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特集：平常時・災害時の衛生対策

＜総説＞

日本産たばこ主流煙の化学分析法と測定結果

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Determination of Chemical Substances in Mainstream of Japanese Cigarettes

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抄録

わが国では，2005年に「たばこの規制に関する世界保健機関枠組条約」に締結し，条約に基づきたばこ対策が実施されている．しかしながら，対策後の受動喫煙の状況変化やたばこより発生する各種化学物質の正確な実態調査などの化学的数値の積み上げは少ない．なかでもたばこと喫煙において重要な喫煙者に関する数値が乏しく，特に，喫煙者が取り込む主流煙成分測定結果が非常に少ない状況であり，国内で販売されているたばこ外箱表示には，タール及びニコチン量のみが記載されている．しかしながら，この主流煙にはニコチンばかりでなく，発がん性を有する有害化学物質が粒子・ガス成分に多数含有されている．粒子成分には，IARCの発がん性リスク一覧においてグループ1に分類されたたばこ特異的ニトロソアミン類と多環芳香族炭化水素なども含有される．また，ガス成分には，グループ1に分類されたホルムアルデヒドをはじめとするアルデヒド類なども含まれる．これまでたばこ主流煙は，国際標準化機構が定める喫煙法で捕集されてきたが，ヒトの喫煙行動（代償性補償喫煙）に近い喫煙法（カナダ保健省提案）で捕集すると主流煙の化学成分量は増加する．今後，上記喫煙法での新たな主流煙測定結果が報告されることを期待する．

キーワード：たばこ，喫煙者，主流煙，発がん性，化学物質，代償性補償喫煙

Abstract

Japan executed the tobacco control program under the Framework Convention on Tobacco Control (FCTC) in 2005. However, after the program was carried out, scientific data about the assessment of passive smoking has been limited and there have been few accurate surveys of the various chemical substances contained in tobacco smoke. Additionally, there has been a lack of scientific evidence of smoking and smoker. In particular, data on chemical substances in a mainstream of tobacco smoke is not publicized in Japan. Therefore only tar and nicotine yields have been printed on domestic cigarette packages. However, the mainstream contains not only nicotine, but various carcinogens in a particle matter and in a gaseous phase. Among the particle matter 4-(Methylnitrosamino)-1-(3-pyridyl)-1-butanone, *N*'-nitrosonornicotine and benzo[*a*]pyrene classified as IARC Group 1 substances (human carcinogens) have been reported. Also, the gaseous phase contains carbonyl compounds such as formaldehyde classified as an IARC Group 1 substance. Up to the present date, International Organization for Standardization (ISO) method has been used to collect the substances of the particle matter and the gaseous phase in the mainstream with smoking machine. But many studies have showed that the amount of chemical substances in mainstream increases through

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a human smoking behavior that is similar to the modified method (Health Canada Intense smoking method: HCI) that is called "compensatory smoking". We hope that the amount of chemical substances in mainstream will be measured using HCI and that many reports will be published.

Keywords: tobacco, smoker, mainstream, carcinogen, chemical substance, compensatory smoking

Ⅰ．はじめに

わが国は，2005年に「たばこの規制に関する世界保健機関枠組条約（たばこ規制枠組条約）」に締結した．この条約の目的は，「たばこの使用及びたばこの煙にさらされることの広がりを継続的かつ実質的に減少させるため，締結国が自国においてならびに地域的及び国際的に実施するたばこの規制のための措置についての枠組みを提供することにより，たばこの消費及びたばこの煙にさらされることが健康，社会，環境及び経済に及ぼす破壊的な影響から現在及び将来の世代を保護すること」と記されている．わが国は，2006年からはニコチン依存の根拠をもとに禁煙治療に対する保険適用が開始されている．加えて2009年には，「受動喫煙防止対策のあり方に関する検討会報告書」がとりまとめられるなど受動喫煙に対する対策がまとまりつつある．さらに神奈川県では，本年（2010年）4月より「神奈川県公共的施設における受動喫煙防止条例」を施行するなど，受動喫煙に対する規制もおこなわれている．また，2010年10月1日からは，たばこ1本あたりの税が引き上げられるなど，たばこ及び喫煙者への関心が高まっている．

喫煙者は生体内にニコチンを摂取するために，喫煙行動を繰り返す．このニコチンは体内で速やかに代謝され，コチニンとなる．そのため多くの喫煙者は，一日に10－20本のたばこを喫煙すると考えられ，ニコチン依存度が高まることでさらに喫煙本数も増加する．喫煙者がたばこ末端（吸い口）より生体内に取り込む煙を主流煙と呼ぶ．この主流煙にはニコチンばかりでなく，発がん性を有する有害化学物質が粒子・ガス成分に多数含有されている．このため，北米カナダでは，たばこ外箱にタール，ニコチン，一酸化炭素（CO），ホルムアルデヒド，シアン化水素，ベンゼンの測定値が表示されている．一方，わが国はたばこの外箱側面に，たばこ事業法施行規制に基づき「財務大臣の定める方法により測定したたばこ煙中に含まれるタール量及びニコチン量」が印字されている．この数値は，国際標準化機構（International Standardization Organization; ISO）の定める測定法に基づいて，機械喫煙装置を用いて主流煙をガラス繊維フィルターへ捕集後，化学分析した結果である[1-3]．この喫煙方法は，1回の吸煙量が35 mL，そのときの吸煙時間が2秒，吸煙間隔が1分，そしてたばこフィルター部分に設けられた通気孔が開放と指定されている．この吸煙法によるたばこ1本あたりの吸煙回数は，6－8回となる．このとき粒子成分は，喫煙装置に設置したガラス繊維フィルターに捕集され，一方，ガス成分は測定対象物質ごとに捕集法が確立している．

本論文では，喫煙者が吸い込む主流煙に含まれている化学物質について，過去の先行研究をもとに説明する．また，低タール・低ニコチンたばこ喫煙者はより多くのニコチンを生体内に取り込もうと喫煙行動をするために喫煙時の吸煙量が多くなる傾向がある．この喫煙行動を採用した喫煙法（HCI法）がカナダ保健省によって提唱されている[4]．このHCI法を用いて捕集したニコチン量とISO法によって測定した結果も合わせて報告する．

Ⅱ．ニコチン

ニコチンは，たばこの葉中に含有されるアルカロイドであり，依存性の高い物質である．平成20年度の国民栄養・調査によると現在習慣的に喫煙している者で，たばこをやめたいと思う者は，男性28.5%女性37.4%であり，さらに禁煙を試みたことがある者は，男性52.1%，女性57.0%であった．以上のことからも禁煙することが簡単ではないことが伺える．またニコチンは，「毒物及び劇物取締法」で医薬用外毒物に指定されているが，発がん性は確認されていない．一般的に主流煙のニコチン測定は，ガスクロマトグラフィー（GC）を利用したISO10315に準拠して行なわれている．ニコチンの濃度は，たばこ外箱に表示されているとおりである．

Ⅲ．たばこ特異的ニトロソアミン（TSNA）

主流煙には，ニトロソアミン類が含まれており，特にtobacco specific N－nitrosamines（TSNA）として4種が存在する．TSNAはたばこ葉のアルカロイドであるnicotine, nornicotine, anatabine, anabasineと亜硝酸や硝酸が反応して，4－(Methylnitrosoamino)－1－(3－pyridyl)－1－butanone (NNK)，N'－nitrosonornicotine (NNN)，N'－nitrosoanatabine (NAT) とN'－nitrosoanabatine (NAB) が生成される[5]．また，たばこの発酵，製造過程により生成されるものや，たばこの燃焼時に熱合成により生成されるものもある[6]．4種のTSNAのうち，NNKとNNNはIARCの発がん性リスク一覧においてグループ1（The agent is carcinogenic to humans.；ヒトに対する発がん性が認められる）に，NATとNABはグループ3（The agent is not classifiable as to its carcinogenicity to humans.；ヒトに対する発がん性が分類できない）に分類されている．

たばこ主流煙中TSNAの測定は，主にガスクロマトグ

ラフィー／熱エネルギーアナライザ（GC/TEA）を用いて行われている[7,8]．近年は，検出感度が良く選択性の高い高速液体クロマトグラフィー／タンデム型質量分析計（LC/MS/MS）を用いて実施されている[6,9]．TSNAは主に粒子成分に含まれるため，まず機械喫煙装置によって喫煙させて発生した主流煙をガラス繊維フィルターで採取する．この採取フィルターから振盪抽出後，抽出液を直接LC/MS/MSに供する方法がある[10]．しかし，溶液中の夾雑物がLC/MS/MSのイオン化を抑制，または測定結果を高めてしまうこともある[11]ため，液－液抽出法や固相抽出法を組み合わせて夾雑物を除去後，LC/MS/MSに供する方法[12]も検討されている．これまでの日本産たばこのTSNA測定は，GC/TEAを使用した報告がほとんどである．旧厚生省は1999－2000年に国産たばこの測定を実施しており，その結果をTable 1に示す[13]．この結果は，TSNA量は，たばこ外箱に表示されたタール量に依存せず，かならずしもタール量が有害性の指標ではないことを示唆している．

Ⅳ．多環芳香族炭化水素（PAH）

多環芳香族炭化水素類(polycyclic aromatic hydrocarbons, PAH）は，一般的に炭化水素のみで構成された環状不飽和化合物（芳香環）が2つ以上縮合したものの一群を指し，有機物質の不完全燃焼により発生することが知られている．発生したPAHは環境状況（気温，湿度等）により気体だけでなく粉塵に吸着した状態などで大気中に拡散していくことも報告されている．このようなPAHの代表的なものとしてBenzo[a]pyrene（BaP）があり，これら化合物はIARCの発がん性リスク一覧においてグループ1に分類されている．たばこ主流煙には，BaPをはじめとする10種ほどのPAHが含有されている[14]．PAHの測定法は，主にHPLC/蛍光光度法（HPLC/FLD）やガスクロマトグラフィーと質量分析を組み合わせた方法（GC/MS）が多用されている[15－17]．また，主流煙中BaP測定法については，公定法が作成されており，ISO法ではGC/MS法[17]が，カナダ保健省の公定法では，HPLC/FLD[16]が採用されている．国産たばこの測定結果は，Table 1に示すとおりである．BaPは，TSNAと異なりたばこ外箱表示タール量に依存して，増加することが確認された．なお，この結果は，Hyodoらと同様の傾向であった[18]．

Ⅴ．アルデヒド類

たばこ主流煙中に含まれるアルデヒド類のうちIARCで発がん性が評価されているものはホルムアルデヒドとアセトアルデヒドの2種類である．ホルムアルデヒドはIARCのグループ1に分類され，アセトアルデヒドはグループ2Bに分類される．主に主流煙中のアルデヒド類は，燃焼によって発生し，ガス成分に含有される．

アルデヒド類やケトン類（カルボニル化合物）の分析方法として，最も広く使用されているのは2,4－ジニトロフェニルヒドラジン（DNPH）による誘導体化法である．これは，DNPHがアルデヒド類やケトン類と選択的に反応し，対応する2,4－ジニトロフェニルヒドラゾン誘導体を生成することを利用している．DNPH誘導体化法はAllen[19]とBrady[20]により最初に報告された．この方法の特徴は，様々なアルデヒド類，ケトン類を同時に分析できることである．空気中のカルボニル化合物の捕集には，当初DNPHの酸性溶液のインピンジャー法[21]が行われていたが，現在はDNPHを含浸させた担体を用いた固体捕集法が主流となっている．固体捕集法に用いられるDNPHの担体には，XAD-2[22,23]，シリカゲル[24,25]，ガラスビーズ[26]，オクタデシルシラン結合シリカゲル[27]，フロリジル[28]，ガラスファイバーフィルター[29]など多くの種類が使われてきたが，最近は，シリカゲルが最も多く使われており，DNPHを含浸させたDNPH-silicaが主流である．このDNPH-silicaを捕集剤とし，分析には紫外吸収検出器を備えた高速液体クロマトグラフィー（HPLC）を用いる方法が，世界各国の公定法に採用されている[30]．この方法で著者らはたばこ主流煙中のアルデヒド類を分析し，ホルムアルデヒドやアセトアルデヒド以外8種類のアルデヒド類を測定している[31]．特に，毒性の高いアクロレイン，グリオキサール，メチルグリオキサールが検出，定量可能であった．環境たばこ煙（ETS）に含まれるアルデヒド類の分析にはDNPH-silicaを充填したカートリッジが使われ

Table 1 Amounts of TSNA and BaP in mainstream smoke of Japanese cigarettes

Cigarette Brands	Nicotine (mg/cig.)	Tar (mg/cig.)	NNN (ng/cig.) mean	SD	NNK (ng/sig.) mean	SD	NAT (ng/cig.) mean	SD	NAB (ng/cig.) mean	SD	BaP (ng/cig.) mean	SD
Frontier Lights	0.12	1.4	21.9	± 1.56	N. D.		18.5	± 1.97	N. D.		2.15	± 0.133
Mild Seven Extra Lights	0.30	3.2	45.6	± 3.16	27.7	± 1.91	44.4	± 3.49	9.9	± 1.26	3.72	± 0.198
Mild Seven Super Lights	0.44	5.2	47.8	± 2.20	28.1	± 4.13	55.5	± 2.69	11.7	± 1.62	5.51	± 0.479
Marlboro Menthol Lights	0.60	7.5	125	± 4.36	89.9	± 5.61	120	± 8.54	19.6	± 0.45	6.43	± 0.442
CABIN Mild	0.66	8.7	116	± 6.94	52.8	± 3.56	84	± 3.86	15.7	± 0.55	8.87	± 0.678
Mild Seven	0.96	11.8	81	± 4.28	47.7	± 3.54	96.2	± 6.51	15.9	± 1.09	11.4	± 0.576
Seven Stars	1.44	16.3	65	± 6.07	40.3	± 3.19	77.6	± 4.58	13.1	± 1.87	14.6	± 0.984

Smoking protocol is ISO regimen. Source modified from reference 13.
N. D. : Not detected

Table 2 Amounts of carbonyls in mainstream smoke vapors of Japanese cigarettes

Cigarette brands	Formaldehyde mean (μg/cig.)	SD	Acetaldehyde mean (μg/cig.)	SD	Acetone mean (μg/cig.)	SD	Acrolein mean (μg/cig.)	SD	Propionaldehyde mean (μg/cig.)	SD	Crotonaldehyde mean (μg/cig.)	SD	Methyl ethyl ketone mean (μg/cig.)	SD	Butyraldehyde mean (μg/cig.)	SD
Frontier Lights	3.46	± 0.047	112	± 17.7	82.5	± 5.81	9.93	± 1.09	11.8	± 1.36	2.33	± 0.367	13.6	± 1.35	12	± 0.962
Mild Seven Extra Lights	7.64	± 0.691	228	± 11.9	142	± 4.63	18.9	± 1.2	21.4	± 0.892	2.23	± 0.191	23	± 2.04	14.4	± 0.872
Mild Seven Super Lights	11.3	± 0.976	301	± 34.2	161	± 16.4	22.5	± 3.14	28.1	± 3.08	4.08	± 0.394	26.5	± 3.8	16.3	± 1.24
Marlboro Menthol Lights	15.8	± 1.75	397	± 60.6	212	± 20.2	34.8	± 4.44	36.8	± 2.08	5.66	± 0.535	44.4	± 2.36	26.8	± 2.58
CABIN Mild	19.3	± 1.07	491	± 26.8	249	± 9.27	37.4	± 1.71	45	± 2.53	5.37	± 0.371	42.5	± 4.5	25.5	± 1.85
Mild Seven	37.9	± 2.22	560	± 50.7	295	± 26.8	47.6	± 4.05	44.4	± 2.96	12.3	± 0.624	55	± 5.23	35.4	± 2.12
Seven Stars	70.7	± 5.51	766	± 51.2	362	± 19.2	73.8	± 5.16	72.3	± 5.35	18.3	± 1.29	72.9	± 1.13	42.6	± 1.63

Source modified from reference 13.

るが，主流煙の分析にはDNPH酸性溶液のインピンジャー法[32]が用いられている．この理由は，一般に，たばこ主流煙に含まれるアルデヒド類の濃度が非常に高く，通常のカートリッジ法で分析すると，DNPHを全て消費してしまい破過するためである．しかし，インピンジャー法は，操作が煩雑であるばかりでなく，感度が低いため，高濃度のアセトアルデヒド等は分析できるが，低濃度の物質は測定できない欠点がある．たとえ低濃度でも，毒性の高いアルデヒド類がたばこ煙に存在する可能性もあるので，今後，高感度分析法の開発が必要である．最後に，国産たばこのアルデヒドの測定結果をTable 2に示す．

Ⅵ．HCI法によるニコチン測定結果とその比較

カナダ保健省は，ヒトの喫煙行動を考慮した喫煙法（HCI法）を提案している．この喫煙方法は，1回の吸煙量が55 mLでそのときの吸煙時間が2秒，吸煙間隔が30秒，そしてたばこフィルター部分に設けられた通気孔が閉鎖と指定されている．我々の研究グループは，過去に日本人喫煙者を対象としてその吸煙量を調査したところ，ニコチン表示量が0.6 mg未満のたばこを吸う喫煙者は，平均で58.4 mLの吸煙量であり，0.6 mg以上の場合は，同値50.0 mLであった[33]．上記研究結果から喫煙者は，ISO法よりもHCI法に近い喫煙行動を行なうことを示唆した．これは，低タール・低ニコチンたばこを喫煙する喫煙者がニコチンを体内に取り込むために「代償性補償喫煙」を行った結果であると考えられる．次にEndo[34]らによる国産たばこ10銘柄におけるISO，HCI法で捕集した主流煙中のニコチン測定結果をTable 3に示す．たばこ外箱表示が0.6 mg未満のたばこは，喫煙法をHCIにするとニコチン量は，0.89 − 1.4 mg/cig.となった．この結果から吸煙量の増加にともなってニコチン量も増加することが証明された．また，この研究で最もニコチン表示量が高いたばこをHCI法で捕集し，ニコチン量を測定したところ2.21 mg/cig.であり，ニコチン表示量が最も低いたばこのHCI法による捕集後のニコチン量0.89 mg/cig.と比較すると2.5倍ほどであった．ここで外箱ニコチン表示量の差は12倍であるが，喫煙法による違いは12倍以下であった．このことから喫煙法によって，ニコチン量は，たばこ外箱表示量とは異なる結果となることが確認され，また実際の喫煙においてもニコチン摂取量が変化するものと考えられた．

Table 3 Amounts of nicotine in mainstream smoke of Japanese cigarettes

Cigarette brands	Nicotine (mg/cigarette) ISO Mean	SD	HCI Mean	SD
Pianissimo One	0.20	± 0.01	0.89	± 0.01
Mild Seven One	0.19	± 0.03	0.97	± 0.07
Mild Seven Extra Lights	0.32	± 0.02	1.28	± 0.19
Caster Mild	0.47	± 0.06	1.29	± 0.07
Mild Seven Super Lights	0.53	± 0.05	1.40	± 0.08
CABIN Mild	0.63	± 0.03	1.46	± 0.08
Mild Seven Lights	0.66	± 0.05	1.52	± 0.09
Mild Seven Original	0.78	± 0.12	1.81	± 0.17
HOPE	0.95	± 0.06	2.04	± 0.04
Seven Stars	1.11	± 0.13	2.21	± 0.20

Source modified from reference 34.

現在，わが国では，低タール・低ニコチンたばこがたばこ販売本数においてその多くを占めている．今後は，喫煙者が代償性補償喫煙を行なうことを考えて，HCI法による主流煙の捕集やニコチン以外のTSNA，PAH，アルデヒド類の測定結果がより多く公表されることを期待している．また，毎年，新しい銘柄のたばこが発売されているが，これら国産たばこの測定報告が，非常に少ないのは残念である．加えて，本報告で紹介することが出来なかった重金属をはじめとする有害化学物質の測定がさらに実施されることを期待する．

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Improved Measurement of Ozone and Carbonyls Using a Dual-Bed Sampling Cartridge Containing *trans*-1,2-Bis(2-pyridyl)ethylene and 2,4-Dinitrophenylhydrazine-Impregnated Silica

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We have previously developed a new method using *trans*-1,2-bis-(4-pyridyl)ethylene (4-BPE) and 2,4-dinitrophenylhydrazine (DNPH) for the simultaneous determination of ozone and carbonyls in air using a two-bed cartridge system. In this method, 4-BPE was used to capture ozone. 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 by using *trans*-1,2-bis-(2-pyridyl)ethylene (2-BPE) in place of 4-BPE. The efficiency of the reaction of ozone with 2-BPE 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 and 120 min, respectively. In the 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. 2-BPE exhibits a maximum reaction efficiency of 84% at 32% relative humidity (RH), while 4-BPE attains a maximum reaction efficiency of 82% at 49% RH. Humidity has much less influence on the reaction of 2-BPE with ozone. Above 18% RH, 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 concentrations of ozone and carbonyls by the improved 2-BPE/DNPH method corresponded with the mean value by an ozone auto analyzer in an air monitoring station and a DNPH cartridge coupled with a KI-ozone scrubbing cartridge.

For the measurement of carbonyl compounds, a selective and sensitive method based on derivatization with 2,4-dinitrophenylhydrazine (DNPH) and subsequent high-performance liquid chromatography (HPLC) separation has been widely used in active[1] and diffusive[2−4] sampling methods. Because of the importance of this method, it has been introduced as a standard procedure by several national standardization bodies. However, recent research has resulted in the identification of chemical interferences caused by the presence of ozone[5−7] or nitrogen dioxide[8] in the air sample. Ozone at high concentrations has been shown to interfere negatively by reacting with both DNPH and its carbonyl derivatives (2,4-DNPhydrazones) in the cartridge.[5,9] The extent of interference depends on the temporal variations of both the ozone and the carbonyl compounds and the duration of sampling. Significant negative interference from ozone was observed even at concentrations of formaldehyde and ozone typical of clean, ambient air (i.e., 2 and 40 ppb, respectively). The most direct solution to ozone interference is to remove the ozone before the sample stream reaches the sampling cartridge. This process entails constructing an ozone denuder[1] or scrubber and placing it in front of the cartridge. Ozone scrubbers (cartridges filled with granular potassium iodide) are also available from suppliers of precoated DNPH. These scrubbers exhibit optimum performance when the ambient air contains a minimum of 15% relative humidity. When ambient atmospheric sampling is conducted at higher humidity, potassium iodide in the ozone scrubber is likely wetted by atmospheric moisture. The wetted potassium iodide can trap carbonyls before they reach the DNPH sampling media. Moreover, potassium iodide dissolved by airborne moisture can migrate into the DNPH cartridge and react to form unknown compounds.

We have developed a new method for the simultaneous determination of ozone and carbonyls in air using a two-bed cartridge system (BPE/DNPH-cartridge).[10] Each bed consists of reagent impregnated silica particles. The first contains *trans*-1,2-bis-(4-pyridyl)ethylene (BPE), while the second contains 2,4-

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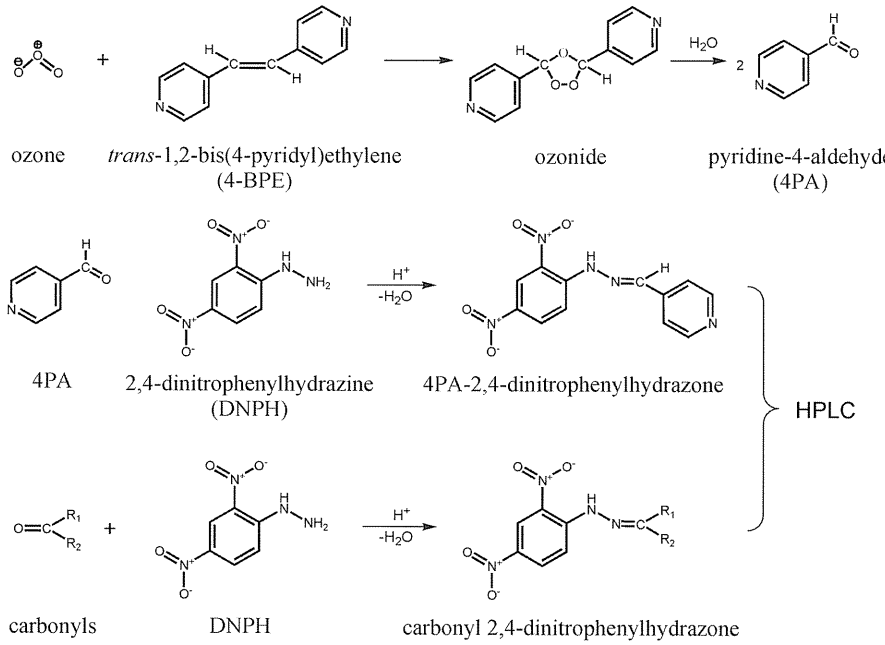
Scheme 1



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 to produce pyridine-4-aldehyde. Airborne carbonyls pass unimpeded thorough the BPE and are trapped in the second bed by the DNPH-coated silica particles. They produce carbonyl 2,4-DNPhydrazones. These reactions are outlined in Scheme 1.

DNPH and carbonyl 2,4-DNPhydrazones are not influenced by ozone because of effective trapping by the BPE. Extraction is performed in the direction reverse to air sampling. When solvent is eluted through the BPE/DNPH cartridge, excess DNPH is washed into the BPE bed where it reacts with pyridine-4-aldehyde and forms the corresponding 2,4-DNPhydrazone derivative. All of the 2,4-DNPhydrazones derived from airborne carbonyls and pyridine-4-aldehyde (derived from ozone) are completely separated and measured using HPLC. The use of a BPE/DNPH cartridge has made possible the simultaneous determination of ozone and carbonyls, which provides many advantages over traditional methods. However, some problems such as low solubility of pyridine-4-aldehyde DNPhydrazone, long reaction time, and influence of humidity were observed in BPE/DNPH methods using *trans*-1,2-bis-(4-pyridyl)ethylene. In this study, the BPE/DNPH method was improved by using *trans*-1,2-bis-(2-pyridyl)ethylene instead of *trans*-1,2-bis-(4-pyridyl)ethylene.

EXPERIMENTAL SECTION

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 a 150 mm length × 4.6 mm i.d. stainless steel tube (Supelco Inc., Bellefonte, PA) packed with Ascentis Express C18, 2.7 μm particles. The mobile phase mixture was acetonitrile/water (55:45 v/v) containing 5 mmol/L ammonium acetate. The column temperature was 40 °C, and the injection volume was 10 μL. The ozone analyzer (OA-683, Kimoto Electric Co., Ltd., Japan) was calibrated with a standard reference photometer of the National Institute of Standards and Technology (NIST). Standard ozone gas was generated using an ozone generator (model 1410, Dylec, Inc., Japan) equipped with an air purifier unit (model 1400, Dylec, Inc.). Two air pumps (SP-100 Dual GL Sciences Inc., Saitama, Japan) and wet gas meter (WS D-1A; Shinagawa Co., Tokyo, Japan) were used for the collection of air samples. Humidity and temperature of standard ozone gas were recorded using a TR-72U data logger (T&D Corporation, Japan).

The 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). 2,4-Dinitrophenylhydrazine hydrochloride (>98%), *trans*-1,2-bis(2-pyridyl)ethylene (>97%) and *trans*-1,2-bis(4-pyridyl)ethylene (>98%) were from Tokyo Kasei Co. Ltd. (Tokyo, Japan). Acetonitrile (HPLC grade, >99.9%), 2-pyridinecarboxaldehyde (pyridine-2-aldehyde, 99%), 3-pyridinecarboxaldehyde (pyridine-3-aldehyde, 98%), 4-pyridinecarboxaldehyde (pyridine-4-aldehyde, 97%), phosphoric acid (85% solution in water), hydrochloric acid (37%), and ammonium acetate (99.999%) were from Sigma-Aldrich Inc., St. Louis, MO. Rezorian ozone scrubbers (3 mL/1.5 g of potassium iodide) were from Supelco Inc. Silica gel (spherical, 60/80 mesh, 120 Å mean pore size) was from AGC Si-Tech. Co., Ltd. (Fukuoka, Japan). DNPH-coated silica particles were diverted from commercially available DSD-DNPH (Sigma-Aldrich Inc., St. Louis, MO).

Synthesis of Pyridine-2-aldehyde (2PA), Pyridine-3-aldehyde (3PA), or Pyridine-4-aldehyde (4PA) 2,4-DNPhydrazone Derivative. First, 2,4-dinitrophenylhydrazine hydrochloride (2.3 g) was dissolved in ethanol (400 mL), and hydrochloric acid (5 mL) was added. Three milliliters of 2PA, 3PA, or 4PA was then added with continuous stirring. After a few minutes, the resulting precipitate was recovered by filtration and washed with water (3 × 500 mL), and then 10 mmol/L sodium carbonate (500 mL) was added. After a few minutes, the resulting precipitate was recovered by filtration and washed with water (3 × 500 mL). Finally, the washed precipitate was recrystallized from acetonitrile and dried for 3 h at 105 °C.

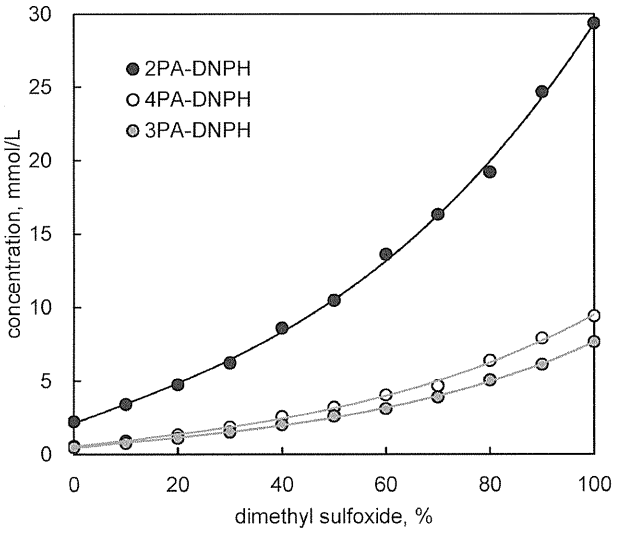


Figure 1. Solubility of isomeric pyridine aldehyde 2,4-DNPhydrazones in various solutions of dimethyl sulfoxide and acetonitrile.

BPE-Coated Silica Particles. Silica gel (50 g) was washed with water (3 × 500 mL) and acetonitrile (2 × 500 mL). To the washed silica gel was added 500 mg of *trans*-1,2-bis(2-pyridyl)ethylene (2-BPE) or *trans*-1,2-bis(4-pyridyl)ethylene (4-BPE) in acetonitrile with continuous stirring. The solvent was then evaporated to dryness at 40 °C under vacuum using a rotary evaporator. 2-BPE was used after recrystallization with acetonitrile.

BPE/DNPH Cartridge for Collection of Ozone and Carbonyls. BPE-coated silica particles (130 mg) and DNPH-coated silica particles (270 mg, containing 38 μmol phosphoric acid) were packed into a polyethylene cartridge (Rezorian tube, Supelco Inc., Bellefonte, PA) and stored in a refrigerator at 4 °C. BPE and DNPH contents of BPE/DNPH cartridge are 7.1 and 6.6 μmol, respectively. The air sample was drawn through the cartridge from the BPE bed to the DNPH bed. Extraction was performed in the reverse direction to air sampling. The solution eluted through the BPE/DNPH-cartridge, containing 2,4-DNPhydrazones derivatized with various carbonyls including pyridine aldehyde formed from ozone, was analyzed by HPLC.

RESULTS AND DISCUSSION

BPE/DNPH-Cartridge Extraction and HPLC Analysis. 4PA-2,4-DNPhydrazone is difficult to dissolve in acetonitrile and dissolves in dimethyl sulfoxide at relatively high concentration.[10] Each of the pyridine aldehyde-2,4-DNPhydrazone isomers, including 2PA-2,4-DNPhydrazone, 3PA-2,4-DNPhydrazone, and 4PA-2,4-DNPhydrazone, were added with continuous stirring to various mixtures of dimethyl sulfoxide and acetonitrile until no dissolved material remained at 20 °C. Then, the concentrations of pyridine aldehyde 2,4-DNPhydrazones in the saturated solutions were measured by HPLC. Figure 1 shows the solubility of pyridine aldehyde 2,4-DNPhydrazones in mixtures of dimethyl sulfoxide and acetonitrile.

In order to maintain high extraction efficiency, it is necessary to elute BPE/DNPH cartridges with acetonitile containing dimethyl sulfoxide. After considering the solubility and elution of 2PA-2,4-DNPhydrazone, a solution containing 25% dimethyl sulfoxide in acetonitrile was used as the eluent in this study.

The spectral profile of the 2PA-2,4-DNPhydrazone derivative is very similar to that for 4PA-2,4-DNPhydrazone.[10] They exhibit

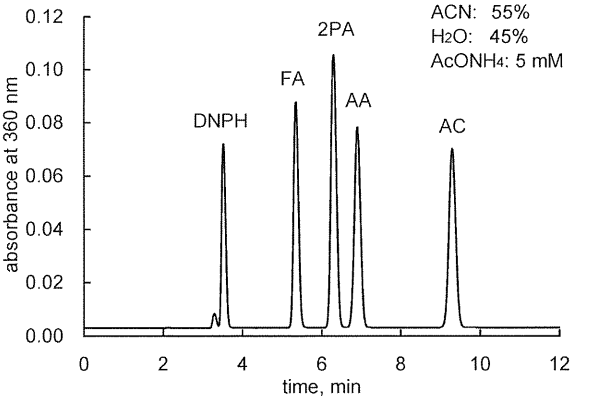


Figure 2. Chromatogram of pyridine-2-aldehyde and other carbonyl 2,4-DNPhydrazones.

long maximum absorption wavelengths (378 nm) and large absorption coefficients (3.4×10^4 L/mol/cm).

Analytical conditions for pyridine-2-aldehyde- and C_1-C_3 carbonyl-DNPH derivatives were examined using an Ascentis Express C18 column. Figure 2 shows the chromatogram of a standard mixture containing pyridine-2-aldehyde (2PA), formaldehyde (FA), acetaldehyde (AA), and acetone (AC) 2,4-DNPhydrazones (20 μmol/L). The pyridine-4-aldehyde (4PA) derivative is not separated from this mixture as it coelutes with the formaldehyde derivative (FA). Under these HPLC conditions, *E*- and *Z*-stereoisomers of acetaldehyde DNPhydrazone were not separated and appeared as a single peak.

Reactivity of 2-BPE and 4-BPE. The reactivity of BPE with ozone is diminished in the presence of acids, but the reactivity of pyridine aldehydes with DNPH is enhanced under acidic conditions. In our initial experiments, we used silica impregnated with DNPH-hydrochloride and made no effort to remove hydrogen chloride. Using ion chromatography measurements, we discovered that volatile hydrochloride in DNPH-impregnated silica migrated into the basic BPE-silica bed of the dual-bed sampling cartridge. Nonvolatile phosphoric acid in DNPH-silica did not migrate into the BPE-silica. For this reason, DNPH-silica containing no hydrochloride was used in this study. Air containing ozone was sampled using the 2-BPE/DNPH and 4-BPE/DNPH cartridges at a flow rate of 1 L/min for 1 h. The cartridges were then eluted with 3 mL of 25% dimethyl sulfoxide in acetonitrile solution or 25% dimethyl sulfoxide in acetonitrile solution containing 0.1% phosphoric acid. Figure 3 shows the changes in pyridine aldehyde DNPhydrazones concentration over time as the eluent solutions were allowed to set at room temperature.

Under all experimental conditions investigated in this study, the reaction rate of 2PA with DNPH is faster than 4PA. With the use of nonacidic eluent, the reaction of 4PA with DNPH was very slow. The use of nonhydrochloride DNPH required 500 min to complete the reaction with 4PA. The reaction of 2PA with DNPH was complete in 120 min. The reaction of PA with DNPH was enhanced by catalytic acid. With the use of acidic eluent (0.1% phosphoric acid), the reaction rate of both 2PA and 4PA increased dramatically. For 2PA, 2,4-DNPhydrazone formation was complete within 15 min. Thus, the most efficient eluent for the 2-BPE/DNPH dual-bed cartridge is 25% dimethyl sulfoxide in acetonitrile solution containing 0.1% phosphoric acid.

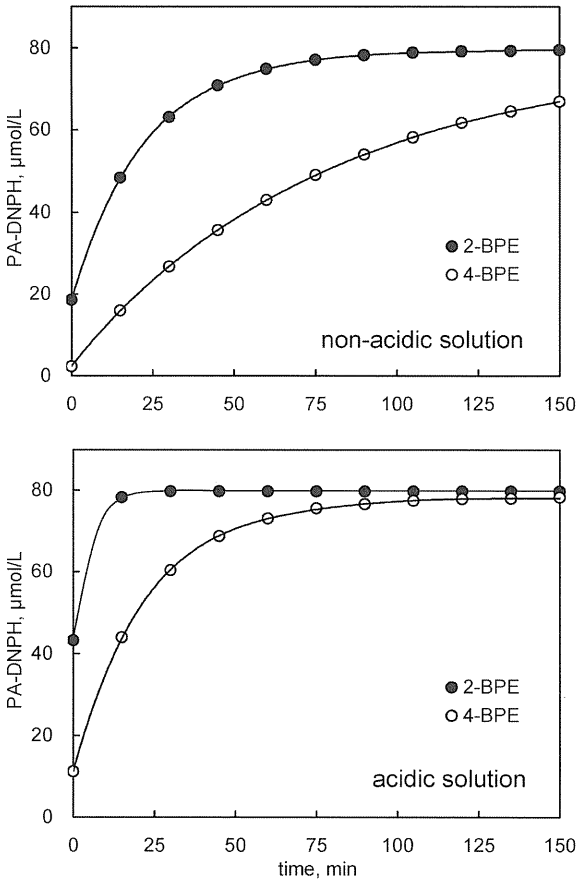


Figure 3. Changes in carbonyl concentrations with time in eluate of 25% dimethyl sulfoxide in acetonitrile solution (upper panel) and 25% dimethyl sulfoxide in acetonitrile solution containing 0.1% phosphoric acid (lower panel).

Comparison of the BPE/DNPH Method with a UV-Absorption Ozone Analyzer. Standard ozone gas was generated using an ozone generator and then mixed with humidified pure air. Humidified standard ozone gas, controlled to 49 ± 2% relative humidity, was introduced into the 500 mL manifold and then drawn to an ozone analyzer. The prepared standard ozone gas was also drawn through parallel 2-BPE/DNPH and 4-BPE/DNPH cartridges at flow rates of 500 mL/min for 1 h. Following sampling, the cartridges were eluted with 3 mL of 25% dimethyl sulfoxide in acetonitrile solution containing 0.1% phosphoric acid. After 3 h, eluents were analyzed by HPLC. Figure 4 shows the relationship between ozone concentrations measured using the UV-absorption ozone analyzer and BPE/DNPH samplers. Ozone concentrations measured using the 2-BPE/DNPH and 4-BPE/DNPH samplers were lower compared to the ozone analyzer. In the case of 2-BPE/DNPH, there was a linear relationship in the range of 0−300 ppb with the coefficient of determination of 0.9998. In the case of 4-BPE/DNPH, there was a linear relationship in the range of 0−100 ppb. However, there was less agreement with the ozone analyzer above 100 ppb. The slope of the regression line for the relationship between BPE/DNPH and the ozone analyzer results indicates that the BPE/ozone reaction efficiency is estimated at 84%.

The reaction of BPE with ozone requires water for the hydrolysis of ozonide (Scheme 1). It is possible that adsorbed water in BPE-silica may be sufficient to hydrolyze the ozonide. However, the moisture in the sampled air may be needed. Standard ozone gas was generated and mixed with humidified

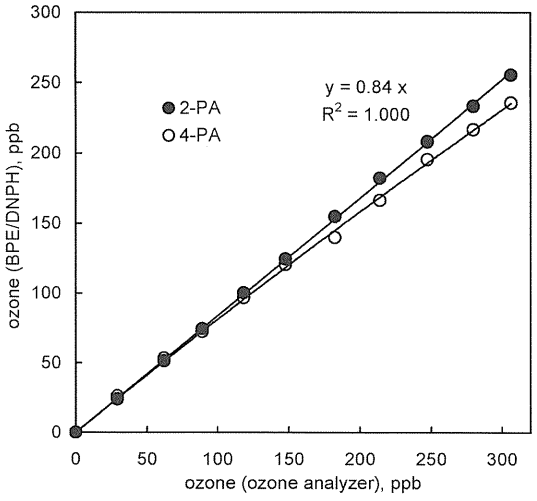


Figure 4. Relationship between UV-absorption ozone analyzer and BPE/DNPH methods.

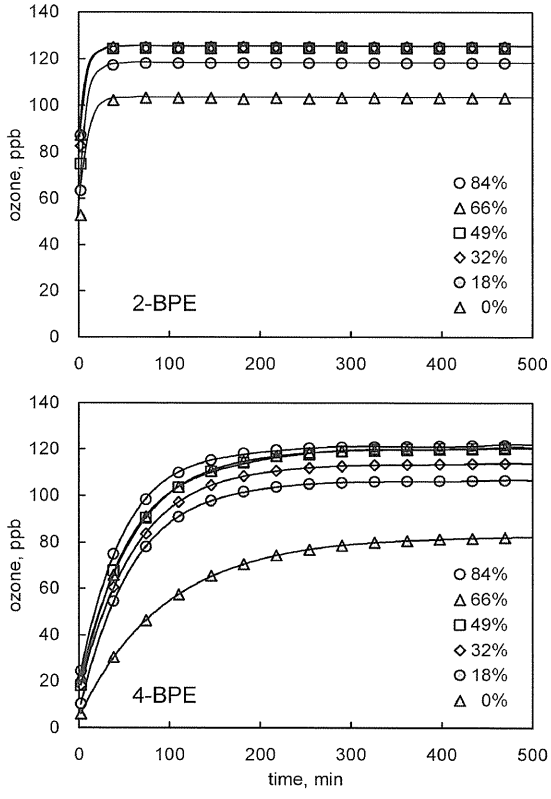


Figure 5. Changes in reactivities of ozone with 2-BPE or ozone with 4-BPE at a wide range of relative humidities. Concentrations of standard ozone gases were controlled to 147 ppb (146−149 ppb).

pure air. Humidified ozone gas (150 ppb) was controlled to 0, 18, 32, 49, 66, or 84% relative humidity, introduced into the 500 mL manifold and drawn to an ozone analyzer. The prepared standard ozone gas was also drawn through parallel 2-BPE/DNPH and 4-BPE/DNPH cartridges at the flow rates of 500 mL/min for 1 h. Following sampling, the cartridges were eluted with 3 mL of 25% dimethyl sulfoxide in acetonitrile solution containing 0.1% phosphoric acid. After 3 h, the eluents were analyzed by HPLC. Figure 5 shows the changes in reactivities of ozone with 2-BPE or ozone with 4-BPE at a wide range of relative humidities.

In the absence of atmospheric moisture (0% relative humidity), the reaction efficiencies of 2-BPE and 4-BPE with ozone are quite low, ranging from 55% for the 4-BPE/ozone reaction to 70% for

Table 1. Concentrations of Ozone and Carbonyls Measured in Ambient Air Collected by a DNPH Cartridge Coupled with a KI-Ozone Scrubber (KI) and a 2-BPE/DNPH Cartridge (2BPE)[a]

	weather conditions			2-BPE/DNPH, KI/DNPH					AMS		
				formaldehyde		acetaldehyde		ozone	ozone		
date (2009)	weather	temp (°C)	RH (%)	2BPE	KI	2BPE	KI	2BPE	mean	minimum	maximum
January 10−11	fair/fair	4.6	46	1.8	na[b]	1.7	na[b]	63	63	4	84
January 11−12	fair/fair	3.9	53	4.1	na[b]	3.2	na[b]	33	38	4	86
January 17−18	fair/fair	6.3	60	3.3	na[b]	3.1	na[b]	30	28	2	80
January 24−25	clouds/clouds	3.4	85	1.5	na[b]	1.4	na[b]	35	36	4	64
February 7−8	fair/fair	7.5	62	3.0	na[b]	4.2	na[b]	38	41	4	84
February 14−15	fair/fair	16	63	2.8	na[b]	2.9	na[b]	62	66	26	130
February 21−22	fair/fair	5.3	44	2.2	2.1	2.0	2.0	39	43	4	100
February 28−1	clouds/clouds	7.3	61	1.6	1.3	1.5	1.3	57	57	12	88
March 1−2	rain/clouds	6.1	86	1.5	0.9	1.5	1.0	43	46	4	88
March 20−21	clouds/fair	7.0	53	1.8	1.5	1.9	1.9	61	63	8	100
March 21−22	fair/clouds	14	58	1.6	1.5	1.2	1.3	85	88	58	100
March 28−29	clouds/fair	7.3	54	2.0	1.9	1.8	1.9	53	57	4	100
April 4−5	clouds/rain	14	71	2.4	na[b]	2.2	na[b]	84	85	14	130
April 11−12	fair/clouds	18	64	3.7	3.4	2.5	2.3	110	120	36	180
April 18−19	clouds/clouds	13	64	3.1	3.0	2.2	2.0	69	75	16	160
April 25−26	rain/fair	13	97	2.1	0.9	1.9	0.8	74	77	36	110
May 2−3	fair/clouds	20	63	2.8	2.6	2.8	2.7	130	130	88	160
May 3−4	clouds/clouds	20	64	2.1	1.9	1.4	0.9	120	110	96	130
May 4−5	clouds/rain	20	67	2.1	1.8	1.5	1.2	90	86	42	120
May 9−10	fair/fair	22	71	3.4	3.0	2.6	2.3	85	88	58	130
May 23−24	clouds/rain	23	68	3.6	3.4	3.2	2.8	91	96	38	110
May 30−31	rain/clouds	19	92	1.8	1.0	1.2	0.5	46	47	28	64

[a] Air collections were performed simultaneously at Chiba City's air monitoring station (AMS), January−May 2009. Concentration units are in micrograms per cubic meter. [b] na, not available.

the 2-BPE/ozone reaction. However, the reactivity of both 2-BPE and 4-BPE with ozone increase with increasing relative humidity. 2-BPE exhibits a maximum reaction efficiency of 84% at 32% RH, while 4-BPE attains a maximum reaction efficiency of 82% at 49% RH. Humidity has much less influence on the reaction of 2-BPE with ozone as compared to 4-BPE. Above 18% RH, the reaction efficiency of 2-BPE with ozone is in the range 80−84%. Clearly, 2-BPE is the more useful reagent for ozone analysis.

Measurement of Real Ambient Air. Ambient air was collected between February and May 2009 using different DNPH cartridges at Chiba City's air monitoring station. These included parallel samplings of a DNPH cartridge coupled with a KI-ozone scrubbing cartridge and a two-bed 2-BPE/DNPH cartridge. The ozone auto analyzer at the air monitoring station (AMS) records every 1 h mean value obtained from every 1 min data. Ambient air was sampled at a flow rate of 100 mL/min for 24 h. The measured concentrations of ozone and carbonyls, together with the weather conditions, are listed in Table 1. The ozone concentrations measured by 2-BPE/DNPH method were corrected with the reaction efficiency of 0.84. Although the ambient ozone concentrations recorded during 24 h time-periods fluctuated considerably, the ozone concentrations measured using the 2-BPE/DNPH samplers agreed reasonably well with the mean concentrations measured by the ozone auto analyzer in AMS.

When sampling was performed during high humidity periods (March 1−2 and April 25−26), the concentrations of carbonyls collected with the KI-DNPH sampling train were lower compared to carbonyls collected with 2-BPE/DNPH cartridges. We believe that carbonyls were trapped in the ozone scrubber as a consequence of the potassium iodide being wetted by atmospheric moisture. Moreover, it was observed that dissolved potassium iodide migrated into the DNPH cartridge and the yellow DNPH color changed to reddish brown. It is suggested that migrated potassium iodide oxidized DNPH and 2,4-DNPhydrazones. Many agencies frown on the use of the scrubbers for ambient carbonyl measurements and require the denuder, preferably glass with potassium iodide.

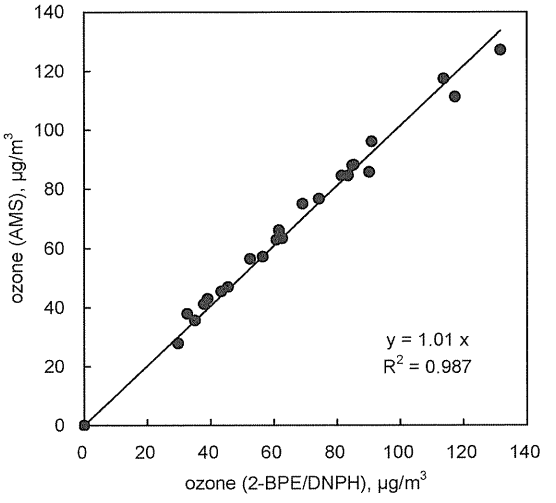


Figure 6. Relationship between the 2-BPE/DNPH method and the ozone auto analyzer in AMS.

Figure 6 shows the comparison between the 2-BPE/DNPH method and an ozone auto analyzer in the air monitoring station concerning ozone concentration. The relationship between the 2-BPE/DNPH method and the ozone auto analyzer showed excellent linearity with a coefficient of determination 0.987.

As above-mentioned, 2-BPE performs as an ozone scrubber too. When 2-BPE/DNPH cartridge is limited to the measurement of formaldehyde and acetaldehyde without ozone measurements, an alternative is to elute the samples with acetone in methanol,

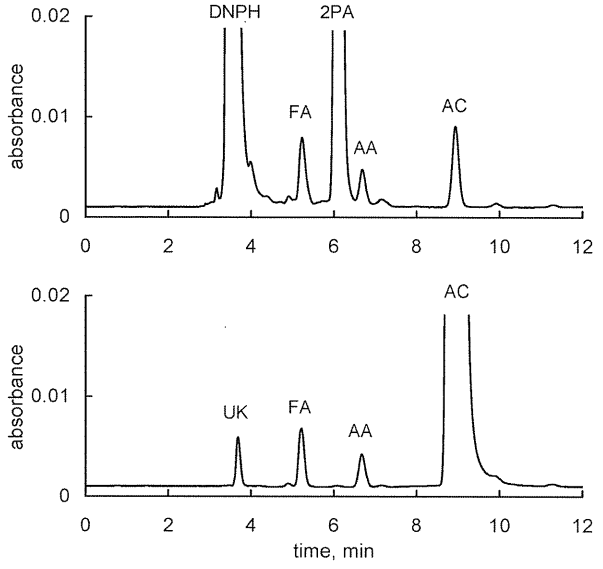


Figure 7. Chromatographic profiles of pyridine-2-aldehyde and other carbonyl 2,4-DNPhydrazones. The upper panel shows the case of using dimethyl sulfoxide/acetonitrile (25:75 v/v) containing 0.1% phosphoric acid as the eluent. The lower panel shows the case of using acetone/methanol (50:50 v/v) as the eluent.

the elution using acetone is very applicable. Two parallel air samplings were performed using the 2-BPE/DNPH cartridge at a flow rate of 100 mL/min for 24 h. One cartridge was eluted with 3 mL of 25% dimethyl sulfoxide in acetonitrile solution containing 0.1% phosphoric acid at a flow rate of 1 mL/min. Another cartridge was subsequently eluted with 3 mL of mixed acetone/methanol (50:50 v/v). After the derivatization reaction was allowed to proceed for 30 min, the eluates were analyzed by HPLC. Figure 7 shows the chromatographic profiles of pyridine-2-aldehyde (2PA), formaldehyde (FA), acetaldehyde (AA), and acetone (AC) 2,4-DNPhydrazones in the solutions extracted from air samplers.

As mentioned above, PA-2,4-DNPhydrazones are difficult to dissolve in acetonitrile. When acetone is used as the eluent for the 2BPE/DNPH-cartridge, all residual unreacted DNPH in the cartridge is reacted with acetone and produces the equivalent amount of acetone DNPhydrazone derivative. Pyridine-2-aldehyde, formed from 2-BPE with ozone, has no opportunity to react with DNPH. As a result, the chromatographic profile shows very simple peaks without DNPH and PA-2,4-DNPhydrazone. An unknown peak (UK) appeared before the FA peak. This unknown peak exhibited a maximum absorption wavelength of 341 nm, suggesting a lower fatty acid 2,4-dinitrophenylhydrazide derivative such as formic acid.[11] If the BPE/DNPH cartridge is used for the limited measurement of formaldehyde and acetaldehyde, the use of acetone as the eluent is very applicable.

CONCLUSIONS

A BPE/DNPH cartridge utilizing *trans*-1,2-bis-(2-pyridyl)ethylene provides several advantages over an analogous cartridge containing *trans*-1,2-bis-(4-pyridyl)ethylene. These include improved elution of pyridine aldehyde from the cartridge, the faster reaction of pyridine aldehyde with DNPH, and less dependence on ambient humidity. The use of the 2-BPE/DNPH cartridge permits the simultaneous determination of ozone and airborne carbonyls. A separate ozone-scrubbing cartridge is not necessary because BPE performs this function. Moreover, air sampling can be performed effectively during either high or low humidity conditions. The 2-BPE/DNPH method corresponded with the mean value measured by an ozone auto analyzer in the air monitoring station. The carbonyl concentrations measured using BPE/DNPH cartridges agreed with those made using the DNPH cartridge coupled with a KI-ozone scrubbing cartridge. The exception was high humidity conditions where the wetted KI-ozone scrubber artificially lowered the measurement of airborne carbonyls.

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Determination of acrolein and other carbonyls in cigarette smoke using coupled silica cartridges impregnated with hydroquinone and 2,4-dinitrophenylhydrazine

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ABSTRACT

A new method for the determination of acrolein and other carbonyls in cigarette smoke using a dual cartridge system has been developed. Each cartridge consists of reagent-impregnated silica particles. The first contains hydroquinone (HQ) for the inhibition of acrolein polymerization, while the second contains 2,4-dinitrophenylhydrazine (DNPH) for the derivatization of carbonyls. Smoke samples were firstly drawn through the cartridge containing HQ-impregnated silica (HQ-silica) and then through the DNPH-impregnated silica (DNPH-silica). Acrolein in the sample was completely trapped in the first HQ-silica cartridge. Some other airborne carbonyls were also trapped by the HQ-silica, and those that pass through were trapped in the second DNPH-silica cartridge. Extraction was performed in the reverse direction to air sampling. When solvent was eluted through the dual-cartridges, excess DNPH was washed into the HQ bed where it reacted with acrolein and other trapped carbonyls to form the corresponding hydrazone derivatives. All of the hydrazones derived from airborne carbonyls were completely separated and measured using high-performance liquid chromatography. This HQ-DNPH-method can be applied for the determination of acrolein and other α,β-unsaturated aldehydes, such as crotonaldehyde, in cigarette smoke.

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1. Introduction

Cigarette smoking causes 30% of all cancer deaths: the smoke contains more than 3500 chemicals and at least 50 of these are carcinogens [1,2]. Carbonyls, including acrolein (propenal), are among the compounds present at high levels. Long-term exposure to carbonyl compounds, such as formaldehyde and acetaldehyde, is known to increase the risk of asthma [3] and cancer [4]. Accurate carbonyl measurements are therefore important both for determining the formation mechanism of carbonyls and for evaluating their implication in human health. Acrolein, is not currently a suspected human carcinogen as, to date, no studies have been conducted to observe its carcinogenic effects on human cells. However, studies in rats have shown an increase in cancerous tumors from ingestion but not inhalation, and Feng et al. [5] have recently suggested a connection between acrolein in cigarette smoke and an increased risk of lung cancer. This emerging evidence suggests a need for an efficient technique for acrolein measurement.

For the analysis of carbonyl compounds including acrolein, their specific reaction with 2,4-dinitrophenylhydrazine (DNPH), forming the corresponding 2,4-dinitrophenylhydrazones, is one of the most important qualitative and quantitative methods in organic analysis. This was published by both Allen [6] and Brady [7] in the 1930s. The main advantage of the DNPH-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 [8–10] or with acidic solid sorbents using a DNPH-coated cartridge.

A number of cartridge devices containing solid sorbents coated with DNPH have previously been introduced for sampling aldehydes in air. The solid sorbents include XAD-2 [11,12], silica gel [13,14], glass beads [15], octadecylsilane bonded silica gel [16], Florisil [17], and glass fiber filters [18]. More recently, DNPH-coated silica gel has been widely used for a standard procedure by several national standardization bodies [19]. 2,4-Dinitrophenylhydrazone derivatives extracted from solid sorbent are usually separated by means of high-performance liquid chromatography (HPLC) and detected using UV spectrophotometry at 360 nm (depending on the absorption maximum of the hydrazones). However, for the analysis of α,β-unsaturated aldehydes such as acrolein and crotonaldehyde, numerous problems inherent in the methodology have been reported, including the instability of the acrolein DNPhydrazone (ACR-D) during collection and storage [20–23]. Contradictory data for the technique are found in the literature [20,24–27] and

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severe problems have been observed in inter-laboratory comparisons [21].

Tejada was the first to describe the occurrence of chemical interference in the determination of acrolein using the DNPH-method [20]. During storage of samplers coated with acidified DNPH, the ACR-D peak disappeared and was replaced by an unknown reaction product in such a manner that the sum of the peak areas appeared to be invariant with time. Possanzini and DiPalo [24,25] identified two peaks for both acrolein and crotonaldehyde as syn- and anti-isomers. Risner and Martin [26], as well as Risner [27], traced the poor recovery observed during the determination of acrolein back to the formation of a dimer between two acrolein molecules (2-formyl-3,4-dihydro-2H-pyran) before derivatization. Progress has been made in resolving these limitations, such as using mass spectrometry instead of UV detection, but the instability of the ACR-D under the conditions necessary for collection from air had not yet been overcome.

Decomposition of ACR-D may be prevented by collecting acrolein away from the DNPH. In this study, an improved method which enables the determination of acrolein in addition to previously measurable carbonyls in air, and therefore cigarette smoke, using a dual cartridge system has been developed.

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 photo-diode array detector. The analytical column was an Ascentis Express RP-Amide, 2.7 μm particle size, 150 mm × 4.6 mm i.d. (Supelco Inc, Bellefonte, PA, USA). The column temperature was 40 °C and the injection volume was 10 μL. Two analytical conditions were adopted. The first was used in isocratic mode for rapid C_1–C_3 aldehyde derivative analysis and the mobile phase mixture was acetonitrile/water (60/40, v/v) containing 5 mmol/L ammonium acetate. The flow rate of the mobile phase was 0.6 mL/min. The second was used in gradient mode for C_1–C_{10} aldehyde derivative analysis. Solution A of the mobile phase mixture was acetonitrile/water (40/60, 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 8 min, followed by a linear gradient from 100% A to 100% B in 37 min and then held for 15 min. The flow rate of the mobile phase was 0.7 mL/min.

The smoking machine model LM1/PLUS (Borgwaldt Technik GmbH, Hamburg, Germany) was used for collection of cigarette smoke. Air pump (SP-100 Dual GL Sciences Inc., Saitama, Japan) and wet gas meter (WS D-1A; Shinagawa Co., Tokyo, Japan) were used for the collection of air samples.

The 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). The 2,4-dinitrophenylhydrazine hydrochloride (>98%) was obtained from Tokyo Kasei Co., Ltd., (Tokyo, Japan). The acetonitrile (HPLC grade, >99.9%), ethanol (>99.5), hydroquinone (>99%), phosphoric acid (85% solution in water), and ammonium acetate (99.999%) were from Sigma–Aldrich Inc., (St. Louis, MO, USA). Silica gel (spherical, 60/80 mesh, 120 Å mean pore size) was from AGC Si-Tech. Co., Ltd. (Fukuoka, Japan).

2.2. Preparation of a DNPH-impregnated silica cartridge (DNPH-cartridge) and a hydroquinone impregnated silica cartridge (HQ-cartridge)

Two types of DNPH-silica particles, for sampling low and high levels of carbonyls, were prepared. DNPH-silica: silica gel (50 g) was washed with water (3× 500 mL), methanol (2× 500 mL), and lastly acetonitrile (2× 500 mL). 2,4-Dinitrophenylhydrazine hydrochloride (0.25 g for low-level carbonyls and 1 g for high-level carbonyls) and phosphoric acid (0.5 mL for low-level carbonyls and 1 mL for high-level carbonyls) were dissolved in 200 mL acetonitrile. This solution was added to the washed silica gel (50 g), the mixture was stirred and the solvent was evaporated to dryness at 40 °C under vacuum on a rotary evaporator.

Hydroquinone (HQ)-silica: silica gel (50 g) was washed with water (3× 500 mL), methanol (2× 500 mL), and lastly acetonitrile (2× 500 mL). Then the solvent was 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. HQ (0.05 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.

DNPH-silica (270 mg) and HQ-silica (270 mg) were packed into separate polyethylene cartridges (Rezorian tube, 1 mL, Supelco Inc., Bellefonte, PA) and stored in a refrigerator at 4 °C.

2.3. Collection and analysis of cigarette smoke and air sample

Before collecting a sample, an HQ-cartridge and a DNPH-cartridge were connected.

In the case of analyzing mainstream cigarette smoke, test cigarettes were prepared at 22 °C temperature and 60% humidity. Mainstream smoke constituents were collected under the conditions of 35 mL puff volume, 2-s puff duration, and 60-s puff interval according to ISO machine-smoking conditions [28]. A coupled cartridge was connected to the back of the filter, and cigarette smoke was drawn through the coupled cartridge from the HQ-cartridge to the DNPH-cartridge.

In the case of analyzing an air sample, air was drawn through a coupled cartridge from the HQ-cartridge to the DNPH-cartridge at flow rates of 0.1–1000 mL/min. After collection, the coupled cartridges were extracted with acetonitrile (containing 1% phosphoric acid) in the reverse direction to air sampling until the total volume of solution was 4.5 mL. After 10 min, the eluate solution was added with ethanol (0.5 mL) and was analyzed by HPLC. If the extraction was not performed immediately, the HQ-DNPH-cartridge set was decoupled and the individual cartridges were capped with stoppers.

3. Results and discussion

3.1. Decomposition of acrolein in the DNPH-cartridge

Acrolein standard gas (20 ppm) was drawn through DNPH-cartridges at a flow rate of 100 mL/min for 10 min. The DNPH-cartridges were then stored at 35 °C. At various times, the DNPH-cartridges were eluted with acetonitrile until the total volume of solution was 5 mL. The eluates were then immediately analyzed in isocratic mode using an HPLC instrument equipped with an autosampler set to 4 °C. Acrolein in air reacts with DNPH in the cartridge to form ACR-D. As DNPH-cartridge storage time increased, the peak of ACR-D decreased and unknown peaks appeared. Fig. 1 shows the chromatogram of the sample following 2.4 h storage at 35 °C.

ACR-D (λ_{max} = 372 nm) and four unknown peaks, a, b, c and d, were detected in the chromatogram. The maximum wavelengths of ACR-D, a, b, c and d were 373, 356, 350, 353 and 350 nm, respectively. Other unknown products may not have been extracted from the DNPH-cartridge. During the collection of acrolein, the intake side of DNPH-silica (about 1 mm thickness) changed color from yel-

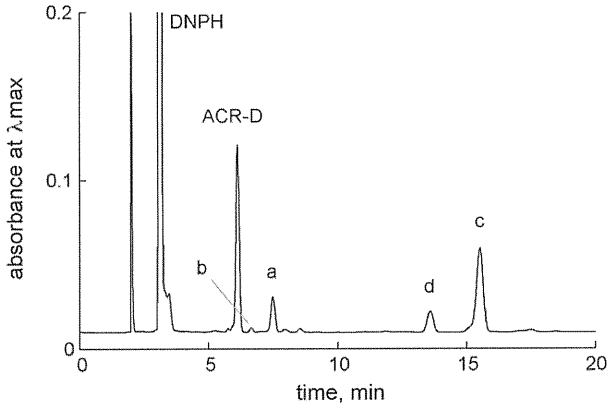

Fig. 1. Chromatographic profile of ACR-D and unknown peaks in the eluate of the DNPH-cartridge 2.4 h following acrolein standard gas sampling.

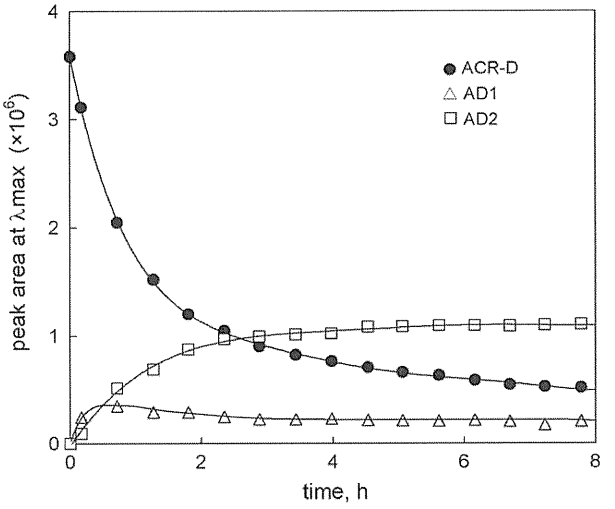

Fig. 3. The decomposition of ACR-D and the formation of adducts (AD1, AD2) in a DNPH-cartridge at 35 °C over time.

low to red. The red color remained after extraction. Unknowns a and b, and c and d are double peaks. It has been suggested that a and b, and c and d are geometric isomers [29–32], assigned AD1′ and AD1, and AD2′ and AD2 respectively, about the C=N double bond, because the peak area ratios of AD1′:AD1 and AD2′:AD2 are constant in this experiment. Schulte-Ladbeck et al. [22] reported that the unstable hydrazones react with excess reagent to form adducts (AD1, AD2 and their isomers). Fig. 2 illustrates the reaction of ACR-D with DNPH. ACR-D reacts with excess DNPH generating adduct (AD1). Further reaction between AD1 and ACR-D results in formation of AD2.

The decomposition reactions are observed both in the DNPH-cartridge and in the eluate with acetonitrile; however, faster decomposition is observed in the DNPH-cartridge. Fig. 3 shows the changes in HPLC peak areas of ACR-D and its adducts as the DNPH-cartridge is allowed to stand at 35 °C.

ACR-D concentration decreased rapidly on standing to 86% after 10 min, 29% after 2.4 h and 12% after 10 h. AD1 increased rapidly at first, dropped slightly after 1 h, then maintained a constant level. The major decomposition product is AD2, which increased in concentration while the ACR-D concentration decreased.

3.2. Decomposition of acrolein derivative in the acetonitrile eluate

An unused DNPH-cartridge was eluted with acetonitrile until the total volume of solution was 4.95 mL. ACR-D acetonitrile solution (50 μL, 1 mmol/L) was then added to the eluate. The sample was analyzed immediately and re-analyzed every 1.7 h for a total

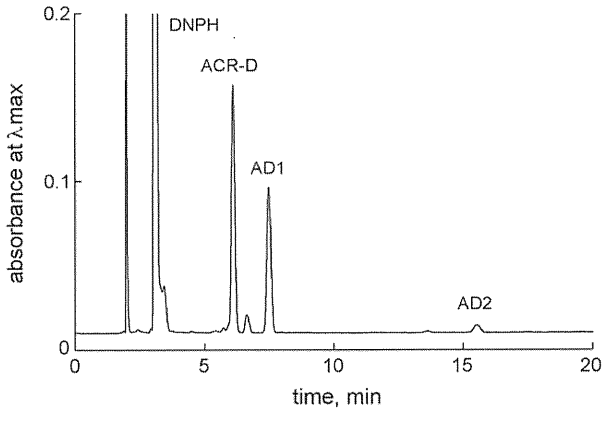

Fig. 4. Chromatographic profile of the DNPH-cartridge eluate of the acetonitrile solution after 60 h at 25 °C.

of 60 h by HPLC in isocratic mode using an instrument equipped with an autosampler set to 25 °C. Fig. 4 shows the chromatogram of the sample following standing for 60 h.

ACR-D and its adducts (AD1 and AD2) were detected in the chromatogram. The decomposition of ACR-D in the eluate is accelerated with the addition of acid. Fig. 5 shows the changes in the levels of ACR-D and AD1 in the eluate at various concentrations of phosphoric acid.

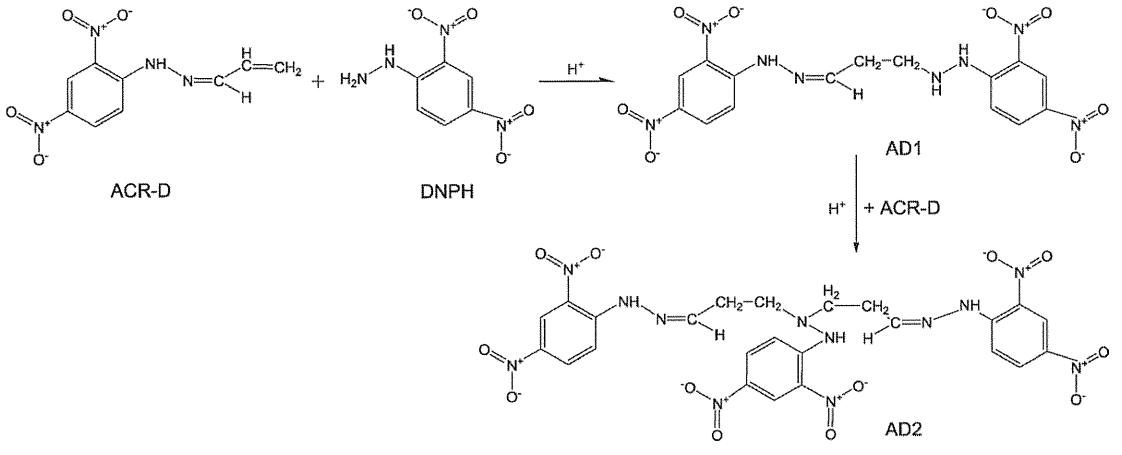

Fig. 2. Decomposition of ACR-D with DNPH.

In the case of the reaction in the DNPH-cartridge, the major decomposition product was AD2 and the minor decomposition product was AD1. By contrast, AD1 is the predominant decomposition product in the acetonitrile eluent solution; AD1 concentration increased in direct proportion to the decrease in ACR-D concentration. ACR-D concentration decreased gradually with time to 89% after 10 h and 64% after 60 h when no acid was added to the eluate. Adding phosphoric acid accelerated the decomposition of ACR-D to the extent that a 50% concentration decrease was measured following 12 h in the presence of 1% phosphoric acid.

3.3. Inhibition method for the decomposition of acrolein derivative in the DNPH-cartridge

The decomposition of ACR-D can be prevented by collecting acrolein using an alternative medium, followed by derivatization with DNPH just before analysis. The use of a dual sampling cartridge system comprised of silica gel and DNPH-silica results in the acrolein vapor being trapped by the silica. Acrolein is degraded by a reaction with hydroxyl radicals [33,34], and it has been shown in previous work that free radicals are generated in mainstream cigarette smoke [35]. Therefore, hydroquinone (HQ), a radical-trapping reagent, was used to inhibit acrolein decomposition. Several coupled HQ-DNPH-cartridge sets were prepared. Acrolein standard gas (20 ppm) was drawn through the coupled cartridges from the HQ-cartridge to the DNPH-cartridge at a flow rate of 100 mL/min for 10 min. After collection, the coupled cartridges were separated and were capped with stoppers. The HQ-cartridges and the DNPH-cartridges were stored at 35 °C. At various times, an HQ-cartridge and a DNPH-cartridge were reconnected. Elution was performed from the DNPH-cartridge to the HQ-cartridge with acetonitrile (containing 1% phosphoric acid) until the total volume of solution was 4.5 mL. After 10 min, ethanol (0.5 mL) was added to the eluate solution. This was analyzed by HPLC using an autosampler set to 4 °C. In contrast to the decomposition seen in Fig. 3, products such as AD1 and AD2 were not detected and the level of ACR-D stayed relatively constant over 8 h (Fig. 6).

In the case of separate storage of the HQ-silica and DNPH-silica cartridges, decrease of the ACR-D was not observed. The separated HQ-cartridge was stable for at least 2 weeks. However, in the case of coupled-cartridge storage, ACR-D concentration decreased gradually with time during storage at 35 °C. It was found that acrolein trapped in HQ-silica migrated into the DNPH-cartridge of the dual sampling cartridge causing decomposition. Therefore, it is necessary to separate the cartridges when analysis is not carried out immediately after sample collection.

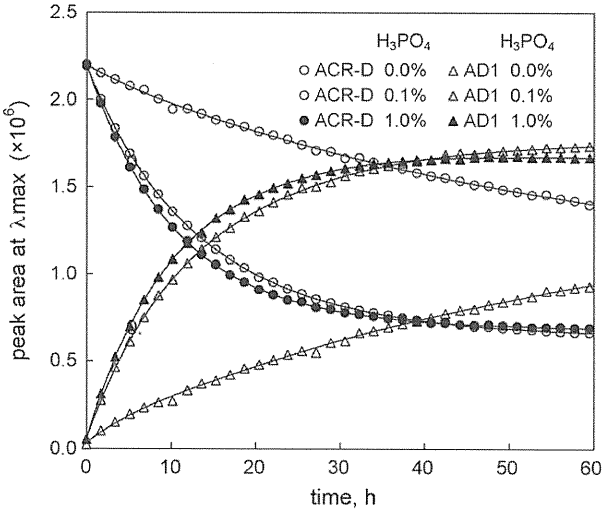

Fig. 5. Decrease of ACR-D and increase of AD1 with time in the acetonitrile eluate at various concentrations of phosphoric acid.

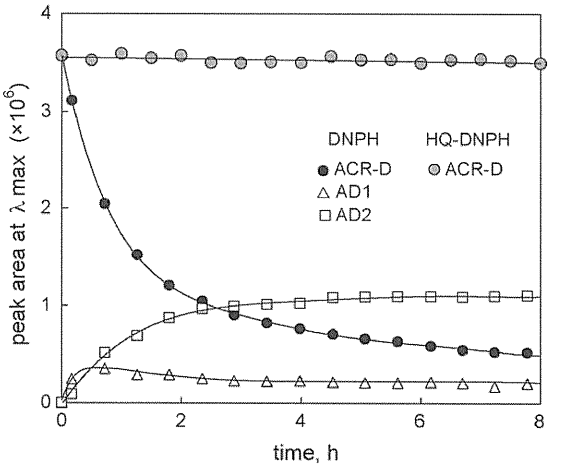

Fig. 6. Changes in ACR-D in a HQ/DNPH dual cartridge method and a DNPH-cartridge method with time at 35 °C over time.

3.4. Inhibition method for decomposition of acrolein derivative in the eluate

Acrolein adsorbed onto the HQ-cartridge is extracted with acetonitrile through the DNPH-cartridge, meaning acrolein reacts with DNPH in the acetonitrile eluate. The reaction of acrolein and DNPH is relatively slow unless accelerated with catalytic acid. Unused DNPH-cartridges were eluted with acetonitrile containing various concentrations of phosphoric acid until the total volume of solution was 4.95 mL. ACR solution in acetonitrile (50 μL, 1 mmol/L) was then added to the eluate. This was immediately analyzed by HPLC in isocratic mode using an instrument equipped with an autosampler set to 25 °C. Fig. 7 shows the reaction rate between acrolein and DNPH in the acetonitrile eluate from DNPH-cartridges.

When the eluent from the DNPH-cartridge contained no acid, the derivatization reaction in the eluate was slow and complete in 60 min at 25 °C. The reaction rate for DNPH derivatization increased with an increase in the concentration of phosphoric acid. The most efficient eluent was acetonitrile containing 1% phosphoric acid and the derivatization reaction could be finished within 5 min. As stated above (Fig. 5), ACR-D was rapidly decomposed when 1% phosphoric acid was used as eluent. Unused DNPH-cartridges were eluted with acetonitrile or ethanol containing 1% phosphoric acid until the total volume of solution was 4 mL. In an attempt to combat the acid effect, ethanol was added to these solutions at 0, 10, 20 and 90% concentration levels. ACR-D acetonitrile solution (50 μL, 1 mmol/L) was then added to the eluate. The sample was immediately analyzed and was analyzed every 1.7 h for a total of 60 h by HPLC in isocratic mode using an instrument equipped with an autosampler set to 25 °C. Fig. 8 shows the changes of ACR-D and its adduct (AD1) with time in the eluate containing ethanol.

The decomposition of ACR-D was depressed by adding ethanol to the DNPH eluate. This suggests that the addition reaction of DNPH to ACR-D proceeds readily in polar aprotic solvents such as acetonitrile, but is inhibited in protic solvents such as ethanol.

3.5. Limit of detection, limit of quantitation and reproducibility

The limit of detection (LOD) and limit of quantitation (LOQ) of a HQ-DNPH coupled cartridge method was calculated using linear regression theory [36]. 5 mL of carbonyl standard gas (20 ppm, 5 components) was introduced into the HQ-DNPH coupled cartridges and analyzed using the analytical conditions described above. The

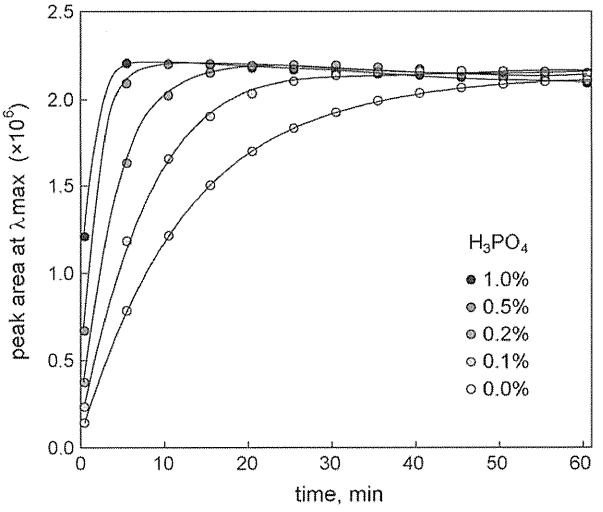

Fig. 7. Rate of reaction of acrolein and DNPH in the eluate with various concentrations of phosphoric acid.

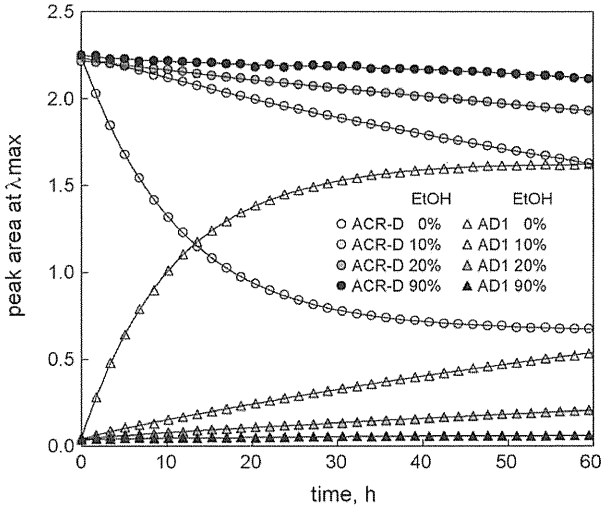

Fig. 8. Decrease of ACR-D and increase of AD1 with time in the acetonitrile eluate containing various concentrations of ethanol.

Table 1
LOD, LOQ and reproducibility of HQ-DNPH coupled cartridge method.

Compounds	LOD, μg	LOQ, μg	RSD, %
Formaldehyde	0.015	0.050	1.9
Acetaldehyde	0.034	0.11	1.8
Acetone	0.020	0.067	2.1
Acrolein	0.074	0.25	1.2
Propionaldehyde	0.059	0.20	2.1

LOD and LOQ were calculated as being three times the standard deviation obtained from the data of 10 replicate measurements (Table 1). The reproducibility of DNPH coupled cartridge-HPLC analysis was estimated from data of 10 samplers spiked with 1000 mL of carbonyl standard gas (20 ppm, 5 components). The relative standard deviation (RSD) is shown in Table 1.

3.6. Analysis of cigarette smoke

3.6.1. Normal cigarette

The mainstream smoke vapors of three brands of cigarettes (A, tar 6 mg, nicotine 0.5 mg; B, tar 12 mg, nicotine 1.0 mg; C, tar 8 mg, nicotine 0.7 mg) were collected in HQ-DNPH coupled cartridges by a smoking machine in accordance with ISO machine-smoking conditions [28] and then analyzed using the aforementioned HQ-DNPH-method. DNPH-cartridges for high-level carbonyl analysis were used. Table 2 shows the carbonyl compounds generated from cigarettes. Many carbonyls including acrolein were detected in cigarette smoke. For reference, after collection, the collected HQ-cartridge was eluted with an unused DNPH-cartridge and the collected DNPH-cartridge was eluted individually. From this experiment it was found that all carbonyls, except acetaldehyde, were completely (>99%) trapped in the HQ-cartridge and not detected in the DNPH-cartridge. Most of the acetaldehyde (89%) was trapped in HQ-cartridge, with the remainder (11%) trapped in DNPH-cartridge.

3.6.2. Electronic cigarette

The HQ-DNPH coupled cartridge was connected to the electronic cigarette (The Plemium Smoker, EPI International Co., Ltd., Tokyo Japan) and air was drawn through at a flow rate of 500 mL/min. DNPH-cartridges for low-level carbonyl analysis were used. After collection, the coupled cartridge was kept for 10 min or 16 h at room temperature and was eluted with acetonitrile containing 0.1% phosphoric acid in the reverse direction to air sampling. The eluate was analyzed by HPLC operating in the gradient mode. For comparison, a DNPH-cartridge was used simultaneously for collection. Fig. 9 shows the chromatograms of formaldehyde DNPhydrazone (FA), acetaldehyde DNPhydrazone (AA), acetone DNPhydrazone (AC), acrolein DNPhydrazone (ACR-D), propanal DNPhydrazone (PA), butanal DNPhydrazone (BA), glyoxal DNPhydrazone (GO), and methylglyoxal DNPhydrazone (MG) in the eluate of the HQ-DNPH coupled cartridge and the DNPH-cartridge.

Various kinds of carbonyls, including acrolein, were detected in the electronic cigarette sample. Complete separation of the ACR-D peak and the acetone DNPhydrazone peak was achieved on an RP-

Table 2
Amounts of carbonyls in mainstream smoke vapors of cigarettes, $n = 10$ μg/cigarette.

Compounds	A			B			C		
	Puffs: 7.3–8.0			Puffs: 7.5–8.6			Puffs: 6.0–7.0		
	Mean	Min.	Max.	Mean	Min.	Max.	Mean	Min.	Max.
Formaldehyde	2.0	1.4	3.1	2.4	1.4	5.5	3.3	2.4	5.1
Acetaldehyde	270	230	320	520	490	580	380	310	430
Acetone	78	60	97	140	130	150	130	110	150
Acrolein	19	16	22	36	30	44	18	12	24
Propanal	20	16	25	42	39	47	29	24	34
Crotonaldehyde	3.4	2.1	4.3	9.4	7.1	11	7.4	5.9	8.9
Butanal	12	9.2	15	23	21	27	26	20	31
i-Pentanal	4.5	3.0	5.8	11	8.7	12	7.3	6.1	8.6
Pentanal	0.3	0.2	0.4	0.8	0.6	1.3	0.6	0.4	0.8
Glyoxal	0.2	0.0	0.2	0.1	0.0	0.2	0.5	0.4	0.7
Methylglyoxal	0.3	0.1	0.4	0.4	0.3	0.5	0.9	0.5	1.7

Table 3
Concentrations of carbonyls generated from the electronic cigarette, mg/m^3.

Compound	HQ-DNPH		DNPH	
	10 min	17 h	10 min	17 h
Formaldehyde	8.3	7.9	8.1	7.6
Acetaldehyde	11	9.2	10	9.3
Acetone	2.9	3.0	2.6	2.4
Acrolein	9.3	9.2	6.4	0.3
Propanal	8.0	7.4	8.3	7.6
Butanal	1.5	1.7	1.7	2.4
Glyoxal	1.3	1.3	1.2	1.6
Methylglyoxal	4.5	4.2	4.3	4.7

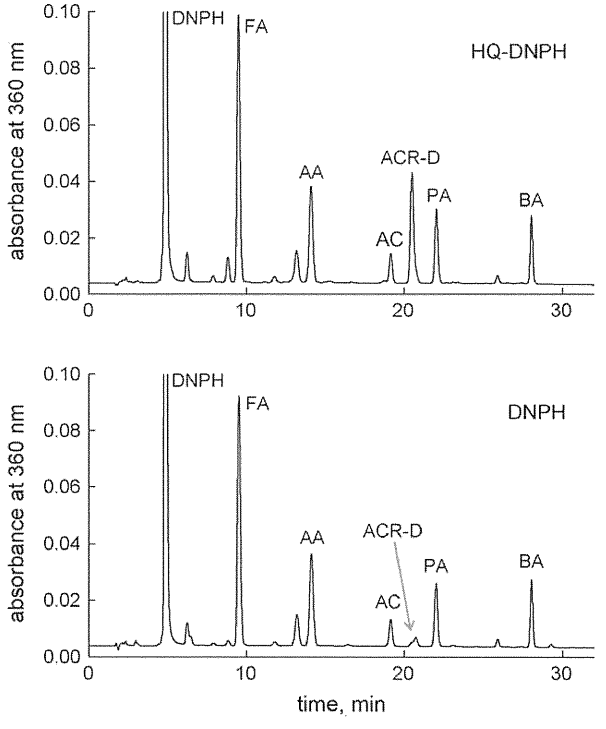

Fig. 9. Chromatographic profiles of acrolein and other carbonyl DNPhydrazones in the eluate of the HQ-DNPH coupled cartridge and DNPH-cartridge 17 h after sample collection.

Amide column with a mobile phase consisting of acetonitrile/water. In the case of the HQ-DNPH coupled cartridge, a large ACR-D peak was detected. In the case of the DNPH-cartridge, ACR-D was greatly diminished in peak area. Table 3 shows the concentrations of carbonyls generated from the electronic cigarette.

With the exception of the acrolein concentration, the HQ-DNPH-method is in good agreement with the traditional DNPH-method. The acrolein concentration measured by the DNPH-method was about one thirtieth of that measured by the HQ-DNPH-method; therefore, the use of the traditional DNPH-method for analysis of acrolein may lead to erroneous results.

The electronic cigarette, introduced recently to the marketplace, is a battery-powered device that provides inhaled doses of nicotine by heating a nicotine-chemical solution into a vapor. Many legislation and public health investigations are currently pending in many countries due to its relatively recent emergence. High concentrations of hazardous pollutants such as formaldehyde, acetaldehyde and acrolein were detected by using the HQ-DNPH-cartridge.

4. Conclusions

In the analysis of acrolein using a traditional DNPH-cartridge, ACR-D is decomposed rapidly in the DNPH-cartridge and forms DNPH and ACR-D adducts. Therefore, decomposition of ACR-D is prevented by collecting acrolein in a separate cartridge from DNPH. In this study, a dual cartridge system has been developed. Each cartridge consists of reagent-impregnated silica particles. The first contains hydroquinone (HQ) for the inhibition of acrolein polymerization, while the second contains 2,4-dinitrophenylhydrazine (DNPH) for the derivatization of carbonyls. Air samples are drawn through the cartridge first through the HQ-impregnated silica (HQ-silica) and then through the DNPH-impregnated silica (DNPH-silica). Acrolein in the air sample is completely trapped in the first HQ-silica cartridge without addition of DNPH. ACR-D decomposition also occurs, albeit more slowly, in acidified acetonitrile solution. This process can be inhibited by the addition of a protic solvent such as ethanol. The HQ-DNPH-method can be successfully applied to the determination of 2-alkenals such as acrolein or crotonaldehyde in air and cigarette smoke.

Acknowledgments

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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.

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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 monitoring 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].

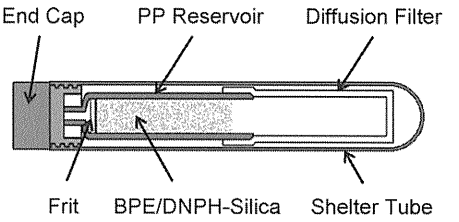

Fig. 1. Schematic representation of the DSD-BPE/DNPH sampler.

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