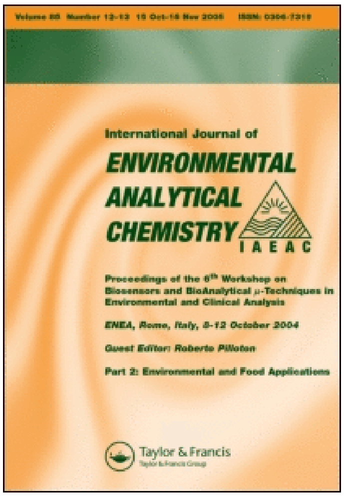

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Determination of Nitro-Polynuclear Aromatics in Ambient Aerosol Samples†

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A method has been developed for determining polynuclear aromatic hydrocarbons (PAHs) and their nitro-derivatives (nitro-PAHs) at nanogram to picogram levels in ambient aerosol samples using derivatization–capillary gas chromatography (CGC) with electron capture detection (ECD). Analysis of nitro-PAHs is achieved by direct extraction of ambient particulate samples into cyclohexane followed by CGC-ECD determination. PAHs may be similarly analyzed following conversion into one or more nitro-derivatives in aqueous acidic nitrate solutions under conditions simulating aerosol droplet conditions. Nitro-derivatives may be prepared from PAH solutions or by mixing dried PAH extracts on filters with nitric acid solution.

Degradation of benzo(a)pyrene is reduced and more nitro-PAH formed if the sample is derivatized on particulate-laden filters vis-a-vis clean filters, whereas the reserve occurs for pyrene—more nitropyrene formed on clean filters. Results suggest strongly that nitro-derivatization of adsorbed PAHs during atmospheric transport is feasible. Analyses of ambient aerosol samples from urban and rural areas to determine levels of nitro-PAHs and their parent compounds have been conducted.

KEY WORDS: PAHs, nitro-PAHs, aerosol samples, derivatization-GC, capillary GC, ECD.

INTRODUCTION

For many years it has been known that extracts of the organic carbon component of airborne particulate matter are frequently carcinogenic[1] and this activity has often been ascribed to polynuclear aromatic hydrocarbons (PAH). Recent studies using the Ames/salmonella tests have indicated that organic extracts of airborne particles also exhibit mutagenic activity,[2] and that most of the mutagenicity of extracts of particulate matter from

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mobile sources is due to compounds other than PAH.[3] Thus the finding that PAH may be converted into direct acting mutagens by exposure to photochemical smog[2,4] has provoked much interest in identifying the mutagenic species.

It is now established that mononitro-derivatives of PAH (nitro-PAH) may be formed from PAH sorbed onto airborne particulate matter upon exposure to NO_2 plus nitric acid,[4-7] and in some cases nitro-PAH are present in particulate matter from mobile sources.[8-10] Examples of nitro-PAH which have been identified include nitro-derivatives of pyrene, benzo(a)pyrene and perylene in photochemical smog aerosols,[2,4] and nitro-pyrene in particulate matter from diesel exhaust[9,10] and in xerographic toner materials.[11,12] Reviews of progress in the study of nitroarene formation in airborne particulate matter[13] and of their determination in diesel particulate matter[14] have recently appeared.

In summary, major portions of the mutagenicity of these samples have been attributed to the small amounts of nitro-PAH present. Hence it is necessary to develop improved analytical methods, especially for airborne particulate samples, to determine picogram levels of nitro-PAH in complex matrices in relatively large numbers of samples. This will permit examination of some fundamental questions:

a) What is the human exposure level to nitro-PAH?
b) Are nitro-PAH formed *in situ* by reaction of the parent PAH, or
c) Are nitro-PAH solely an artifact of the filter methods used?

In this paper we report on a method (nitro-derivatization followed by capillary GC analysis with electron capture detection (CGC–ECD)) which is capable of determining low nanogram and picogram levels of nitro-derivatives of several common PAH. Stability studies of pyrene (Py) and benzo(a)pyrene (BaP) adsorbed on filter materials have been conducted, and conditions for nitration of these and several other PAH have been optimized. Analysis of airborne particulate matter for Py, BaP and their nitro-derivatives has been conducted with samples from several urban and rural areas. Nitro-BaP and nitropyrene have been tentatively identified by CGC–ECD in some of these samples, and confirmation of peak identities by gas chromatography–mass spectrometry (GC–MS) is being conducted.

EXPERIMENTAL

Materials

Reagent grade nitric acid, sulfuric acid and sodium nitrate were used in nitration reactions conducted in sealed glass vials. HPLC-Grade cyclohexane was used for extractions from solution and direct extractions from filter samples, except reagent grade benzene was used in a few experiments. Filter materials used for procedural tests were prepared from tissue quartz (Pallflex QA0, Pall Corp.) by the method of Leahy *et al.*[15] Some of the airborne particulate samples were collected on high purity glass fiber filters. PAH standards were obtained as solutions (0.2 mg PAH/ml) in methanol or methylene chloride and were diluted to 0.04 mg PAH/ml with cyclohexane standard before use. 9-Nitroanthracene (0.223 mg/ml in cyclohexane) was used as an internal standard.

Instrumentation

ALL PAH and nitro-PAH analyses were conducted using a Hewlett–Packard Model 5710A gas chromatograph with a Model 18740B capillary inlet system, and with a ^{63}Ni electron capture detector (Model 18713A). Some confirmatory GC analyses were done using the H–P Model 18774A nitrogen–phosphorus flame ionization detector. All chromatographic data were recorded and reduced using a Varian Model CDS-111 data system. Confirmation of the identity of peaks used in CGC–ECD determinations was accomplished by GC–MS-computer analysis using a Hewlett–Packard Model 5985A GC–MS system with H–P Model 2113 data system. Peak identification was achieved on the GC–MS with larger PAH and nitro-PAH samples, but employing the same extraction and nitration procedures as in the CGC–ECD determinations. In all cases, the GC separation was performed on a 30 m × 0.22 mm id fused silica capillary column coated with DB-5 liquid phase (J & W Scientific). Capillary injection was performed using the splitless mode with a 60 sec delay. Capillary column pressure was 20 psi with 12 cc/min of 95% argon–5% methane make-up gas added just upstream from the ECD.

Procedures

Nitro-derivatives of all polynuclear aromatic hydrocarbons (PAH) tested were prepared by adding 5–50 μl portions of 0.04 mg/ml solutions of diluted PAH to a small vial, adding 550 μl of nitric acid or 200–250 μl sodium nitrate–sulfuric acid mixtures, and stirring on a hand-held mixer for 2 min with glass beads added to promote mixing. Thereafter the reaction mixture was neutralized with KOH, then extracted with 500 μl of cyclohexane and 1- or 2-μl aliquots of the organic extract injected into the CGC–ECD for determination of the nitro-PAH formed. In later analyses the neutralization step was replaced by a dilution step using 500 μl H_2O.

An alternate procedure was also tested. A portion of the diluted PAH was applied to a small circular portion ($\sim$1.4 cm^2) of quartz filter medium, the solvent removed by evaporation, and aqueous nitric acid applied to the dried sample. Extraction and analysis proceeded as per the liquid samples above. In a few cases the reaction was conducted using PAH samples dried onto the quartz filters, by contacting the samples with HNO_3 vapor above concentrated or fuming nitric acid in a closed vial (with benzene as extracting solvent).

Direct analyses for nitro-PAH compounds in ambient particulate matter collected on filters were conducted by extracting a portion of the filter ultrasonically for 60 min with cyclohexane and injecting an aliquot of the extract into the CGC–ECD system. Only nitropyrene and nitrobenzo(a)pyrene (assumed to be the 1-nitro- and 6-nitro-isomers, respectively, based on the Dewar reactivity scales)[16-18] were identified in the samples based on retention times and quantitated based on peak areas. Due to the complexity of the CGC–ECD chromatograms, triplicate analyses for the parent PAH by nitration reaction were conducted: one filter portion was reacted and analyzed directly, one after spiking with 0.1 ng Py, and a third after spiking with 0.2 ng BaP. A fourth portion was extracted and analyzed for 1-nitro-Py and 6-nitro-BaP directly. In nearly all cases (including all ambient sample analyses) an internal standard (9-nitroanthracene) was added to normalize the peak areas prior to quantitative analysis.

Calibration curves were prepared by addition of varying amounts of the standard Py or BaP solution with reaction, extraction, and CGC–ECD analysis conditions identical to those used for the samples. Further cross-checks of the calibration data were conducted by varying the injection volume for individual nitropyrene preparations as well as varying the amount of pyrene starting material.

Storage condition studies were performed by applying Py or BaP samples to clean or particulate-loaded filter portions, evaporating off the solvent, then storing the samples in the dark in closed containers. Air was not excluded during the storage period. Particulate-loaded filter samples were selected to contain of the order of 10–100 μg of elemental carbon/cm^2 of filter surface; carbon analysis was by the thermo-evolution technique of Tanner *et al.*[19]

RESULTS AND DISCUSSION

Optimization of nitration conditions

Initially it was desired to establish the appropriate nitration conditions for mono-nitration of several PAH to be studied. An optimum acid concentration (for aqueous reactions in mixtures of sodium nitrate and sulfuric acid) was anticipated since di- and tri-nitro derivatives might well be formed in more concentrated acids; mono-nitration was desired due to the difficulty of eluting the higher derivatives on the capillary column used even at the upper temperature limit of the liquid phase. In later experiments the optimization procedure was repeated for PAH samples applied to the quartz filters. Representative plots are shown in Figure 1 of response (normalized peak area of the nitro-PAH peak) vs. concentration (wt. %) of sulfuric acid. There is an indication from this data that more easily nitrated compounds such as BaP produce mononitro-derivatives at maximum concentration in more dilute acid than less reactive compounds (e.g., Py) under the same conditions. Also, it appears that the maximum response is at lower concentrations for PAH applied to filters (although the yield may be lower), since less reactive chrysene on filters yields maximum nitro-derivative response at the same acid concentration as does the more reactive pyrene in solution.

A summary of the optimization experiments for all of the PAH examined is given in Table I. Based on the 5-class electrophilic reactivity scheme reported by Nielsen *et al.*,[13] which in turn is based on the pioneering localization energy calculations of Dewar and coworkers,[16-18]

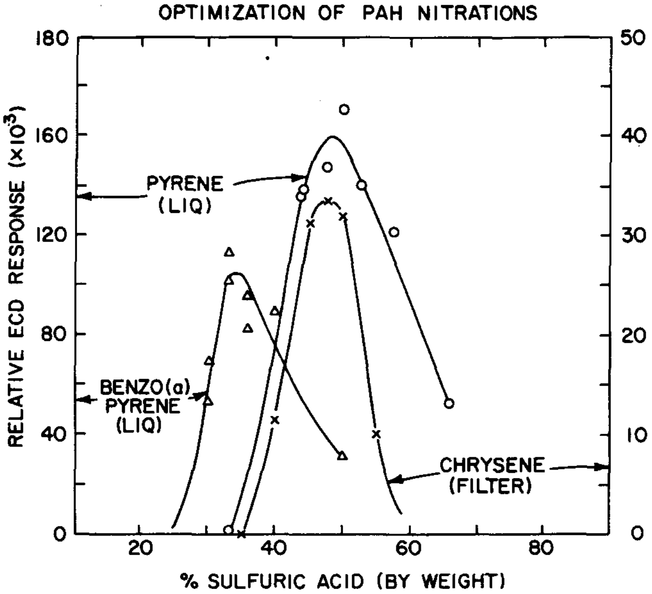

FIGURE 1 Optimization of PAH nitration conditions; O = pyrene, liquid phase nitration; △ = benzo(a)pyrene, liquid phase; x = chrysene, nitration of filter-applied sample.

TABLE I

Optimization of nitration conditions.

Compound	(% H_2SO_4 by weight)		Relative response (X10^3)	
	Liq.	Filter	Liq.	Filter
Benzo(k)fluoranthene	50	NR[a]	39	—[b]
Benzo(b)fluoranthene	NR	NR	—	—
Benzo(ghi)perylene	NR	NR	—	—
Fluoranthene	60–65	50–52	18	31
Pyrene	48–52	25–30	160	180–220
Chrysene	57	48–50	192	33
Benzo(a)pyrene	30–35	≤25	105	~35[c]

[a] NR = No peak was observed indicating no reaction or peak not eluted from column.

[b] No data was obtained for these conditions.

[c] Response on clean filters is highly variable (see storage data, Fig. 2).

the reactivity of the PAH examined toward nitration should decrease in the order: anthracene, BaP > Py, benzo(ghi)perylene > chrysene > benzo-fluoranthenes (b,k), fluoranthene. Nitration of anthracene was studied only briefly in this work since 9-nitroanthracene was selected as internal standard. It appears that the reactivities observed, based on the acid concentrations required for mononitration, are generally consistent with the known reactivities for both solution and filter reactions. The nitration of benzo(k)fluoranthene does occur at lower acid concentrations than expected in solution but no nitro product was observed for filter reactions. It would appear that benzo(b)fluoranthene is unreactive up to 70% H_2SO_4, but we surmise, in contrast, that benzo(ghi)perylene is being nitrated but that the nitro-drivative is not eluted even at the maximum column temperature permitted.

Stability studies

Although PAH are relatively stable in air in the dark,[20] the reactivity of certain PAH toward sub-ppm levels of ozone is known.[21] This, coupled with early observations that PAH are more stable in the sorbed state than in pure form,[22] necessitates a careful evaluation of the lifetimes of PAH under the sampling, storage, and analysis conditions employed in a particular study. Attempts to determine PAH and their nitro-derivatives in ambient samples by the technique reported here is further complicated by the observed photochemical decomposition of nitroarenes to the corresponding quinones and other products.[23]

Studies were thus conducted to establish the stability of the parent PAHs, BaP and Py, on clean and particulate-loaded quartz filter material. Loaded filters were obtained from weekly high-volume filter samples collected on the roof of our BNL laboratory. Duplicate 0.4 ng samples of Py or 1.0 ng samples of BaP were applied to the filters and stored in closed containers in the dark (air not excluded) until nitration, extraction and CGC–ECD analysis procedures were performed.

The results of the stability studies are shown in Figure 2, from which the following inferences may be made. Under the storage conditions used BaP samples are more stable (based on subsequent yield from the nitration reaction) when applied to particulate-laden filters. In contrast higher nitration yields are obtained for Py samples applied to clean filters. In addition, there is a significant negative slope for Py samples applied to both clean and loaded filters indicating decomposition with time, whereas no significant slope is observed for the BaP samples.

Initially, there was considerable difficulty in obtained reproducible results for BaP applied to clean filters and this is confirmed by the greater scatter in the response of values given in Figure 2 for BaP. It appears from these results that BaP is stabilized to a greater degree with respect to oxidative decomposition by ambient particulate matter than is Py. In

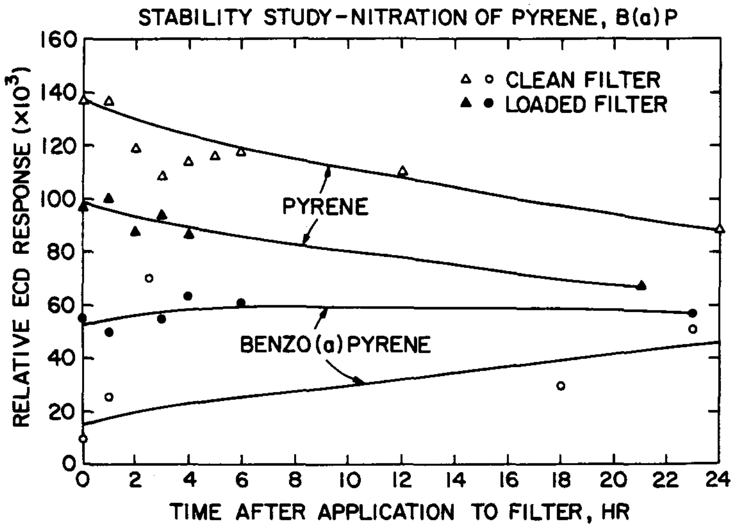

FIGURE 2 Stability study of nitration of pyrene and benzo(a)pyrene on clean and particulate-loaded filters; triangles = pyrene, circles = benzo(a)pyrene; open circles = clean filters, filled circles = loaded filters.

addition we observed that pyrene will continue to slowly decompose on both clean and loaded filters after collection when stored in the dark at room temperature. Storage at −20°C or below and rapid analysis of samples after collection is recommended. A final observation is that calibration curves (such as those presented below) performed with PAH applied to clean filter media may not be accurate for some compounds due to differing rates of decomposition and differing yields, both of which may depend on the nature and amounts of co-collected particulate matter.

Calibration curves

Linear calibration curves have been obtained for both pyrene and BaP samples using both the liquid solution-nitration procedure and the procedure of reacting the PAH after application to inert quartz filters. These curves are obtained by plotting the ECD chromatographic peak areas of the nitro-Py and nitro-BaP product peaks at ~16 min and ~23 min, respectively versus the amount of PAH(ng) reacted in solution or on the filter. Representative plots for Py calibrations are shown in Figure 3 and the regression statistics for these plots in Table II. It is clear that pyrene may be derivatized to what appears to be 1-nitropyrene reproducably in solution and from filters.

BaP samples in solution and from filters may be nitrated to form what we presume to be the 6-nitro-BaP derivative with normalized response curves consistent with the lower response observed in the optimization plots (Figure 1). As noted above there is considerably more scatter ($r \sim 0.9$ or less) in the plots of response vs. ng BaP applied to clean filters due to the instability of BaP toward oxidative degradation when stored on that media.

TABLE II
ECD calibration curve for nitropyrene

Best-fit regression data				
Curve	Slope	*Y*-intercept	*r*	*N*
A	66.8	−0.8	0.989	9
B	54.9	−2.2	0.971	8
C	53.5	−1.7	0.987	10
D	49.3	0.7	0.978	20

A = Composite calibration data for 10/81, clean filters
B = Calibration data for 10/21/81, clean filters
C = Calibration data for 12/16/81, clean filters
D = Calibration data for 12/16/81, particulate-loaded filters

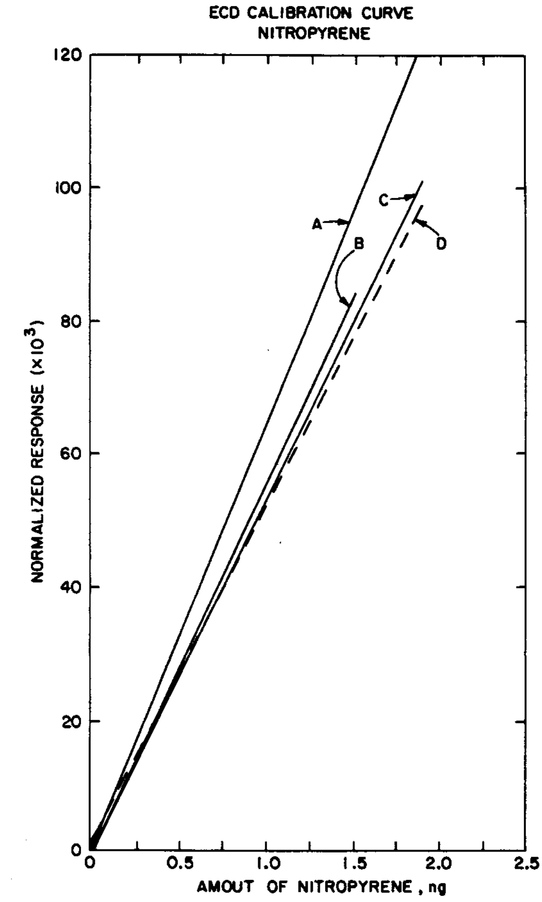

FIGURE 3 Calibration curve for nitropyrene obtained by nitration of pyrene applied to clean (curves A, B, C) and particulate-loaded (curve D) filters; response is peak-area-normalized to the internal standard (9-nitroanthracene). See Table II for regression statistics on these curves.

Detection limits, based on spiking ambient particulate samples with Py or BaP and nitrating with 25% HNO_3 followed by the normal extracting CGC–ECD analysis procedure, were about 20 and 60 pg, respectively, for Py and BaP when ~0.7 cm^2 of filter was analyzed.

Preliminary results from ambient samples

Ambient hi-vol samples were collected at the BNL site on a weekly basis and analyzed for Py, BaP and their nitro-derivatives by the procedures outlined. Only in a few cases were Py and BaP detected at near the detection limit ($\sim 1\,pg/m^3$) and no nitro-derivatives were detected by direct extraction. It is presumed that the long collection times and the clean nature of the BNL sampling site during most periods combined to reduce the amount of Py and BaP on the filter to negligible levels, principally by oxidative decomposition during sampling. In addition, the samples were not generally stored in the dark at low temperatures ($\leqq 20°C$), storage conditions that now appear to be required for retention of Py, BaP, and possibly their nitro-derivatives after formation/collection.

An additional set of ambient particulate samples was obtained from 3 urban sites and one rural site in New Jersey and were stored properly at $\leqq -20°C$ prior to analysis. The results from these samples were as follows: pyrene was tentatively identified by reaction on all of the urban and rural samples, and what is apparently 1-nitropyrene was identified by direct extraction in only 5 of 17 urban and 3 of 5 rural samples. Benzo(a)pyrene was identified by reaction in 7 of 17 urban samples and 1 of 5 rural samples; what is probably 6-nitro-BaP was identified by direct extraction in only 2 of 17 urban samples and was not found in 5 rural samples. Highest ambient levels of Py and BaP were in the $100\,pg/m^3$ range ($1\,pg = 10^{-12}\,g$). These levels are lower than usually found in ambient samples and additional studies to evaluate reaction efficiencies using the techniques described above are desirable.

Work is in progress to definitively identify the chromatographic peaks used for analysis of Py and BaP as the mononitro-derivatives using capillary GC–MS analysis. It is not essential for analytical purposes to identify the particular nitro isomers formed by means of the multiple MS techniques employed by Schuetzle *et al.*,[9] and Newton *et al.*[10] However, the preliminary identification of the analytical peaks as nitrogen compounds by GC with a nitrogen-sensitive FID will be confirmed by GC–MS prior to subsequent reports detailing ambient levels of Py, BaP and their nitro-derivatives.

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