Σ30N, Shibata Scientific Technology Ltd., Tokyo, Japan) and wet gas meters (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 (BPE, >97%) were purchased from Tokyo Kasei Co. Ltd. (Tokyo, Japan). Acetonitrile (HPLC grade, >99.9%), hydroquinone (HQ, >99%), 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). Rezorian Ozone Scrubbers and LpDNPH Rezorian cartridges 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 [15].

2.2. Preparation of a trans-1,2-bis(2-pyridyl)ethylene-impregnated silica cartridge (BPE-scrubber) and a hydroquinone-impregnated silica cartridge (HQ-scrubber)

Silica gel (50 g) was washed with water (3× 500 mL), methanol (2× 500 mL), and lastly acetonitrile (2× 500 mL). The solvent was then completely evaporated to dryness at 100 °C for 30 min under vacuum on a rotary evaporator. After cooling to room temperature, acetonitrile (200 mL) was added to the washed silica gel. BPE (0.5 g) or HQ (0.1 g) was dissolved in 50 mL acetonitrile. This solution was added to the washed silica gel, the mixture was stirred and the solvent was evaporated to dryness at 40 °C under vacuum on a rotary evaporator. BPE-impregnated silica (130 mg) or HQ-impregnated silica (130 mg) was packed into polyethylene cartridges (Rezorian tube, 1 mL, Supelco Inc, Bellefonte, PA) and stored in a refrigerator at 4 °C.

The commercially available ozone scrubbers used in this study contained 1.5 g of potassium iodide.
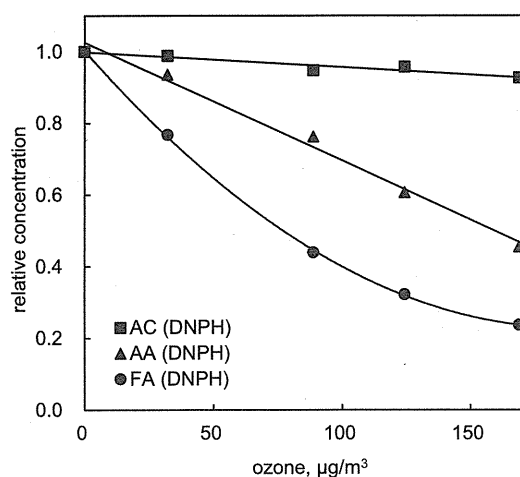
Fig. 2. Changes in measured carbonyl concentrations with the coexistent ozone concentration. DNPH-cartridges were used without an ozone scrubber. FA: formaldehyde; AA: acetaldehyde; AC: acetone.

2.3. Air sampling and analysis

Prior to air sample collection, each KI-scrubber, BPE-scrubber or HQ-scrubber was connected to a DNPH-cartridge to construct a dual-cartridge sampling train (KI-DNPH, BPE-DNPH and HQ-DNPH). Air was drawn through a coupled cartridge pair from the ozone scrubber to the DNPH-cartridge at a flow rate of 200 mL/min. After collection, the coupled cartridges were extracted. In the case of KI-DNPH, the KI-cartridge was discarded and the DNPH-cartridge was eluted with acetonitrile to a final volume of 5 mL. With BPE-DNPH, elution was performed in the reverse direction to air sampling. An intact, coupled cartridge pair was eluted with 30% dimethyl sulfoxide in acetonitrile solution containing 0.085% (v/v) phosphoric acid to a final volume of 5 mL. The HQ-DNPH cartridge pair was also eluted intact and in the reverse direction to air sampling. Acetonitrile was used as the elution solvent to a final volume of 5 mL. After 30 min of elution, the eluates were analyzed by HPLC.

3. Results and discussion

3.1. Decomposition of DNPhydrozones by ozone

An ozone generator was operated in the environmental test chamber set at a temperature of 25 °C and a relative humidity of 50%. The concentrations of formaldehyde, acetaldehyde and acetone in the environmental test chamber were 14, 15 and 8.0 μg/m³, respectively. Air sampling was performed by using a DNPH-cartridge without an ozone scrubber for 24 h at a flow rate of 200 mL/min. After collection, DNPH-cartridges were eluted with acetonitrile and analyzed by HPLC. Fig. 2 shows changes in the concentrations of carbonyl compounds with changes in ozone concentration. Carbonyl concentrations are expressed as relative concentrations in Fig. 2. Concentrations of formaldehyde and acetaldehyde decreased dramatically with increased ozone concentration. When the concentration of ozone is 170 μg/m³, the measured concentrations of formaldehyde and acetaldehyde are 20% and 45%, respectively, of the concentrations measured when no ozone is present. Alternatively, the measured concentration of acetone remained within 5% of the concentration when no ozone was present.
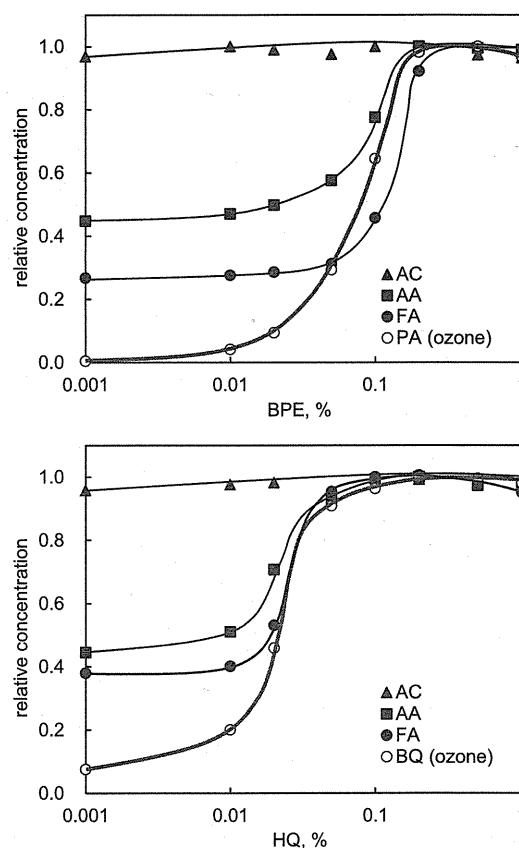

Fig. 3. Changes in the concentrations of carbonyl compounds with the contents of BPE (upper panel) and HQ (lower panel) in the ozone scrubber at a temperature of 25 °C and a relative humidity of 50%. FA: formaldehyde; AA: acetaldehyde; AC: acetone; PA: pyridine-2-aldehyde; BQ: benzoquinone.

3.2. Preferable contents of BPE and HQ in silica gel

An ozone generator was operated in the environmental test chamber set at a temperature of 25 °C and a relative humidity of 50%. The ozone concentration reached a constant value of 140 μg/m³ after 24 h. The concentrations of formaldehyde, acetaldehyde and acetone in the environmental test chamber were 13, 11 and 8.0 μg/m³, respectively. BPE-scrubbers containing 0–1% (0–7.1 μmol) of BPE and HQ-scrubbers containing 0–1% (0–12 μmol) of HQ were connected to DNPH-cartridges. Air sampling was performed for 24 h at a flow rate of 200 mL/min. After collection, BPE-DNPH-cartridges were eluted with 25% dimethyl sulfoxide in acetonitrile solution containing 0.085% (v/v) phosphoric acid and HQ-DNPH-cartridges were eluted with acetonitrile. After 30 min following elution, the eluate was analyzed by HPLC. Fig. 3 shows changes in the relative concentrations of carbonyl compounds with the loading of BPE (upper panel) and loading of HQ (lower panel) in the ozone scrubber.

In the case of BPE-DNPH cartridge, concentrations of formaldehyde, acetaldehyde, acetone, and pyridine-2-aldehyde increased with increasing BPE concentration, and reached a maximum value when the BPE concentration exceeded 0.5% (3.6 μmol). HQ-DNPH cartridges exhibited similar behavior. Measured concentrations of carbonyls reached a plateau when the HQ concentration exceeded 0.1% (1.2 μmol). HQ may react more efficiently with ozone than BPE. Based on the data presented in Fig. 3, appropriate ozone scrubbers with 130 mg silica support packing should contain 1% BPE-cartridge and 0.2% HQ-cartridge.
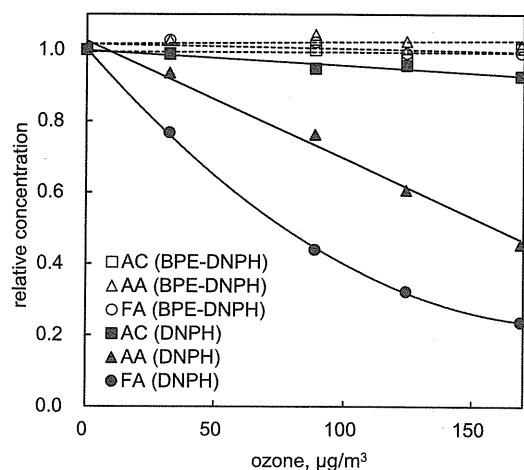
Fig. 4. Changes in measured carbonyl concentrations with the coexistent ozone concentration. DNPH-cartridges were used with BPE-scrubber and without an ozone scrubber. FA: formaldehyde; AA: acetaldehyde; AC: acetone.

Under the same conditions described in Fig. 2, KI-DNPH, BPE-DNPH and HQ-DNPH methods showed good performance in ozone removal and analysis of carbonyls. Broken lines of Fig. 4 show changes in measured carbonyl concentrations with the coexistent ozone concentration by using BPE-DNPH. Decreases of formaldehyde, acetaldehyde and acetone were not observed at a wide range of ozone concentrations (0–170 $\mu g/m^3$). HQ-DNPH and KI-DNPH exhibited similar behavior to BPE-DNPH.

3.3. Influence of humidity on the ozone scrubbers

The environmental test chamber was set to a temperature of 25 °C and relative humidity was varied from 3% to 97%. The concentrations of ozone, formaldehyde, acetaldehyde and acetone in the environmental test chamber were 70, 40, 12 and 9.0 $\mu g/m^3$, respectively. Air sampling was performed by using KI-DNPH, BPE-DNPH and HQ-DNPH for 24 h at a flow rate of 200 mL/min. Fig. 5 shows the chromatographic profiles of the eluates eluted from KI-DNPH, BPE-DNPH and HQ-DNPH cartridges. In the case of KI-DNPH, when the relative humidity exceeded 80%, KI in the KI-scrubber was gradually wetted and changed to yellow in color (KI$_3$). The liquefied, wet KI migrated into the DNPH-cartridge where the DNPH-silica was discolored to brown. DNPH was decomposed by the wet KI and the DNPH peak was not detected in the chromatogram. Peak abundance of FA-D, AA-D and AC-D was diminished to 25%, 15%, 2%, respectively, relative to the same peaks when BPE-DNPH or HQ-DNPH was used. It is suggested that carbonyl compounds dissolve in the wet KI because carbonyl compounds are polar, hydrophilic and water-soluble. In the case of BPE-DNPH and HQ-DNPH, decomposition of DNPH was not observed and large unreacted DNPH peaks were detected. Peak abundance of FA-D, AA-D and AC-D is of the same order of magnitude as
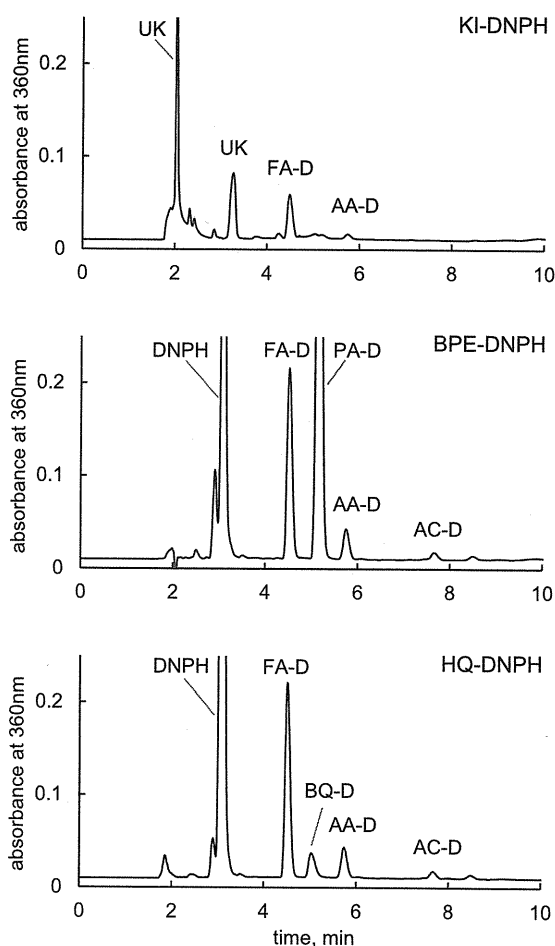

Fig. 5. Chromatographic profiles of DNPhydrazones obtained from KI-DNPH (upper panel), BPE-DNPH (middle panel) and HQ-DNPH (lower panel) methods. FA-D: formaldehyde DNPhydrazone; AA-D: acetaldehyde DNPhydrazone; AC-D: acetone DNPhydrazone; PA-D: pyridine-2-aldehyde DNPhydrazone; BQ-D: benzoquinone DNPhydrazone; UK: unknown compound.

those measured by both BPE-DNPH and HQ-DNPH under dry conditions.

The large PA-D peak in the BPE-DNPH chromatogram is the derivative derived from PA and DNPH. PA is formed by the reaction of BPE with ozone [15]. Therefore, it is possible to determine ozone concentration by measuring PA concentration quantitatively [15]. By the same token, BQ-D peak in the HQ-DNPH is the derivative derived from BQ and DNPH (Fig. 6). Ozone reacts with HQ completely to form BQ, however, partial subsequent reaction with DNPH also occurs. Ozone concentration can be determined by summing BQ-D and underivatized BQ concentrations.
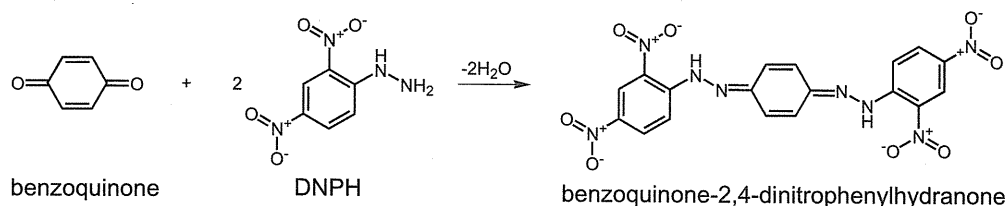

Fig. 6. Reaction of benzoquinone with DNPH.

4. Conclusions

For measuring carbonyl compounds using DNPH-cartridge, a potassium iodide has been widely used as ozone scrubber. However, when air sampling is performed under conditions of high humidity, moist potassium iodide in the KI-scrubber traps carbonyls before they reach the DNPH-cartridge. Moreover, wet KI reacts with I_2 to form KI_3 and this oxidative reagent moves to the DNPH cartridge and destroys the DNPH and DNPhydrazone derivatives. BPE-DNPH and HQ-DNPH methods have the advantage of air sampling under high humidity conditions without the problems associated with the hygroscopic nature of potassium iodide, and besides, these methods allow the simultaneous measurement of ozone and carbonyl compounds.

Acknowledgements

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REGULAR ARTICLE

Factors in genetic susceptibility in a chemical sensitive population using QEESI

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Abstract

Objectives Inherited impairment of xenobiotic metabolism is a postulated mechanism underlying environmentally associated pathogeneses such as multiple chemical sensitivity (MCS). Using the Quick Environmental Exposure and Sensitivity Inventory (QEESI), we defined people who have a strong response to chemical substances as "chemical sensitive populations (CSP)." The aim of this study is to evaluate the condition of subjects sensitive to chemicals and to analyze their genotypes in order to identify susceptibility factors in CSPs in Japanese populations.

Methods A total of 1,084 employees of Japanese companies were surveyed using the QEESI, history of MCS, and sick house syndrome. The common genotypes of the participants were analyzed for *glutathione S-transferase (GST) M1, GSTT1, aldehyde dehydrogenase2 (ALDH2),* and *paraoxonase1 (PON1)* in order to identify factors in the susceptibility to sensitivity to chemicals.

Results Four subjects had history of diagnosis of MCS; no subjects had diagnosis of sick house syndrome. The subjects were divided into four levels according to scores of 0, 1–19, 20–39, and 40 or more on three of the QEESI subscales. In addition, we used the MCS criteria by Hojo to differentiate between cases (CSP) and controls. No significant differences in the allelic distribution of genetic polymorphisms in the *GSTM1, GSTT1, ALDH2* or *PON1*

genes were found among the four levels of each subscale, or between cases and controls.

Conclusions Our findings suggest that the common genotypes of *GSTM1, GSTT1, ALDH2,* and *PON1* are of little importance to CSP in a Japanese population.

Keywords Genetic susceptibility · Multiple chemical sensitivity (MCS) · Idiopathic environmental intolerance (IEI) · Quick Environmental Exposure and Sensitivity Inventory (QEESI) · Logistic regression analysis

Introduction

Multiple chemical sensitivity (MCS), also known as idiopathic environmental intolerance [1], has been described as disabling multi-organ symptoms triggered by multiple exposures to chemicals. A number of hypotheses concerning the etiology and pathophysiology of MCS have been proposed [2], including impaired ability to metabolize toxic chemicals [3] and psychological mechanisms [4].

There is no widely accepted instrument to measure general chemical intolerance, and no objective criteria for identification of chemicals contributing to MCS, but Miller and Prihoda [5, 6] developed the Quick Environmental Exposure and Sensitivity Inventory (QEESI), which has its origins in the Environment Exposure and Sensitivity Inventory [7]. The QEESI is a reliable and valid screening instrument for chemical intolerance that consists of five subscales: chemical sensitivity, other chemical sensitivity, symptom severity, life impact, and masking index. The Japanese version of the QEESI was translated by Ishikawa and Miyata in 1999 [8].

The present study was designed to determine whether the results of the QEESI reveal a genetic difference for

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specific enzymes, primarily those possibly associated with chemical detoxification: glutathione S-transferase (GST) M1, GSTT1, aldehyde dehydrogenase 2 (ALDH2), and paraoxonase 1 (PON1).

The GSTs are a family of multifunctional enzymes and play a central role in detoxification of toxic and carcinogenic electrophiles. The polymorphic GSTs catalyze conjugation of glutathione to a variety of electrophilic compounds, including formaldehyde. Absence of activity of GSTM1, a μ class enzyme which detoxifies the reactive metabolites of benzo[a]pyrene and other polycyclic aromatic hydrocarbons, is due to homozygous deletion of the gene [9]. A similar polymorphism of the GSTT1 gene, encoding the θ class enzymes, has been described [10]. GSTT1 metabolizes various potential carcinogens, such as monohalomethanes, which are widely used as methylating agents, pesticides, and solvents [9].

Although GSTs are presumed to be involved in the first step of formaldehyde metabolism, it is still not clear which GST molecular species is responsible for formaldehyde metabolism. In addition, GST cytosolic activity in olfactory epithelium, the highest among extrahepatic tissues [11], is of particular interest in MCS, where the role of odorous triggers is important.

Acetaldehyde is one of the important chemicals that induce sick house syndrome and MCS [12]. Approximately half of the Japanese population lack ALDH2 activity because of a structural point mutation in the ALDH2 gene. This genetic polymorphism, which is seen in Asians, including Japanese, but not in Caucasians, results in catalytic deficiency of aldehyde metabolism [13]. However, there are few studies regarding MCS and genetic polymorphism among Asians.

PON1 is known to be polymorphic in humans, with two isoforms displaying distinct hydrolyzing activities. The Arg192 isoform hydrolyzes paraoxon rapidly, whereas the Gln192 isoform acts slowly [14]. PON1 genes were associated with Gulf War Syndrome [15], and PON1 reacts with toxic organophosphorus compounds [16].

Methods

Subjects

The present study was conducted from August to October 2003 at two companies (company A, an integrated circuit manufacturing company; company B, a paper pulp producing company) in Kyushu, in the south of Japan. The participants numbered 1,310 people from company A (males 936, females 374) and 891 from company B (males 778, females 113). Those who replied to the questionnaires (90.2%) and furthermore agreed to give genetic samples

(52.2%) were included. Finally, a total of 1,084 subjects (49.3%) who had purified DNA in good condition, including 502 subjects from company A (males 390, females 112) and 582 from company B (males 579, females 3), were eligible for this study (Table 1).

Table 1 Demographic characteristics of the subjects

	All subjects
Sex	
Male	969 (89.4%)
Female	115 (10.6%)
Total	1,084
Average age (range), years	
Male	42.2 ± 8.9 (19–63)
Female	32.3 ± 6.3 (23–67)
Total	41.2 ± 9.1 (19–67)
Smoking (>once/week)	
Male	572 (59.0%)
Female	31 (27.0%)
Total	603 (55.6%)
Drinking (>once/week)	
Male	742 (76.6%)
Female	43 (37.4%)
Total	785 (72.4%)
History of diagnosis	
Multiple chemical sensitivity	
Male	2 (0.2%)
Female	2 (0.2%)
Total	4[a] (0.3%)
Sick house syndrome	
Male	0 (0%)
Female	0 (0%)
Total	0 (0%)
Allergic disease	
Male	212 (21.9%)
Female	47 (40.9%)
Total	259[a] (23.9%)
Chemical sensitivity ≥40	
Male	60 (6.2%)
Female	8 (7.0%)
Total	77 (7.1%)
Other chemical sensitivity ≥25	
Male	29 (3.0%)
Female	5 (4.3%)
Total	34 (3.1%)
Symptom severity ≥40	
Male	68 (7.0%)
Female	6 (5.2%)
Total	74 (6.8%)

[a] Three subjects had both multiple chemical sensitivity and allergy disease

Instruments

We used the QEESI (Japanese version) [8] for the survey described above. The QEESI consists of five subscales: the chemical sensitivity subscale measures the extent to which certain odors or exposures make one sick, the other chemical sensitivity subscale measures the extent to which a variety of other exposures make one sick, the symptom severity subscale refers to the extent to which one experiences certain symptoms, the life impact subscale measures the extent to which the sensitivity affects certain aspects of life, and the masking index measures whether there are ongoing exposures from routinely used products. Unlike other studies in which the subjects were patients, the participants in our study were collected from the general population. We selected 3 of the subscales, eliminating life impact and masking index. Each subscale has ten questions, and each question has a possible score of 0–10. Therefore, the total possible score was 0–100. All study subjects completed a self-reporting questionnaire which covered history of MCS, sick house syndrome, and allergic disease, drinking history, and smoking history.

As defined by Miller and Prihoda [5, 6], scores on the QEESI reveal three levels of symptom: low, medium, and high. The criteria for chemical sensitivity and symptom severity are low = 0–19, medium = 20–39, and high = 40–100. The criteria for other chemical sensitivity are low = 0–11, medium = 12–24, and high = 25–100. It has been reported that these three subscales can distinguish individuals with high susceptibility and a control group using cutoff values [5].

Hojo et al. [17] designed a study to establish a new cutoff value for Japanese using the QEESI for screening of MCS patients. According to that study, one difference from patients in America was that in Japanese patients the other chemical sensitivity subscale had low sensitivity and low specificity. They concluded that the other chemical sensitivity subscale should be excluded when applying the QEESI to evaluate subjective symptoms in Japan. The new cutoff values for Japanese subjects were determined to be ≥ 40 for the chemical sensitivity subscale, ≥ 20 for the symptom severity subscale, and ≥ 10 for the life impact subscale. Using their criteria, we divided our subjects into two groups according to the score achieved (Table 2). Individuals with chemical sensitivity score ≥ 40 and symptom severity score ≥ 20 were defined as chemical sensitive population (CSP) (cases), while individuals with moderate or no symptoms were classified as nonsensitive (controls, chemical sensitivity score <39 or symptom severity score <19).

Table 2 Demographic characteristics of cases and controls defined by QEESI score

	Cases (CSP)[a] (n = 47)	Controls (n = 1,037)	P value
Sex			
Male	41 (87.2%)	928 (89.5%)	
Female	6 (12.8%)	109 (10.5%)	0.62[b]
Average age, years			
Mean ± SD	44.2 ± 8.8	41.1 ± 9.1	0.69[c]
Median (range)	44 (30–59)	41 (19–67)	
Smoking status (>once/week)	18 (38.3%)	585 (56.4%)	0.01[b]
Drinking status (>once/week)	32 (68.1%)	753 (72.6%)	0.50[b]

Cases (chemical sensitive population, CSP): chemical sensitivity score ≥ 40 and symptom severity score ≥ 20. Controls: chemical sensitivity score ≤ 39 or symptom severity score ≤ 19

[a] Classification in cases and controls according to Hojo et al. [17]

[b] P value, chi-square test. P < 0.05, difference significant

[c] P value, Student's t-test. P < 0.05, difference significant

Genotyping

Genomic DNA was isolated from peripheral leukocytes by proteinase K digestion, phenol/chloroform extraction, and ethanol precipitation. A multiplex polymerase chain reaction (PCR) method was used to detect the presence or absence of GSTM1 and GSTT1 [18].

Absence of GSTM1 and GSTT1 is due to homozygous deletion of these hereditary genes, termed the null genotype. The genotypes of ALDH2 (rs671) were identified by the method of Harada and Zhang [13] as the homozygous genotype of normal ALDH2 (*1/*1), the homozygous genotype of inactive ALDH2 (*2/*2), and the heterozygous genotype of normal and inactive ALDH2 (*1/*2). The genotype of the Gln192Arg (rs662) polymorphism of the PON1 gene was determined essentially as described previously [19].

Statistical analysis

Relative associations between the CSP and controls were assessed by calculating crude odds ratios (ORs) from contingency tables. Corresponding chi-square tests were carried out on the cases and controls. In logistic regression analysis, ORs with corresponding 95% confidence intervals (CI) were calculated. P values smaller than 0.05 were considered significant. Statistical analysis was carried out using SPSS version 19.