

# Reports

## N-Nitroso Compounds: Evidence for Their Presence in Airborne Particles

**Abstract.** Chemical, infrared, and thermal energy analyses have provided evidence for the presence of the N-nitroso functional group in extracts of airborne particles. The total molar N-nitroso concentrations in New York City air are equivalent to the total concentrations of polycyclic aromatic hydrocarbons. Since 90 percent of the N-nitroso compounds that have been tested are carcinogens, the newly discovered but untested materials may represent a significant environmental hazard.

Airborne particulate organic matter has been shown in several bioassays to be carcinogenic or mutagenic (1). Although the polycyclic aromatic hydrocarbons (PAH) generally have been the focus of concern, early studies demonstrated the presence of other active materials (2). Many recent studies have focused on potent mutagens in ambient samples (3); however, the Ames test as normally used is not sensitive to all classes of mutagenic or carcinogenic compounds (4). One such class of potent carcinogens is the *N*-nitrosamines. The discovery of vapor-phase nitrosamines in urban air (5) led us to investigate whether *N*-nitroso compounds are present in airborne particles. We report here evidence for the apparent presence of such compounds.

We used a modified Griess colorimetric method (6, 7) to test for the presence of *N*-nitroso compounds. Samples of respirable ( $\leq 3.5 \mu\text{m}$ , 50 percent collection efficiency) particulate matter collected in New York City were extracted ultrasonically twice with dichloromethane at 0°C (6). Potential interferences were removed by sequential extractions with 0.2*N* NaOH (removal of acids, phenols, nitrates, and nitrites) and 0.2*N* H<sub>2</sub>SO<sub>4</sub> (removal of amines and bases); the samples were then subjected to a fractional distillation and change of solvent to methanol with a Kuderna-Danish evaporator (8).

The procedure was calibrated with the following series of standard compounds: *N,N*-dimethylnitrosamine (DMNA), *N,N*-dibutylnitrosamine (DBNA), *N*-nitrosopiperidine, *N*-nitrosopyrrolidine, and *N*-nitrosodiphenylamine (DPNA). The responses were collinear for all compounds tested, and the regression line

obtained for calibration with 62 standard solutions was as follows:  $A = 3.44 \times 10^6 \text{ mole}^{-1} (M) + 2.61 \times 10^{-2}$  (correlation coefficient  $r^2 = 0.99$ ), where  $A$  is the absorbance and  $M$  is the number of moles of *N*-nitrosamine. The qualitative and quantitative lower limits of detection, calculated according to the method of Currie (9), were 0.014 net absorbance unit above blank (or  $2.3 \times 10^{-9}$  mole per sample) and 0.040 net absorbance unit (or  $6.6 \times 10^{-9}$  mole per sample), respectively. The mean filter-reagent blank was  $0.032 \pm 0.004$  absorbance unit. The percentages of 1 to 5  $\mu\text{g}$  of DMNA and 20 to

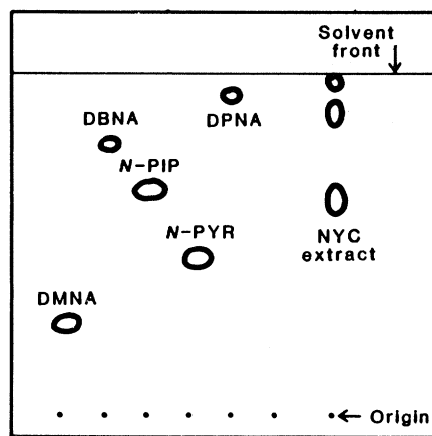


Fig. 1. Thin-layer chromatogram of standard and unknown *N*-nitrosamines [silica-gel plates with hexane-ether-dichloromethane solvents (4:3:2 by volume)]. Standard *N*-nitrosamines were *N,N*-dimethylnitrosamine (DMNA), *N,N*-dibutylnitrosamine (DBNA), *N*-nitrosopiperidine (*N*-PIP), *N*-nitrosopyrrolidine (*N*-PYR), and *N*-nitrosodiphenylamine (DPNA). The unknowns were contained in an extract from a sample of particulate matter collected in New York City (NYC). Colored spots were observed by development with Griess or Preussmann reagents (10).

60  $\mu\text{g}$  of DPNA added to blank filters that were recovered were  $60 \pm 11$  percent and  $84 \pm 5$  percent, respectively. We also tested the recovery of DPNA by adding known amounts to filter strips already loaded with samples and evaporating the solvent. After correction for the sample values, the recoveries were 48, 53, and 65 percent for 20-, 59-, and 99- $\mu\text{g}$  additions, respectively (100, 300, and 500 pmole). Measurements on ambient samples indicated that over 100 pmole of unidentified *N*-nitroso compounds were present per cubic meter of air.

We used both thin-layer chromatography (TLC) (Fig. 1) and high-performance liquid chromatography (HPLC) (Fig. 2) to separate the compounds present in the methanol solution from the initial separation procedure. The presence of *N*-nitroso groups was indicated by positive responses with both the Griess and Preussmann reagents (10). Two or three spots were found with TLC in the samples tested, and the  $R_F$  values were in the range of 0.6 to 0.65, 0.87 to 0.92, and 0.98 to 1.0.

Prior to HPLC separation, we separated the *N*-nitroso compounds in the methanol solution from aliphatic and PAH classes by development of the silica-gel plates with cyclohexane. The *N*-nitroso compounds remained at the origin and appeared more polar than aliphatic or PAH compounds, which had  $R_F$  values of 0.8 to 0.9 and 0.3 to 0.5, respectively. Separations with HPLC then gave three fractions that were consistently positive by the Griess reaction (Fig. 2).

Infrared spectra were obtained for these fractions by use of Fourier transform infrared spectroscopy (11). The spectra of the C1 + C2 and C3 fractions showed the bands expected for *N*-nitroso functional groups, that is, in the regions 1450 to 1470  $\text{cm}^{-1}$  (N=O stretching) and 1020 to 1118  $\text{cm}^{-1}$  (N-N stretching) (12). Bands at 1738 and 1620  $\text{cm}^{-1}$  in both spectra indicated the presence of carbonyl and amide functional groups (13). Bands typical of aromatic compounds, in the regions 3000 to 3100  $\text{cm}^{-1}$  (aromatic C-H stretching), 1500 to 1600  $\text{cm}^{-1}$  (aromatic C-C stretching), and 675 to 870  $\text{cm}^{-1}$  (out-of-plane C-H bending), were absent. Absorption was observed in the region 2855 to 2950  $\text{cm}^{-1}$ , typical of tetrahedral C-H stretching. Although the spectra indicate the presence of *N*-nitroso, amide, and aliphatic hydrocarbon functional groups, the purity of the fractions has not been determined.

For confirmation, we tested a C1 + C2 fraction from the TLC-HPLC cleanup, using a thermal energy analyzer

(TEA), which is extremely sensitive and has good specificity for the *N*-nitroso functional group (14, 15). Injection without chromatographic separation confirmed the presence of *N*-nitroso groups at a level 30 times the blank response (15). No response was obtained by a gas chromatography-TEA method; *N*-nitroso structures could not be demonstrated by gas chromatography-mass spectrometry in our laboratory or in a second laboratory.

The combined results of Griess and Preussmann tests, infrared spectra, and TEA responses indicate the apparent presence of the *N*-nitroso functional group. The infrared spectra further suggest the possible presence of *N*-nitrosamides. The gas chromatographic work with several detectors, including mass spectrometry at two laboratories, suggests that these compounds have relatively low volatility or are thermally unstable. In the absence of compound isolation and structure identification, we cannot be certain of the specific presence of *N*-nitroso compounds. Nonetheless, the combined evidence for their presence is extremely strong.

Despite the lack of structure information, we have attempted to rule out the possibility of sampling and analytical artifacts. Addition of 1000 to 1100  $\mu\text{g}$  of  $\text{NaNO}_3$ ,  $\text{NaNO}_2$ , or diphenylamine and combinations of these compounds to blank filters did not increase the absorbance of blanks. These materials either

individually or in combination did not interfere in the recovery of DPNA. Additions of 1500  $\mu\text{g}$  of nitrobenzene and *p*-nitrophenol or 1000  $\mu\text{g}$  of *p*-nitrosophenol and 2-nitroso-1-naphthol did not change the recovery of 10  $\mu\text{g}$  of DBNA. The nitro compounds showed a slight response to the Griess reagent at levels two orders of magnitude higher than would be expected in ambient air samples. We also added mixtures of nitrite, nitrate, amine, and C-nitroso compounds to sections of filters prior to sampling. No increase was observed in the absorbances for the treated as compared to untreated areas. Thus, artifacts do not appear to be formed to any significant extent during the sampling and analysis.

Weekly samples were analyzed using the modified Griess colorimetric method. The concentrations of *N*-nitroso compounds found for New York City samples taken from 1978 to 1980 averaged 140  $\text{pmole/m}^3$  ( $< 20$  to 350) uncorrected for any recovery factor. The average for a rural forested area in Tuxedo, New York, was 43  $\text{pmole/m}^3$  ( $< 20$  to 100). Fine *et al.* (16, p. 295) have reported the detection of "several as yet unidentified nonvolatile nitroso compounds" in soils. Since deposition of airborne particles is often a source of contaminants in urban soil, the two findings may be related.

The average benzo[*a*]pyrene concentration in New York City from 1977 to 1979 was 5.4  $\text{pmole/m}^3$  (1.35  $\text{ng/m}^3$ ) (17).

About 90 percent of some 300 *N*-nitroso compounds tested have been found to be carcinogenic (18). If the compounds found in this study are as potent as the compounds evaluated in animal studies, then they may be more hazardous than benzo[*a*]pyrene, since the molar concentration ratio of the two materials is about 26 to 1. Thus the *N*-nitroso compounds are about equal in concentration to the total PAH complex and are potentially equal in carcinogenic potency. This estimate that the hazard from this class of compounds may equal that for total PAH indicates the need to determine the structures of the *N*-nitroso materials and to test their biological activity.

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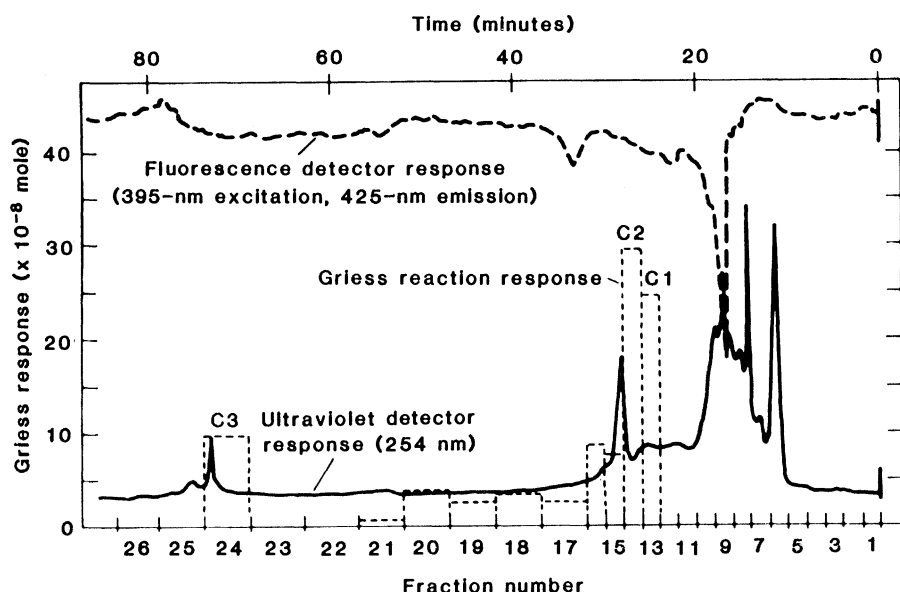


Fig. 2. High-performance liquid chromatographic separation of unknown *N*-nitroso compounds. The colorimetric, photometric, and fluorometric detection responses are shown. A  $\mu$ Bondapak C-18 column was used (30 cm by 3.9 mm). Solvent composition was programmed from 20 to 80 percent acetonitrile in water (pH = 4, phosphoric acid) over 20 minutes, was held isocratic (constant solvent composition) for 22 minutes, and then was programmed from 80 to 100 percent acetonitrile in 1 minute, and was held at 100 percent acetonitrile for 20 minutes. The flow rate was 1.0 ml/min.

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## Spontaneous Vesicles Formed from Hydroxide Surfactants: Evidence from Electron Microscopy

**Abstract.** *Dialkyldimethylammonium hydroxide surfactants are highly soluble in water and form spontaneous stable vesicles. These vesicles can be grown to size with added acid, and appear to provide an ideal membrane mimetic system for the study of fusion and ion transport. These phenomena are a consequence of strong hydration forces that are not necessarily limited to the hydroxide ions. The forces can be used to design a variety of model systems whose behavior differs from that of systems in which double-chained surfactants form insoluble liquid crystalline phases in water and unstable vesicle suspensions on prolonged sonication.*

We report here on vesicle suspensions that form spontaneously, are stable and fairly monodisperse, and can be grown to a prescribed size by titration with acid. These are prepared from the double-chained ionic surfactant didodecyldimethylammonium hydroxide  $[(C_{12}H_{25})_2(CH_3)_2NOH]$ . The existence of such spontaneous vesicles is of interest for the following reasons. (i) Vesicles prepared by the usual sonication or solvent extraction methods are unstable and eventually revert to the (lowest free energy) liquid crystalline state from which they emerged (1, 2). This led to the belief that all vesicles, unlike micelles or liquid crystals, are a peculiar nonequilibrium state of matter to which the usual theories of self-assembly based on statistical mechanics cannot be applied. According to theory (3), it should be possible to produce some stable vesicle systems by suitable design of surfactant (to control bilayer curvature) and choice of suspending medium (to control interaggregate forces). Our system appears to fulfill these two criteria. (ii) Vesicles have been suggested as model systems (1) for photocatalysis and ion transport mechanisms in membranes. The inability of investigators to show that vesicles can be stable has inhibited progress in these areas. (iii) The particular hydroxide vesicles described here provide dramatic evidence for the existence of strong hydration forces characteristic of the hydroxide ion. These are qualitatively different from forces associated with other anions. This observation, along with the demonstration of hydration forces (4) for cations (5), extends the

boundaries of classical colloid science to hitherto inaccessible regions including biological macromolecules and surfaces (6).

Didodecyldimethylammonium hydroxide is readily prepared by gently mixing the insoluble bromide surfactant with hydroxide ion-exchange resin (Rexyn 201, Fisher Scientific) at 25°C, filtering off the supernatant, and repeating the exchange process a second time (2). Typically, 3 g of the bromide surfactant are added to 50 ml of resin (previously rinsed with several batches of water and drained of excess water) in a 250-ml stoppered Erlenmeyer flask. After the surfactant has been thoroughly mixed with the damp resin, 50 ml of water are added and the slurry is gently mixed with a magnetic stirring bar. The resin is separated from the surfactant solution by passing through filter paper and the resin is rinsed with about 20 ml of water. After the second exchange, a small portion of the solution is checked for completeness of exchange by acidifying with nitric acid ( $pH \sim 4$ ) and adding silver nitrate. The exchange process yields about 1.5 g of surfactant (0.05M).

For the exchange and filtering operations, we used a polyethylene glove bag filled with  $CO_2$ -free air. Other than gentle mixing and gravity filtration, the solutions are not subjected to any stirring or agitation. Measurements with  $pH$  paper give values ranging from 11 to 13, depending on surfactant concentration. Our attempts to use  $pH$  electrodes were unsuccessful because of precipitation of the surfactant at the liquid junction of the reference electrode. Most of our studies

with added salt have involved partial neutralization with hydrobromic acid. Preliminary measurements of vesicles neutralized with hydrochloric or hydrofluoric acid indicate that there may be significant differences in vesicle size and stability depending on counterion (7). Unlike the halides which form liquid crystals (and vesicles only on prolonged sonication), the hydroxide surfactant is highly soluble ( $> 1.0M$ ) in water. The supernatant can be dried by lyophilization to a fine white powder which dissolves instantly on addition of water. Freeze-thaw cycles give vesicles that spontaneously reconstitute to the structures illustrated, indicating thermodynamic stability.

We focus here on the direct evidence for vesicles as revealed by cold-stage electron microscopy. Frozen specimens of the dispersions are prepared by trapping a thin layer of liquid, ideally less than half a micrometer thick, between two polyimide film-covered electron microscope grids. These specimens are then plunged into liquid nitrogen and stored in the cryogen until their transfer into the microscope (8). The frozen specimen is transferred into the microscope, a JEOL JEM 100CX, without excessive heating or frost deposition with a special cold-stage transfer module (9) and is kept in the microscope at 95 K. This technique applied to vesicular and liquid crystalline dispersions is free from the artifacts introduced by staining and drying techniques (10). Data from previous work (11) on the contrast mechanism responsible for images of vesicular dispersions enable one to distinguish easily among vesicles, liquid crystals, and globular precipitates found in frozen specimens. However, with this technique we cannot distinguish between unilamellar and multilamellar vesicles.

Figure 1a shows structures observed in a frozen suspension of the hydroxide surfactant ( $1.4 \times 10^{-2}M$ ). These vesicles are fairly monodisperse and have an average diameter of  $300 \pm 80 \text{ \AA}$  ( $\pm$  standard deviation) as determined from measurements on 537 vesicles. Structural features of the ice matrix, such as grain boundaries and defects in the crystalline ice, can also be seen. Partial neutralization (25 percent) with hydrobromic acid results in vesicle suspensions typified by Fig. 1b. The average size here is  $460 \text{ \AA}$ , and the vesicles seem to be less monodisperse. When the vesicles are 50 percent neutralized with HBr, pronounced Tyndall scattering is observed, and some of the vesicles (as characterized by electron microscopy) grow considerably and some become liquid crystals (Fig. 1c).