Determination of *ortho*-phthalaldehyde in air and on surfaces†

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Three sampling and analytical methods have been developed and evaluated for *ortho*-phthalaldehyde (OPA): (1) an HPLC-UV method for OPA in air, (2) a fluorimetric method for OPA on surfaces, and (3) a colorimetric method for OPA on surfaces. (1) The air sampler contains 350 mg of silica gel coated with 1 mg of acidified 2,4-dinitrophenylhydrazine (DNPH). Air sampling may be conducted at 0.03 to 1.0 L min^{-1} for periods up to 8 h. Samples were eluted with ethyl acetate, and the eluents were allowed to stand for 72 h. Analysis was by high performance liquid chromatography (HPLC) with a UV detector set at 369 nm. An unusual phenomenon was the observation that the stability of the sample on a sampler at 3 °C tends to decrease as the total quantity of OPA collected on the sampler decreases. Elution of the samples within 24 h of air sampling is required. The detection limit (LOD) is approximately 0.02 μg of OPA per sample. OPA on surfaces may be collected with strips cut from a sheet of polyvinyl alcohol (PVA wipe). (2) In the surface wipe method with analysis by fluorescence measurement, the strips of PVA wipe were placed into dimethyl sulfoxide. An aliquot was treated with aqueous *N*-acetyl-L-cysteine and ethylenediamine. Analysis was performed with a portable fluorometer (excitation and emission wavelengths = 365 nm and 438 nm, respectively). The LOD is 0.2 μg per sample. (3) In the surface wipe method with visual colorimetric detection, the strips of PVA wipe were placed into 30 : 70 acetonitrile : water. An aliquot was treated with *N*-(1-naphthyl)ethylenediamine in 0.1 M sulfuric acid. After color development, the LOD is approximately 48 μg per sample. These methods have been field tested in a hospital.

Introduction

In health-care settings, *ortho*-phthalaldehyde (OPA) (Fig. 1) is replacing glutaraldehyde as a disinfectant for heat-sensitive medical instruments for several reasons, (1) hospital personnel have complained of health effects due to glutaraldehyde; namely sensitization, occupational asthma, and irritation of the eyes, respiratory tract, and the skin;[1] (2) OPA is effective in sterilization much more quickly than glutaraldehyde;[2,3] (3) OPA has been very effective against strains of mycobacteria resistant to glutaraldehyde;[2] and (4) working with OPA in the hospital setting is much more tolerable than working with glutaraldehyde because the use of OPA leads to much lower air concentrations due to a significantly lower vapor pressure [0.69 Pa (0.0052 mm Hg) at 21 °C *versus* 2000 Pa (15 mm Hg) at 20 °C].[4,5]

OPA, on the other hand, has been found to cause occupational bronchial asthma, and contact dermatitis.[6,7] In addition, OPA stains proteins and unprotected skin gray.[2] These health effects have constituted justification for the National Institute for Occupational Safety and Health (NIOSH) to conduct health hazard evaluations (HHEs) in hospitals in areas where OPA is used; namely, endoscopy units. In order to conduct HHEs for OPA, NIOSH has needed valid sampling and analytical methods.

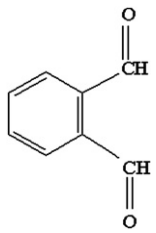

Fig. 1 Molecular structure of *ortho*-phthalaldehyde with two aldehyde groups (MW = 134.13; CAS RN = 643-79-8).

This paper describes three sampling and analytical methods which have been developed and evaluated for OPA: a method for OPA in air and two methods for analysis of surfaces for OPA contamination. The method for OPA in air has been developed with significant improvements over the method of Uchiyama *et al.*[8] Three major weaknesses of the method of Uchiyama *et al.* for OPA are the following: (1) the original method[8] neglects to indicate a serious problem of sample stability because, apparently, no storage studies of fortified air samplers were performed. (2) The original method[8] neglects to mention the air sensitivity of the eluent collected from the air sampler. Without protection of the eluent from air, oxidation of one or more air-sensitive species will occur and recoveries of OPA will be about 65% (losses will be about 35%). (3) The original method[8] neglects to determine recoveries of OPA. Such recoveries can be determined by placing known quantities of OPA in solution into a glass U-tube and

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drawing the OPA vapor into air samplers with a pump. Determination of recoveries from vapor spikes would have shown losses of OPA due to exposure of the eluents to air and, perhaps, other factors. Fundamentally, the OPA surface methods employ fluorimetric and visual colorimetric reaction products for detection. Analysis for the first surface method consists of measurement of the degree of fluorescence of the product with a portable fluorometer; this fluorimetric method is very sensitive. Although the visual colorimetric method is less sensitive, this second method does not require use of expensive instruments for measurement. Surfaces of interest include the surfaces of basins, automatic washers, counter tops, floors and walls. Surface wipe methods are useful to assess potential worker exposure due to contact with surfaces.

Experimental

Materials

S10 LpDNPH cartridges (containing single 350 mg beds of silica coated with 1 mg quantities of acidified DNPH) were purchased from Supelco. Ghost™ Wipes [consisting of a nonwoven polyvinyl alcohol fiber[9] (PVA Wipes)] were purchased from Environmental Express. A portable Fluorometer, TBS model 380, was purchased from Turner Biosystems. Disposable cuvettes, made from polystyrene and methacrylate, were purchased from Perfector Scientific. A UV lamp, model UVGL-25, 4 watts with excitation at 366 nm, was purchased from UVP, Inc.

ortho-Phthalaldehyde, minimum of 97% purity, 4-(dimethylamino)cinnamaldehyde, ≥ 98%, and *N*-(1-naphthyl)ethylenediamine dihydrochloride, 98%, were purchased from Sigma-Aldrich. *N*-Acetyl-L-cysteine, 98%, and 2-carboxybenzaldehyde, 99%, were purchased from Acros Organics. Ethylenediamine, laboratory grade, and methanol (Optima grade) were purchased from Fisher Scientific. *o*-Anisaldehyde, 98%, 4-(dimethylamino)benzaldehyde, 99%, mesitaldehyde, 98%, 1-naphthaldehyde, 97%, 2-nitrobenzene, 98%, salicylaldehyde, 98%, syringaldehyde, 98%, *o*-tolualdehyde, 99%, vanillin, 99%, DNPH 97% and containing 30% water) and ethyl alcohol (reagent containing 5% isopropyl alcohol and 5% methyl alcohol) were purchased from Aldrich Chemical Co. Dichloromethane, pesticide residue grade, ethyl acetate, spectrophotometric grade, and *N*,*N*-dimethylformamide, spectrophotometric grade, were purchased from Burdick & Jackson. DL-Alanine, 98%, *p*-anisaldehyde, 99.5%, benzylamine, 98%, citronellal, 92%, glycine, 98%, 4-hydroxybenzaldehyde, 98%, and urea, 99%, were purchased from Chem Service.

OPA-bis(DNPH)

OPA-bis(DNPH) (Fig. 2) was synthesized from OPA and DNPH in acid solution, washed first with water, then with methanol, and lastly with acetonitrile, air-dried at room temperature, and recrystallized from hot *N*,*N*- dimethylformamide, mp = 301.8–301.9 °C. This derivative melted with explosive force; however, no safety precautions were required when the estimated quantity was less than 1 mg. UV spectrum in 80 : 20 acetonitrile : water showed λ_{max} at 385 nm. The absorbance at 369 nm = ~86% of the maximum (some UV detectors have an upper limit of 369 nm).

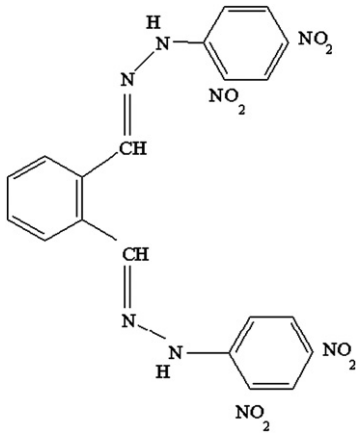

Fig. 2 Molecular structure of OPA-bis(DNPH) (MW = 494.37).

Stock solution of OPA-bis(DNPH)

Due to the extremely low solubility in acetonitrile (about 6 μg mL^{-1} at room temperature),[8] OPA-bis(DNPH) (5 mg) was weighed to five decimal places on a boat fashioned from aluminium foil. The boat was dropped through the neck of a 1 L volumetric flask containing acetonitrile. The acetonitrile was heated to the boiling point with stirring until all of the DNPH derivative had dissolved. This stock solution was stored at room temperature because yellow crystals would have developed under refrigeration.

HPLC

The HPLC was a Waters component HPLC system with a Waters™ 600-MS system controller and a Waters™ 717plus autosampler. The detector was a Shimadzu SPD-10AV VP UV-VIS detector set at its upper limit of 369 nm. The analytical column was an ODS Symmetry™ stainless steel cartridge column, 3.9 × 150 mm, containing 5 μm particles, 19% carbon loading, 100 Å pore size, from Waters Corp. The mobile phase was 80 : 20 acetonitrile : water with a flow rate of 1.0 mL min^{-1}. The injection volume was 20 μL.

Spectra

UV and visible spectra were obtained with a Hewlett-Packard 8453 UV-VIS spectrometer. Fluorescence spectra were obtained with a model 1100 Agilent HPLC with a column bypass and an Agilent G1321A fluorescence detector. The excitation wavelength was 366 nm for the fluorescent surface wipe method.

Recoveries from liquid spikes

S10 LpDNPH samplers were fortified by penetrating the inlets with the needle of a 500 μL syringe and injecting 80 μL of acetonitrile solution of OPA of known concentration (2 to 25 μg mL^{-1}). The fortified samplers were allowed to stand at room temperature for 30 min. The bis(DNPH) derivative was eluted with 6.0 mL of acetonitrile. Recoveries were calculated from the quantities found by HPLC and the quantities used for fortification [1.00 μg of OPA-bis(DNPH) corresponds to 0.271 μg of free OPA].

Storage Studies with liquid spikes on samplers

S10 LpDNPH samplers were fortified with OPA solutions as described above. Fortified samplers were stored at room temperature, 3 °C, and −16 °C for periods ranging from 8 hours to 28 days. Recovery of OPA-bis(DNPH) was accomplished by elution with 6.0 mL quantities of acetonitrile prior to HPLC analysis.

Recoveries from vapor spikes

A glass U-tube equipped with stopcocks (Schwartz drying tube) was connected in series with an S10 LpDNPH sampler and an air pump capable of operating at 1.0 L min^{-1}. The bottom half of the U-tube was immersed in a water bath maintained at 55–60 °C. A known quantity (0.16 to 2.0 μg) of OPA in 80 μL of acetonitrile solution was added to the U-tube by syringe while the pump was in operation. For a close check on the concentration of OPA in acetonitrile solution, an S10 LpDNPH sampler was fortified within a min with a 40 μL aliquot of the same OPA solution from which an aliquot was taken for addition to the U-tube. [A close check was needed due to the instability of the OPA in solution; this instability possibly was due to dissolved oxygen in the solvent.] The pump was allowed to operate for an additional 24 to 50 min, depending on the quantity of OPA. Compounds on the sampler were eluted with 9.0 mL of ethyl acetate, and a fraction of the eluent was allowed to stand undisturbed at room temperature for 72 h in a hermetically sealed 4 mL vial with minimal headspace (~25 μL of air above the eluent was allowed). Recovery was calculated after HPLC analysis. This vapor spike study was repeated with acetonitrile as the eluting solvent.

Breakthrough study

A breakthrough experiment was conducted with the glass U-tube, an S10 LpDNPH sampler, and a pump in series during the course of three hours. The pump was operated at 1.0 L min^{-1}, and the bath temperature for the U-tube was 55–57 °C. OPA (4.0 μg in 100 μL of acetonitrile solution) was added at 36 min intervals until a total of 20 μg had been added. A backup sampler was replaced with a new backup sampler prior to the addition of the next aliquot of OPA solution. The five backup samplers used were analysed by HPLC.

Kinetics studies for formation of OPA-bis(DNPH) from vapor spikes in ethyl acetate and acetonitrile solutions

OPA (1.7 μg in 80 μL of acetonitrile solution) was placed into each of two glass U-tubes which were arranged side-by-side in a water bath at 56 °C. At the same time, three S10 LpDNPH samplers were fortified with liquid spikes of OPA from the same acetonitrile solution (1.7 μg in 80 μL). The U-tubes were connected in series with separate S10 LpDNPH samplers and pumps. The pumps were operated for 49 min. The three samplers fortified with liquid spikes were eluted with acetonitrile for HPLC analysis to determine the actual concentration of OPA in solution. The vapor spike from one sampler was eluted with 9.0 mL of ethyl acetate, and the other was eluted with 7.0 mL of acetonitrile. The eluents from the vapor spikes were divided into 1.5 mL aliquots for storage in separate vials at room temperature. In one plan, aliquots from the same vial of ethyl acetate solution were analysed after storage times of 1, 26, 48, and 72 h at room temperature; likewise, aliquots from the same vial of acetonitrile solution were analysed at the same intervals. In a second plan, vials of ethyl acetate and acetonitrile solutions remained capped and were stored undisturbed until a single analysis was performed in duplicate at one of the designated storage times indicated above. Other undisturbed vials were analysed separately at the other designated storage times. Recoveries were calculated from the analytical results.

Recoveries of OPA from glass surface

Ghost™ Wipes were cleaned by Soxhlet extraction with dichloromethane for 24 h. Strips, 2 × 10 cm, were cut with scissors from dry, cleaned Ghost™ Wipes. Each strip was folded three times in one direction and, then, once in the other direction. A known quantity of OPA (200 μg, 118 μg, or 68.2 μg) in 80 μL of acetonitrile solution was applied to the center of a glass plate (borosilicate window glass), 20 × 20 cm. According to the study design, six samples at each of three levels were prepared; each level differed from the next level by a factor of approximately two. When the fortified spot was still wet, a folded strip of Ghost™ Wipe material was wet with 25 μL of 40 : 60 dimethyl sulfoxide : water (v/v); an area including the spot on the glass plate (approximately 10 × 10 cm) was wiped in a circular motion with the wet strip. Then the strip was placed into a vial of extraction solvent (12 mL of acetonitrile). The same spot on the glass plate was wiped with a second strip which was wet with 25 μL of 40 : 60 dimethyl sulfoxide : water, and the strip was placed into the same extraction solvent. Then the same spot was wiped with a third strip (the third strip was not wet with 40 : 60 dimethyl sulfoxide : water). An S10 LpDNPH cartridge was fortified with a known volume of the extraction solvent. (The extraction solvent was acetonitrile in the special case in which there was a need to apply a liquid spike to an S10 LpDNPH cartridge for determination of OPA concentration; see below for the routine extraction solvents for use in the surface wipe methods.) The OPA-bis(DNPH) was eluted with acetonitrile, and the eluent was analysed by HPLC. The recovery was calculated from the quantity of OPA found.

Controlled study of air sensitivity of OPA

OPA (123 μg in 15 μL of acetonitrile solution) was placed into each of two 20 mL glass scintillation vials which were tilted and rolled to wet the inside walls. The solvent was allowed to evaporate, and the dry solids were exposed to air for 20 and 50 min, respectively. In the same fashion, OPA (12 μg in 15 μL of acetonitrile solution) was placed into a third glass scintillation vial; the dry solid was exposed to air for 90 min. The solid residues in the three vials were dissolved in acetonitrile. The concentrations of OPA in the solutions were determined by fortification of S10 LpDNPH samplers and HPLC analysis.

Procedure for analysing extraction solvent for OPA by the fluorescent method

The extraction solvent for the fluorescent method was 12 mL of dimethyl sulfoxide. A 3 mL aliquot of the extraction solvent was

transferred to another vial to which 100 μL of 11% *N*-acetyl-L-cysteine in water and 50 μL of 4.4% ethylenediamine in water were added. The reaction was complete within about 2 min. The reaction mixture was transferred to a 1 × 1 cm polystyrene cuvette and the degree of fluorescence was read with a portable fluorometer (dimethyl sulfoxide will destroy methacrylate cuvettes in a matter of minutes). Alternatively, the sample was compared with the blank solution under a UV lamp at 366 nm (this alternative method may be employed when a portable fluorometer is unavailable). The weakest fluorescence which was distinguishable from the blank by the unaided eye was at the limit of detection (LOD).

Procedure for analysing extraction solvent for OPA by the visual colorimetric method

The extraction solvent for the visual colorimetric method was 12 mL of 30 : 70 acetonitrile : water (v/v). A 3 mL aliquot of solution was transferred to another vial to which 300 μL of 0.017 M *N*-(1-naphthyl)ethylenediamine in 0.1 M sulfuric acid was added. The solutions were transferred to 1 × 1 cm cuvettes (cuvettes made of either polystyrene or methacrylate were acceptable). A color development time of 30 min at room temperature was selected arbitrarily for convenience of the worker in the field; however, color reactions generally were incomplete in 30 min. The solution was at the limit of detection (at the arbitrary time of 30 min) when the color was three times stronger than that of the blank by examination with the unaided eye.[10]

Results and discussion

Method for OPA in air

Analytical limits in solution. The limit of detection (LOD) and the lower limit of quantitation (LOQ) of OPA in solution were found to be 0.0013 μg mL^{-1} and 0.0045 μg mL^{-1}, respectively [calculated from the actual concentrations of OPA-bis(DNPH)]. For low-level spikes, the LOD is defined as three times the standard error of the least squares calibration curve divided by the slope, and the LOQ is defined as ten times the standard error of the calibration curve divided by the slope.[11,12] It is conceivable that the analytical limits would have been slightly lower provided that a UV detector capable of operating at 385 nm had been employed in lieu of 369 nm. The calibration curve for six standards in the range of 0.005 to 0.025 μg mL^{-1} was linear with $R^2 = 0.993$. The slope, intercept and standard error were 19 952, 57, and 9, respectively. Calibration standards of OPA-bis(DNPH) in acetonitrile solution were found to be stable for at least 26 days during storage at room temperature. This stability was determined by analysis of eight standards at intervals during this period [concentrations of the OPA-bis(DNPH) corresponded to values ranging from 0.015 to 1.32 μg mL^{-1} of free OPA].

Preparation of OPA solution at target concentration of 25 μg mL^{-1}. When an attempt is made to prepare a solution of OPA in acetonitrile with a target concentration of 25 μg mL^{-1}, the typical "found" concentration is lower (found by HPLC analysis of the eluent from a 40 μL liquid spike on an S10 LpDNPH sampler). The apparent cause of the loss of OPA in solution is oxidation by dissolved oxygen in the solvent. Therefore, OPA solutions in acetonitrile with target concentrations in the range of 4 to 25 μg mL^{-1} often are prepared more accurately by consideration of a 10 to 15% loss of OPA during calculations [20 mg of pure OPA (about 17.5 mg after loss) is dissolved in 3.5 mL of acetonitrile, and an aliquot of the solution is diluted serially in two steps to form 3 mL of the solution at the target concentration]. Once the OPA solutions at target concentrations are prepared, the concentrations will tend to decrease over time.

Recoveries of OPA from liquid spikes. Average recoveries of OPA were 99.6% at the 0.16 μg level ($n = 6$, RSD = 0.062), 99.5% at the 0.50 μg level ($n = 6$, RSD = 0.018), and 94.2% at the 2.0 μg level ($n = 5$, RSD = 0.0333). By Bartlett's test, the RSDs were not poolable at the 5% significance level (p value ~0.045). However, this result depended on inclusion of one low recovery value (0.89) at the 0.16 μg level. With this value excluded, the RSDs were poolable (p value = 0.30) with a pooled value of 0.031. However, since there is no reason to exclude the one low value, the RSD of 0.043 (based on all samples) is used.

Recoveries of OPA from vapor spikes. Following elution of vapor spikes with ethyl acetate and storage of the eluents for 72 h at room temperature in air-tight vials, average recoveries ranged from 76 to 93% (see Table 1). With the exception of the recovery of 93% at the 1.0 μg level, there definitely was a trend in recovery as a function of the quantity of OPA applied as vapor spikes. Ethyl acetate eluents containing OPA-bis(DNPH) originating from vapor spikes were found to be stable for at least 18 days at room temperature in one experiment. However, due to the apparent decrease in recovery after 146 h (6.1 days) at room temperature in another experiment (Table 2), it is recommended that air samples and vapor spikes be analysed during the period of 72 to 96 h after elution.

Generally, the relative standard deviation of measurement (RSD) for average recoveries from vapor spikes is acceptable if it is less than 10% (0.1).[10] Two values of RSD in Table 1 are acceptable (0.066 and 0.079). However, two other values, 0.12 and 0.12, are a bit troubling and are difficult to explain (see rows 1 and 3). These two high values of RSD reflect a wide variability in recovery and are results of averaging recoveries from separate vapor spike experiments which have been performed under virtually the same conditions.

Following elution of vapor spikes with acetonitrile and storage of the eluents for 16 h at room temperature, average recoveries of OPA ranged from 76 to 84% (see Table 3). Since there was no trend in a plot of recovery *versus* quantity of OPA found when acetonitrile was the eluting solvent, the five average recoveries

Table 1 Recoveries of OPA with ethyl acetate from vapor spikes[a]

Level of OPA applied/μg	n	Average recovery	RSD
0.16	10	0.76	0.12
0.65	6	0.81	0.079
1.0	6	0.93	0.12
2.0	12	0.86	0.066

[a] The eluents were stored for 72 h before HPLC analysis.

Table 2 Kinetics studies for formation of OPA-bis(DNPH) in ethyl acetate and acetonitrile[a]

	Recovery with ethyl acetate		Recovery with acetonitrile	
Storage time of eluent/h	Analysis of separate aliquots	Analysis of same aliquot	Analysis of separate aliquots	Analysis of same aliquot
1	0.73	0.73	0.54	0.54
26	0.79	0.65	0.65	0.55
48	0.84	0.69	0.72	0.61
72	0.85	0.76	0.76	0.61
146	0.78	—	—	—

[a] The quantity of OPA applied to each U-tube was 1.7 μg.

Table 3 Recoveries of OPA with acetonitrile from vapor spikes[a]

Level of OPA applied/μg	*n*	Average recovery	RSD
0.17	4	0.80	0.083
0.26	3	0.76	0.027
0.56	3	0.83	0.096
0.78	4	0.84	0.021
2.06	4	0.79	0.054

[a] The eluents were stored for 16 h before HPLC analysis.

were averaged to give 80%. It is of interest to note that all values of RSD in Table 3 are less than 10% and reflect results of a single vapor spike experiment at different levels of OPA applied.

Table 2 presents data for kinetics studies for formation of OPA-bis(DNPH) from vapor spikes in ethyl acetate and acetonitrile eluents. Column 2 exhibits the growth in recovery of OPA at the 1.7 μg level; a plateau in recovery of 0.85 was observed for single analyses in duplicate of separate vials containing aliquots of the same original ethyl acetate eluent. These vials had remained capped and undisturbed until the moment of analysis. Yet, when the ethyl acetate solution from a single vial was analysed at 1, 26, 48, and 72 h intervals in duplicate, recoveries remained constant within a narrow range of 0.65 to 0.76 (see column 3).

Likewise, recoveries of OPA reached a plateau of 0.76 in acetonitrile when separate vials containing aliquots of the same original acetonitrile eluent were analysed once in duplicate (see column 4). These vials also had remained capped and undisturbed until the moment of analysis. Yet, when a single vial was analysed at 1, 26, 48, and 72 h intervals in duplicate, recoveries remained constant within a narrow range of 0.54 to 0.61 (see column 5 of Table 2).

The major conclusion from the results in Table 2 is that air might be introduced into a vial at the time the septum cap of the vial is punctured with a needle for analysis. It appears that the air might be interfering with the progress of derivatization due to oxidation of free OPA and, very likely, OPA-mono(DNPH) in solution. It is known that some free OPA is present in the fresh eluent,[8] and that OPA is air-sensitive. In fact, Uchiyama *et al.*[8] already have shown that three OPA species are present in the air sampler after air sampling: OPA-bis(DNPH), OPA-mono(DNPH) and unreacted OPA. After elution of all OPA species from the sampler with a solvent of choice, excess DNPH continues reaction to maximize the yield of OPA-bis(DNPH). In addition, the rates of reaction are solvent-dependent.[8]

Possible major reasons for losses of OPA in recovery studies with vapor spikes include oxidation of OPA by oxygen in the air during air sampling and oxidation of OPA and, very likely, OPA-mono(DNPH) by dissolved oxygen in the eluting solvent. Conceivably, the eluting solvent could pick up oxygen from possible air pockets in the sampler. It is recommended that ethyl acetate from a freshly opened bottle be used for elution of air samples for minimizing the degree of oxidation (a freshly opened bottle of ethyl acetate may contain less dissolved oxygen).

The practice of using a glass U-tube to determine recoveries from vapor spikes is usually valid and can give rise to quantitative recoveries, depending on the compound being studied. For example, vapor spikes of toluene-2,4-diisocyanate (a very reactive compound) which have been collected from a glass U-tube and trapped by reagent-coated glass wool have led to average recoveries of 97 to 99% in the same NIOSH laboratory.[13]

It appears that reaction rates of DNPH were greater in the laboratory of Uchiyama *et al.*[8] than those in the present NIOSH laboratory due to the relative concentrations of DNPH in the eluents. The apparent concentration of acidified DNPH in the laboratory of Uchiyama *et al.* was about 0.5 mg mL^{-1} (2.1 mg of acidified DNPH in about 4.5 mL of acetonitrile); however, the concentrations of acidified DNPH in the present NIOSH laboratory were about 0.18 mg mL^{-1} (1 mg of acidified DNPH in 5.5 mL of acetonitrile) and about 0.12 mg mL^{-1} (1 mg of acidified DNPH in 8.5 mL of ethyl acetate). When a specified volume of solvent is used for elution of species from an S10 LpDNPH sampler, the actual volume of eluent collected is about 0.5 mL less than the specified volume due to retention of solvent by the sorbent.

Choice of elution solvent for air samples. Ethyl acetate is the solvent of choice for elution of air samples (and vapor spikes) because (1) the higher overall yield of OPA-bis(DNPH) in ethyl acetate is dramatic in Table 2, (2) the length of the storage time of ethyl acetate eluent required prior to analysis has been consistent (about 72 h), (3) average recoveries are nearly quantitative (86–93%) at levels near 1 and 2 μg of OPA, and (4) the recovery tends to increase as the level of OPA applied in vapor spike studies increases. This relationship between recovery and level of application of analyte might be expected in the case of solid sorbents.[10]

Acetonitrile is rejected as an elution solvent for a routine analytical method for OPA in air because (1) ethyl acetate has been found to be superior to acetonitrile in terms of recovery in

the kinetics study (see Table 2), (2) average recoveries of OPA greater than 84% have never been observed in this work when acetonitrile is the elution solvent, (3) the length of storage time for maximum yield of OPA-bis(DNPH) has been variable (16 h for the five-level recovery study with results shown in Table 3 and 72 h for the kinetics study shown in Table 2), and (4) an expected trend of increasing recovery with increasing level of application has not been observed.

During use of the same mobile phase for HPLC (80 : 20 acetonitrile : water), the peak for OPA-bis(DNPH) in acetonitrile solution (which is injected) is significantly taller and narrower than the peak for OPA-bis(DNPH) in ethyl acetate solution (which is injected). Yet, the peak areas are virtually the same when the concentrations of OPA-bis(DNPH) are the same. For example, OPA-bis(DNPH at a concentration of 0.030 μg mL^{-1} (expressed as the concentration of free OPA) gives rise to a peak height of 0.029 data system unit and a width of 0.65 min in acetonitrile; yet, the OPA-bis(DNPH) with the same concentration in ethyl acetate gives rise to a peak height of 0.017 data system unit and a width of 0.80 min. No difference between peak areas is detected (peak area = 0.660 data system unit). A consequence of using acetonitrile as the solvent for HPLC standards of OPA-bis(DNPH) and ethyl acetate as the elution solvent for air samples is that HPLC peaks due to OPA-bis(DNPH) must be measured by area and not by height.

Storage studies of OPA-bis(DNPH) on S10 LpDNPH cartridges. Inspection of rows 1, 2 and 3 of Table 4 reveals that the stability of OPA-bis(DNPH) at the relatively high level of 2.0 μg is improved by lower storage temperatures (recovery is higher for a storage temperature of −16 °C than the recovery for storage at 3 °C). Also, inspection of rows 10, 11 and 12 (at the 0.063 μg level at 3 °C) reveals that the curve of recovery *versus* storage time is linear, indicating that the rate of deterioration of OPA-bis(DNPH) was linear. Therefore, it is assumed that rates of deterioration of OPA-bis(DNPH) at the 2 μg level (row 2) and 0.16 μg level (row 7) were linear or nearly linear at 3 °C. The average recovery of 0.752 for storage of samplers at the 2.0 μg level for 12 days at 3 °C (row 4) differs from an expected recovery of approximately 0.90 according to the data in row 2. This apparent deviation from the expected value may be partly due to lot-to-lot variation of the S10 LPDNPH samplers as a possibility.

An usual phenomenon is the observation that the stability of OPA-bis(DNPH) on a sampler tends to decrease as the level of fortification with free OPA decreases (see Table 4). The interpolated average recovery at the 2.0 μg level after 7.0 days of storage at 3 °C is 0.95 (see row 2). The difference between the average recoveries of 0.95 and 0.89 (row 7) for the 2.0 and 0.16 μg levels after 7.0 days of storage at 3 °C was found to be statistically significant at the 5% level (p value < 0.01). By the same token, the degree of deterioration of OPA-bis(DNPH) at the 0.063 μg level (see rows 10, 11, and 12) was found to be significantly greater than that at the 0.16 μg level.

A possible explanation for this phenomenon of increasing rate of sample deterioration with decreasing quantity of OPA on the sampler is that the numerous sites on the surface of DNPH-coated silica exhibit varying degrees of activity. During air sampling, the initial OPA in small quantity entering the sampler will be trapped on the most active sites. These sites may be locations of acid in relatively high concentration (present as a catalyst for derivatization) and protonated hydroxyl groups which are bonded to silica. The great activity of the most active sites will cause deterioration of OPA or a DNPH derivative of OPA by mechanisms involving acid during storage. The next portion of OPA which enters the sampler will be trapped by less active sites, sites where less acid is present; therefore, this portion will exhibit a smaller degree of deterioration during storage.

A conclusion of this sample instability is that one must elute air samples with ethyl acetate within 24 h of sample collection, particularly when the samples are near the LOQ. However, in most cases, one does not know the sizes of the OPA air samples immediately after collection. In addition, those working at the sampling site may object to the noxious fumes and fire hazard of ethyl acetate and the analytical laboratory may be very distant from the sampling site. Therefore, the air samples may be shipped overnight at 3 °C to the analytical laboratory for immediate elution with ethyl acetate.

Other examples are known in which the degree of sample stability depends on the quantity of analyte collected. While 330 μg of hydrazine is stable on another sampler during storage at room temperature, 2.9 μg of hydrazine is unstable on the same type of sampler at room temperature.[14] Similar situations exist for dichloromethane[15] and 1,2-dibromoethane.[16] The mechanisms of deterioration for these examples are unclear, however.

It was found that sometimes an unknown substance caused positive interference which precluded accurate measurement of the HPLC peak for OPA-bis(DNPH) when the fortified S10 LpDNPH sampler was stored for at least two days at any of the three temperatures (see the recoveries marked with one or two asterisks in Table 4). No interference was observed during HPLC analysis of any samples stored for only one day (Table 4).

Since OPA-bis(DNPH) is formed rapidly in quantitative yield from liquid spikes on DNPH-coated silica and since a mixture of compounds [OPA-bis(DNPH), OPA-mono(DNPH) and underivatized OPA] exist on an air sampler used for actual air sampling

Table 4 Average recoveries of OPA-bis(DNPH) after storage

Quantity of OPA on DNPH-coated silica/μg	n	Storage/ days	Storage time temperature/°C	Average recovery	RSD
2.0	8	28.0	25	0.671	0.0627
2.0	6	28.0	3	0.801	0.0194
2.0	2	28.0	−16	1.035	0.0027
2.0	3	12.0	3	0.752	0.0419
0.94	6	14.0	3	1.060	0.0391
0.16	2	28.0	25	<0.30[a]	—
0.16	3	7.0	3	0.888	0.0460
0.16	2	28.0	−16	1.302[b]	0.457
0.090	1	28.0	−16	1.89[b]	—
0.063	3	0.33	3	0.975	0.0717
0.063	5	1.0	3	0.930	0.0391
0.063	2	1.9	3	0.873	0.0206
0.063	3	2.0	3	6.01[b]	0.599

[a] The average recovery of <0.30 is based on a recovery of 0.41 and a "not detected" value with an LOD of 0.01 μg per sample. [b] Positive interference by an unknown substance precluded accurate measurement of HPLC peaks in five samples.

for OPA,[8] it is unclear whether the unknown substance (Table 4) would cause interference during HPLC analysis of actual air samples. Thus, in order to avoid the possibility of interference, a requirement is made for elution of the air samples within 24 h of collection, a storage period for which such interference has not been observed. This possibility of positive interference during HPLC analysis is another reason why the air samples must be eluted within 24 h of collection.

Estimated losses of OPA in air at twenty four hours of sample storage near the LOQ. According to the analytical limits for OPA in solution presented above, the LOD and LOQ for OPA in air are 0.011 and 0.038 μg per 8.5 mL sample of eluent, respectively, after correction for recovery. A plot of percent loss of OPA near the LOD *versus* storage time of liquid spikes on samplers at 3 °C indicates that OPA at levels near the LOD is lost at a rate of approximately 10% for each 24 h period of storage at 3 °C. Therefore, quantities of OPA in air samples found on the S10 LpDNPH samplers which are near the LOD after 24 h of storage at 3 °C may have a negative bias of about 10%. The LOQ in this air method is limited to the lowest level at which the recovery is at least 75% because recoveries below 75% are uncorrectable for accurate measurement.[10] Therefore, the LOQ is 0.16 μg per sample with a recovery of 76% (see row 1 of Table 1). Quantities of OPA found at the LOQ should be corrected for recovery of 76%. Although recoveries of OPA below the LOQ may be as low as about 60%, quantities of OPA below the LOQ may be corrected for recovery for the purpose of making estimates. Consequently, the LOD for OPA in air is approximately 0.02 μg per sample.

Air sensitivity of OPA. Typical of aromatic aldehydes,[17] OPA easily undergoes oxidation in air and will deteriorate during exposures to air during repeated openings of the container. A single vial of OPA (originally at 97% purity) was opened repeatedly during a ten week period. At the end of this period, the purity was estimated at 10%. Therefore, it is recommended that OPA be purchased in small quantities, such as 1 g quantities. When OPA in an opened bottle has deteriorated significantly, other bottles will remain which contain OPA in high purity. Highly pure OPA will dissolve quickly in acetonitrile with little or no residue. However, addition of acetonitrile to impure OPA will result in undissolved white solid remaining.

In the controlled study of air sensitivity described in the Experimental, the 20, 50, and 90 min exposure times of μg quantities of OPA to air resulted in recoveries of 80%, 53% and 0%, respectively. Thus, the rates of oxidation of OPA due to exposure to air are great when the surface area of the OPA is relatively great. Such is the case with finely divided crystals. The results of the controlled oxidation study described above have implications for surface wipe methods for OPA. Namely, the industrial hygienist must collect surface wipes soon after a work procedure involving CIDEX OPA solution in the hospital in order to maximize the quantities of OPA to be found. Otherwise, OPA may not be detected on dry surfaces after a few hours due to air oxidation.

Breakthrough study. At the end of the three hour sampling period, the front sampler exhibited a faint red band which was visible for the first 38% of the bed of DNPH-coated silica, a result after the addition of a total of 20 μg of OPA [the color of pure OPA-bis(DNPH) is a brick red]. In addition, HPLC analysis of the five backup samplers indicated that no breakthrough from the front sampler had occurred. Although the stoichiometric quantity of OPA that would react with the 1 mg of acidified DNPH on the silica bed is 339 μg, the faint red band that covers the first 38% of the bed suggests that introduction of a total of at least 53 μg of OPA would lead to breakthrough from the front sampler. However, an impractically large volume of ethyl acetate would be required to elute the mixture of OPA species. Also, the reduced quantity of unreacted DNPH in the large volume of acetonitrile would suggest a longer reaction time required to maximize the yield of OPA-bis(DNPH) in the eluent.

The upper limit of the method is considered to be 2.4 μg of OPA because an easily handled volume of ethyl acetate (9.0 mL) can elute the corresponding quantity (8.6 μg) of OPA-bis(DNPH). The working range (range from the LOQ to the upper limit) is 0.33 to 5.0 μg m^{-3} (0.060 to 0.91 ppb) for 480 L air samples (8 h samples collected at 1.0 L min^{-1} which are eluted at 0 to 24 h after the completion of air sampling). The estimated LOD for OPA is 0.04 μg m^{-3} (0.007 ppb) for 480 L air samples. The recommended range of air sampling rates is 0.03 to 1.0 L min^{-1}.

Issue of interferences in the air method for OPA. The S10 LpDNPH sampler from Supelco, which contains 1 mg of acidified DNPH on 350 mg of silica, is manufactured under rigorous quality control specifications and is a highly popular sampler for aldehydes and ketones in air. This author has developed and evaluated sampling and analytical methods for formaldehyde, acetaldehyde, propionaldehyde, valeraldehyde, and isovaleraldehyde in air with S10 LpDNPH samplers.[18,19] Although it is possible that other aldehydes and ketones in the sampling environment would be in sufficient quantity to consume the 1 mg of acidified DNPH reagent, it is highly unlikely that the levels of aldehydes and ketones in the hospital environment where OPA is used would be high enough to consume even 10% of the DNPH present during eight hours of air sampling at 1 L min^{-1}. As a rule, approximately two-thirds of the reagent on the sampler bed is consumed by an air contaminant (which reacts with the reagent) at the point of breakthrough.[10] The sampler bed continues to collect the air contaminant after the point of breakthrough.

The S10 LpDNPH sampler has sufficient capacity for collecting background levels of naturally occurring aldehydes and ketones in the atmosphere at ground level during air sampling for 24 hours or more at 1.0 L min^{-1}. At the end of this period, an orange-colored band about 1 mm in thickness can be observed at the front part of the DNPH-coated silica bed; the rest of the bed (about 11 mm in length) will remain bright yellow. The orange-colored band marks the location of DNPH derivatives of numerous naturally occurring aldehydes and ketones.

Since ozone has been observed to consume the DNPH reagent and the DNPH derivative of formaldehyde,[20] it is likely that ozone at sufficient levels (~30 ppbv or higher) can consume a significant portion of each DNPH species on the sampler. The National Ambient Air Quality Standard (NAAQS) for ozone is 120 ppbv, and many areas of the U.S. have failed to meet this

standard. Typically, indoor ozone levels are 20 to 80% of the outdoor ozone levels, depending on the ventilation rate.[21] Because of the high rate of decay of indoor ozone when ventilation rates are very low, typical indoor ozone levels have been found to be less than 10% of outdoor levels when air conditioning is in use.[22] The typical ozone level is about 10 ppbv inside buildings in which air conditioning is in use unless there is a source of ozone inside, such as electrical appliances which produce sparks. Interference by ozone at levels near 10 ppbv is considered to be insignificant for samplers containing DNPH-coated silica, such as Supelco S10 LpDNPH samplers. This method for OPA in air has not been tested with ozone scrubbers placed in front of the inlets of the S10 LpDNPH samplers.

Recoveries of OPA from a glass surface. Average recoveries of OPA were 90% for 200 μg applied to the glass surface ($n = 6$, RSD = 0.039), 83% for 118 μg applied ($n = 6$, RSD = 0.064), and 93% for 68 μg applied ($n = 6$, RSD = 0.088). Pooled RSD = 0.067. These recoveries were found following the use of 2 × 10 cm strips cut from Ghost™ Wipes. These values are considered to be pertinent to both the fluorescent and visual colorimetric analytical methods even when the extraction solvents are different (the extraction solvent for the above values was acetonitrile in lieu of dimethyl sulfoxide or 30 : 70 acetonitrile : water in order that concentrations of OPA could be determined by fortification of S10 LpDNPH cartridges and HPLC analysis).

A glass surface has been employed for the determinations of recovery because glass approaches an ideal surface. An average recovery of >75% is acceptable, but >90% is preferred.[23] Recoveries from slightly rough surfaces are expected to be lower. However, the typical surfaces which would be wiped in a hospital setting are smooth surfaces. Surface wipe methods allow one to assess the potential dermal exposure of workers.[24]

Ghost™ Wipes commonly are used for surface wipes in industrial hygiene surveys. Cleaning of the Ghost™ Wipes by Soxhlet extraction with dichloromethane is useful for reducing the fluorescent contribution by impurities in the fluorescent surface wipe method. However, this cleaning is of no benefit in the visual colorimetric surface wipe method. In addition, the presence of benzalkonium chloride in the Palindust™ wipe was undesirable in either surface wipe method for OPA.

The reason for using three strips from a PVA wipe for each area of a surface is to realize a complete or nearly complete recovery of OPA. Since the first two strips are wet with 25 μL of 40 : 60 dimethyl sulfoxide : water, the area wiped might appear to be wet. The third strip, which is dry, is used to dry the area wiped. This third strip might recover additional OPA. A volume of 12 mL for the recovery solvent is a compromise between a large volume of solution for efficient mixing and a small volume for improved sensitivity.

Methods for OPA on surfaces by fluorescent analysis. The methods for OPA on surfaces by fluorescent analysis employs Roth's fluorimetric method for determining analytes bearing a primary amino group.[25,26] Under basic conditions, the fluorigenic reagents, OPA, a thiol, and a species containing a primary amino group, undergo reaction to form a thio-*N*-alkyl-substituted isoindole. In this work, Roth's fluorimetric method for determining primary amino compounds has been turned around for the determination of OPA in lieu of compounds bearing primary amino groups. The source of the thiol is *N*-acetyl-L-cysteine.[27] Since ethylenediamine bears two primary amino groups, this compound appears to be an excellent choice for a fluorigenic reagent.

Examination of the UV spectrum of the fluorescent thio-*N*-alkyl-substituted isoindole prepared in this method revealed that there was indeed absorption at the excitation wavelength of 365 nm; a small absorption band was observed at 307 nm to 368 nm. The wavelength of maximum emission was 438 nm; the two wavelengths of the single emission band equal to one-half of maximum emission were observed to be 398 and 464 nm.

Water is not recommended as a solvent for this method because the fluorescence of the substituted isoindole product begins to fade almost immediately, apparently due to deterioration of the fluorescent product in water. However, use of dimethyl sulfoxide as solvent for the reaction allows the fluorescence to be stable for at least 24 h. Since the degree of fluorescence is proportional to the original quantity (or concentration) of OPA, a portable fluorometer may be employed for measurement on-site.

The LOD for OPA is estimated at 0.018 μg mL^{-1} or 0.2 μg per 12 mL sample. The calibration curve flattens out at concentrations of OPA of about 80 μg mL^{-1}. Consequently, the upper limit of the method is about 58 μg mL^{-1} or 700 μg per 12 mL sample. Recoveries of three analyst spikes have been 112 to 120% for OPA applied in the range of 2 to 8 μg. Recoveries of three blind spikes have been 95.5 to 109% for OPA applied in the range of 6 to 20 μg. Recoveries in the range of 75% to 125% are acceptable.[10]

It has been concluded that the reaction of OPA with *N*-acetyl-L-cysteine and ethylenediamine is complete in about 2 min or less because excellent calibration curves have been observed by fluorescence measurement as soon as a series of standards in dimethyl sulfoxide solution are mixed with reagents. These observations are consistent with reports of others that mixtures of OPA and *N*-acetyl-L-cysteine with other amino compounds (various amino acids) give rise to maximum fluorescence within 1 to 3 min. of mixing.[25]

While the primary fluorescent method employs a portable fluorometer for quantitation, the procedure for the secondary fluorescent method is the same as the primary one except that (1) a UV lamp emitting at 366 nm replaces the fluorometer and (2) the secondary method is employed mainly as a qualitative method for detection (not for measurement). The LOD of OPA is estimated at 0.01 μg mL^{-1} or 0.12 μg per 12 mL sample. Cuvettes are not necessary for the secondary method because the glass of 20 mL scintillation vials transmits at least 98% of the UV light at 366 nm. It is highly recommended that one avoids placing paper labels or other fluorescent labels onto the walls of the vials because the bright fluorescence will interfere with examination of a weak sample for fluorescence.

Investigation of interference in the surface wipe method by fluorescence. Fourteen test aldehydes were investigated as potential positive interferences in the fluorescence method (see Table 5). Glutaraldehyde, 2-carboxybenzaldehyde, *o*-anisaldehyde, *p*-anisaldehyde, 4-hydroxybenzaldehyde, mesitaldehyde, 1-naphthaldehyde, and syringaldehyde did not cause

fluorescence in the absence of OPA. However, 4-(dimethylamino)benzaldehyde, 4-(dimethylamino)cinnamaldehyde, salicylaldehyde, *o*-tolualdehyde and vanillin caused very weak to strong fluorescence for the quantities indicated in Table 5; when the quantities of salicylaldehyde, *o*-tolualdehyde and vanillin were reduced by ten-fold, these compounds were no longer potential positive interferences. Reduction of the quantity of 4-(dimethylamino)benzaldehyde by ten-fold reduced but did not eliminate the fluorescence. 4-(Dimethylamino)cinnamaldehyde was quite notable; reduction in the quantity by 200-fold from 200 μg to 1 μg reduced the degree of fluorescence from strong to moderate.

Four of the nine aldehydes which were found not to be positive interferences at the initial quantities in Table 5 were tested as potential negative interferences in the fluorescence method for OPA (see the test aldehydes in rows 1, 2, 4 and 12 in Table 5). A negative interference is defined as that substance which causes a negative bias in the measurement of the analyte when actually present. Glutaraldehyde and *o*-anisaldehyde were found not to be negative interferences at the initial levels tested (see Table 6). 2-Carboxybenzaldehyde was a potential negative interference at the 2.7 mg level but not at the 0.0025 mg level. 1-Naphthylaldehyde was found to be a potential negative interference at two levels tested (0.29 and 0.029 mg).

A conclusion can be made that aldehydes are a minimal interference problem in the fluorescence method. Although glutaraldehyde might be used as a disinfectant in the area, glutaraldehyde is neither a positive nor a negative interference at the 7.5 mg level. 2-Carboxybenzaldehyde, which is an expected oxidation product of OPA, might be present in the area of disinfection. If present at the 2.7 mg level, 2-carboxybenzaldehyde would be a negative interference. The other named aromatic aldehydes were tested because it was deemed that aromatic aldehydes would be the most likely compounds to cause interference (in terms of chemical reaction).

Four amino compounds were tested as potential negative interferences in the fluorescent surface wipe method (see Table 7). Amino compounds are obvious potential interferences due to the capacity to form Schiff-base products with aldehydes, including OPA. Mixtures of the amino compounds with OPA in dimethyl sulfoxide solution were allowed to stand for 72 min at room temperature due to the relatively slow reaction rates. Indeed, each of the amino compounds listed in Table 7 was found to be a potential interference.

It can be concluded that amino compounds, including amino acids, can be potential negative interenferences in the fluorescent method, particularly when sufficient reaction time is allowed before addition of *N*-acetyl-L-cysteine and ethylenediamine. Thus, interferences by amino compounds can be minimized by use of the fluorescence test within a few minutes of collection of surface wipes.

Method for OPA on surfaces by visual colorimetric analysis. The visual colorimetric method has been developed as major modifications of the method of Jung *et al.*[28] for determination of urea. The primary concept of the visual colorimetric method in this work is that *N*-(1-naphthyl)ethylenediamine reacts with OPA in an acidic medium to form a colored product or mixture of colored products. The structure in Fig. 3 is hypothesized as a product responsible for the color. Formation of the product in Fig. 3 conceivably takes place as a concerted displacement of the ethylamine species and condensation of OPA with 1-naphthylamine. The extended conjugation of π electrons is responsible for absorption of visible light, and the naphthyl groups contribute vastly to the extended conjugation. Significantly, the presence of urea is not required for the formation of color. When the solvent system is 50 : 50 acetonitrile : water, color development is slow at 37 °C. However, when the solvent system is replaced with 30 : 70 acetonitrile : water, color development is faster and can be accomplished at room temperature. The LOD for the original OPA is estimated at 4 μg mL^{-1} or 48 μg per 12 mL sample for the arbitrarily selected color development time of 30 min.

It is noted that lower LODs can be realized when time for color development is extended to 1 h or, even, 24 h. However, the selection of 30 min is a compromise between a satisfactory LOD and a waiting time which is acceptable to industrial hygienists working on-site.

Table 5 Tests of positive interference in surface wipe method by fluorescence

Test compound	Quantity of test compound[a]/mg	Degree of blue fluorescence[b]	Quantity of test compound/mg	Degree of blue fluorescence
Glultaraldehyde	7.5	None	—	—
2-Carboxybenzaldehyde	2.7	None	—	—
Citronellal	0.20	None	—	—
o-Anisaldehyde	0.33	None	—	—
p-Anisaldehyde	0.20	None	—	—
4-(Dimethylamino)-benzaldehyde	0.20	Weak (purple)	0.02	very weak
4-(Dimethylamino)-cinnamaldehyde	0.20	Strong	0.001	moderate
4-Hydroxybenzaldehyde	0.20	None	—	—
Mesitaldehyde	0.20	None	—	—
1-Naphthylaldehyde	0.29	None	—	—
Salicylaldehyde	0.22	Very weak	0.022	none
Syringaldehyde	0.20	None	—	—
o-Tolualdehyde	0.44	Very weak	0.041	none
Vanillin	0.35	Moderate	0.035	none

[a] Quantity of test compound in 3 mL of dimethyl sulfoxide. [b] By visual observation.

Table 6 Tests of negative interference by aldehydes in surface wipe method by fluorescence

Test compound	Quantity of test compound/mg	Concentration of OPA in 3 mL aliquot/μg mL^{-1}	Degree of blue fluorescence	Effect of test compound
Glutaraldehyde	7.5	0.10	Moderate	No effect
2-Carboxybenzaldehyde	2.7	0.10	None	Neg. Interference
2-Carboxybenzaldehyde	0.0025	0.010	Very weak	No effect
o-Anisaldehyde	0.33	0.020	Moderate	No effect
1-Naphthaldehyde	0.29	0.020	None	Neg. Interference
1-Naphthaldehyde	0.029	0.020	Very weak	Slight, negative interference

Table 7 Tests of negative interference by amino compounds in surface wipe method by fluorescence[a]

Test compound	Quantity of test compound/mg	Concentration of OPA in 3 mL aliquot/μg mL^{-1}	Degree of blue fluorescence	Effect of test compound
DL-Alanine	0.30	0.10	None	Neg. Interference
Benzylamine	0.30	0.10	None	Neg. Interference
Glycine	0.30	0.10	Very weak	Neg. Interference
Urea	0.30	0.10	Reduced	Neg. Interference

[a] Each test amino compound was added to the OPA solution 72 min prior to addition of *N*-acetyl-L-cysteine and ethylenediamine.

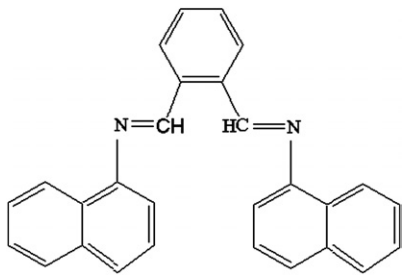

Fig. 3 Hypothesized structure of a colored product with extended conjugation of π electrons.

Fig. 4 A series of cuvettes containing reaction mixtures of OPA and *N*-(1-naphthyl)ethylenediamine. The numbered cuvettes (from 1 to 15) correspond to OPA concentrations of (1) 0.0 μg mL^{-1} for a blank, (2) 0.5 μg mL^{-1}, (3) 1.0 μg mL^{-1}, (4) 2.0 μg mL^{-1}, (5) 4.0 μg mL^{-1} (considered to be the LOD), (6) 6.0 μg mL^{-1}, (7) 12 μg mL^{-1}, (8) 25 μg mL^{-1}, (9) 50 μg mL^{-1}, (10) 100 μg mL^{-1} (11) 250 μg mL^{-1}, (12) 500 μg mL^{-1}, (13) 1000 μg mL^{-1}, (14) 2000 μg mL^{-1}, and (15) 5000 μg mL^{-1}.

According to the UV spectrum of the reaction mixture prepared with an aliquot of OPA in 30 : 70 acetonitrile : water at 8 μg mL^{-1}, there is a small absorption band with λ_{max} of 463 nm. In addition, there is vast absorption at wavelengths below 365 nm. This sample solution appears yellow [consider a shade of yellow intermediate between the shades for cuvettes 6 and 7 in Fig. 4 (corresponding to 6 and 12 μg of OPA per mL, respectively)]. As the original concentration of OPA in solution increases, the limits of the absorption band at 463 nm increase. Consequently, additional wavelengths of visible light are absorbed significantly with increasing concentration of OPA, and fewer wavelengths of visible light are reflected. Thus, the colors which appear to the unaided eye are the colors of light reflected from the reaction mixture. In addition, as the original OPA concentrations increase, the colors shift from yellow to orange, to red, and to very deep red (see Fig. 4).

Due to the examination of the colors visually, this method is considered to be a semiquantitative method. Results of analyst spikes and blind spikes for testing the visual colorimetric method in the range of 6.0 to 250 μg mL^{-1} with standards shown in Fig. 4 have been satisfactory. All "found" concentrations of OPA in four analyst spikes and three blind spikes have been within a factor of 2 of the actual concentrations. The "found" concentration of OPA in the fourth blind spike has been within a factor of 2.5 of the actual concentration.

Table 8 presents the results of tests of positive interference with fifteen separate aldehydes. Only one of the aldehydes, 4-(dimethylamino)cinnamaldehyde, in the quantities indicated (300 and 30 μg) caused formation of color with *N*-(1-naphthyl)ethylenediamine during the 30 min color development time; color was not observed when only 3 μg of 4-(dimethylamino)-cinnamaldehyde was used in the test. Thus, positive interferences by aldehydes generally were minimal.

Tests were run for negative interference by addition of seven of the test aldehydes to separate 3 mL aliquots of 30 : 70 acetonitrile : water solution containing OPA at 12 μg mL^{-1} (see Table 9). Then 300 μL of *N*-(1-naphthyl)ethylenediamine solution was added. After a color-development time of 30 min, all test solutions except one had the typical appearance of being clear and yellow as expected (see Table 9). The test solution containing 300 μg of glutaraldehyde, on the other hand, appeared to be clear and pale brown-orange in color with about the same degree of

Table 8 Tests of positive interference in the surface wipe method by visual colorimetric detection

Test compound	Quantity of test compound[a]/mg	Degree of positive interference	Color of test mixture[b]
Glutaraldehyde	0.30	None	Colorless
2-Carboxybenzaldehyde	0.33	None	Colorless
Citronellal	0.30	None	Colorless
4-(Dimethylamino)-benzaldehyde	0.30	None	Colorless
4-(Dimethylamino)-cinnamaldehyde	0.30	Strong	Orange
4-(Dimethylamino)-cinnamaldehyde	0.03	Moderate	Orange
4-(Dimethylamino)-cinnamaldehyde	0.003	None	Colorless
4-Hydroxybenzaldehyde	0.30	None	Colorless
Mesitaldehyde	0.30	None	Colorless
2-Nitrobenzaldehyde	0.30	None	Colorless
Salicylaldehyde	0.22	None	Colorless
Syringaldehyde	0.30	None	Colorless
o-Anisaldehyde	0.33	None	Colorless
p-Anisaldehyde	0.30	None	Colorless
1-Naphthylaldehyde	0.29	None	Colorless
o-Tolualdehyde	0.41	None	Colorless
Vanillin	0.35	None	Colorless

[a] Quantity of test compound in 3 mL of 30 : 70 acetonitrile : water. [b] Color of test mixture at $t = 30$ min.

Table 9 Tests of negative interference in the surface wipe method by visual colorimetric detection[a]

Test compound	Quantity of test compound/mg	Color of solution	Degree of negative interference
Glutaraldehyde	0.30	Pale, brown-orange	Minor (shift in color)
2-Carboxybenzaldehyde	0.33	Pale yellow	None
Salicylaldehyde	0.22	Pale yellow	None
o-Anisaldehyde	0.33	Pale yellow	None
1-Naphthylaldehyde	0.29	Pale yellow	None
o-Tolualdehyde	0.41	Pale yellow	None
Vanillin	0.35	Pale yellow	None

[a] The concentration of OPA in the 3 mL aliquot of test solution was 12 μg mL^{-1}.

intensity. A shift from the expected color appears to be only a minor negative interference.

Four amino compounds were tested as potential negative interferences in the visual colorimetric test (see Table 10). The amino compounds were added to 3 mL aliquots of OPA solution at 20 μg mL^{-1} in 30 : 70 acetonitrile : water 72 min prior to the addition of *N*-(1-naphthyl)ethylenediamine solution to allow for reaction to take place. Urea was the only test amino compound of the four which was found not to be a potential negative interference

Therefore, it appears that positive or negative interference problems by aldehydes may be very minimal for the visual colorimetric method. Potential negative interference by amino compounds is a reality, particularly when sufficient time for reaction with OPA is allowed prior to the addition of *N*-(1-naphthyl)ethylenediamine.

Table 10 Tests of negative interference by amino compounds in the surface wipe method by visual colorimetric detection[a]

Test compound	Quantity of test compound/mg	Color of solution	Degree of negative interference
DL-Alanine	0.30	Weak yellow	Major
Benzylamine	0.30	Moderate orange	Major
Glycine	0.30	Weak yellow	Major
Urea	0.30	Moderate yellow	Major

[a] Each test amino compound was added to 3 mL of OPA solution (20 μg mL^{-1}) prior to the addition of *N*-(1-naphthyl)ethylenediamine solution.

A possible drawback is that the visual colorimetric method has a significantly higher LOD than the fluorescence method has. Since the levels of OPA on surfaces which cause health effects are unknown, the utility of the visual colorimetric method for OPA is unclear.

Method evaluations at a local hospital. Air sampling was conducted at 1.0 L min^{-1} in the endoscope cleaning room at a local hospital. Measured concentrations of OPA in air in the endoscope cleaning room ranged from 5.6 to 74 μg m^{-3} (1.0 to 13.5 ppb) (see Table 11). For comparison, OPA at 0.1 μg m^{-3} (0.02 ppb) was found in an adjacent office and was not detected in another office. CIDEX OPA test strips (commercial products of Johnson and Johnson) were employed at intervals by the hospital staff to ascertain the strength of the CIDEX OPA solution for disinfection. Disposal of used CIDEX OPA test strips in an open trash can was normal procedure. However, such disposal of test strips resulted in relatively high concentrations of OPA in air (see Table 11). Also, the concentration of OPA in the headspace above an open

Table 11 Results of air sampling for OPA at a local hospital

Location of air sampler	Air volume/l	Concentration in air[a]/μg m^{-3}
Point near automated - washer for endoscopes	441	5.6
Point at second automated - washer for endoscopes	396	6.6
Point 6 cm above trash can - (containing one used - CIDEX OPA test strip)	5.0	74
Point 3 cm from used test - strip during color - development	4.0	35
Point 15 cm above open - tray of CIDEX OPA - solution	13	35
Office adjacent to - endoscope cleaning	377	0.1
Another office adjacent to - endoscope cleaning	473	N.D.

[a] N.D. means "not detected". The LOD for the last sample was 0.09 μg m^{-3}.

Table 12 Results of analysis of surface wipes for OPA at local hospital[a]

Location	Approximate area wiped/cm^2	Quantity of OPA found: Fluorescence method/μg	Quantity of OPA found: Colorimetric method/μg
Floor in front of sink	100	5.0	N.D.
Handles of automated washer	25	5.0	N.D.
Cover for tray containing CIDEX OPA	100	7.2	N.D.
Handles of locker for cleaned endoscopes	20	4.6	N.D.
Handle of gallon bottle of CIDEX OPA	20	2.2	N.D.
Container of OPA test strips	25	3.7	N.D.

[a] N.D. means "not detected". The LOD for the colorimetric method was 48 μg per sample.

container of CIDEX OPA solution was relatively high. Interfering peaks in measuring the HPLC peak for OPA-bis(DNPH) were not observed. In fact, no other DNPH derivatives were observed in the chromatograms. Therefore, the hospital atmosphere appeared to be free from detectable levels of ketones and other aldehydes by the present method for OPA in air. Due to the characteristics of ethyl acetate in the sample eluents, multiple HPLC peaks (five peaks) were observed for the excess DNPH reagent in all chromatograms for air samples.

Table 12 presents the results of analysis of surface wipes in the endoscope cleaning room. In cases in which surface areas were virtually unlimited, an area with dimensions of 10 × 10 cm was wiped. In other cases, one-half of the limited area was wiped for the fluorescent method using the fluorometer, and the remaining area was wiped for the visual colorimetric method. Due to the excellent LOD of 0.2 μg per sample for the fluorescent method, OPA was found in every sample. However, OPA was not detected in any sample by the visual colorimetric method due to the much higher LOD of 48 μg per sample.

Conclusions

Three practical methods for OPA have been developed. The method for OPA in air and one of the surface wipe methods, in particular, the one involving fluorescent detection, should prove to have great utility in industrial monitoring in occupational areas where OPA is employed. The method for OPA in air, the surface wipe method by fluorescence measurement, and the surface wipe method by visual colorimetric detection presently are draft methods and will appear as methods 2025, 9210 and 9211, respectively, in *The NIOSH Manual of Analytical Methods*.

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