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MICRODETERMINATION OF NITRATES AND NITRITES IN SALIVA, BLOOD, WATER, AND SUSPENDED PARTICULATES IN AIR BY GAS CHROMATOGRAPHY

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SUMMARY

A generally applicable method for the analysis of nitrates and nitrites has been used for a wide variety of samples, including human saliva, blood, drinking water, and airborne particulates. Aqueous nitrate ion is first converted to nitrobenzene by reaction with benzene (or another aromatic reactant) in the presence of a catalyst. The nitrobenzene is then quantitated by electron capture gas chromatography (GC–ECD). Nitrite ion and gaseous oxides of nitrogen can be determined also if such samples are treated appropriately prior to GC–ECD analysis. Other reactants such as 1,3,5-trimethoxybenzene can also be used in the place of benzene. The high sensitivity of ECD allows the quantitation of as little as 0.1 ppm (w/w) nitrate in a single drop of saliva or blood.

INTRODUCTION

It is well established that nitrites and secondary amines can react to form the highly carcinogenic nitrosamines, and that such reactions can occur under the conditions present in the human stomach[1]. For this reason the widespread use of nitrites and nitrates in meat processing is cause for concern; furthermore, a significant source of nitrites which find their way into the stomach is saliva itself[2]. Nitrate concentrations in human saliva are generally in the range of 5–60 ppm; and the presence of nitrate-reducing bacteria in the saliva causes nitrite to be formed, resulting in nitrite concentrations in the range of 6–10 ppm[2]. In view of the fact that the average person swallows approximately 1 l of saliva per day, these levels could be significant.

The presence of high concentrations of nitrate in drinking water can cause infant methemoglobinemia[3] and can also be correlated with high incidences of gastric cancer[4]. Furthermore, high levels of oxides of nitrogen in the ambient air in urban areas can be statistically correlated with high cancer rates; it is speculated that NO_x plays a role in the formation of atmospheric nitrosamines[5].

For these reasons and others, development of rapid, simple, and sensitive general techniques for the determination of nitrates and nitrites in a variety of matrices is essential. This paper details the extension and further development of the gas chro-

matographic (GC) technique for the analysis of nitrates that we and others have outlined in recent communications[6,7]. We have now refined and miniaturized the method so that determinations of sub-ppm levels of nitrate or nitrite can be performed with a single drop of saliva or blood. Furthermore, a sensitive method has been developed for measurement of nitrates and nitric acid present in and adsorbed on the surface of suspended particulates in ambient air.

EXPERIMENTAL

Apparatus

Chromatographic analyses were performed on Hewlett-Packard Models 402 and 5750 gas chromatographs, equipped with ^{63}Ni and titanium tritide electron capture detectors (ECD), respectively. The columns used contained 1.5% SE-30 coated on 80–100 mesh Chromosorb G, acid-washed, packed in either 76 cm × 4 mm I.D. glass tubing or 51 cm × 3 mm I.D. heavy-walled Teflon® tubing. Isothermal column temperatures between 100 and 150° were used for various experiments, and carrier gas flow-rates of 40–60 ml/min of argon–methane (90:10) were employed.

24-h airborne particulate samples were collected with a Precision Scientific Hi-Vol air sampler, which meets ASTM standard D2009. Specific ion electrode measurements were made using an Orion Model 93-07 nitrate ion electrode.

Reagents

Reagent grade sulfuric acid and thiophene-free reagent grade benzene were obtained from both Fisher Scientific (Pittsburgh, Pa., U.S.A.) and J. T. Baker (Phillipsburg, N.J., U.S.A.). Precautions noted in our previous paper with regard to sulfuric acid purity should be observed[7]. Both the benzene and the sulfuric acid were used without additional purification. Mallinckrodt (St. Louis, Mo., U.S.A.) Analytical Reagent grade 30% hydrogen peroxide, and nitrobenzene (Chem. Services, West Chester, Pa., U.S.A.) were employed. 1,3,5-Trimethoxybenzene (99%) (Aldrich, Milwaukee, Wisc., U.S.A.) was further purified by sublimation *in vacuo* at 40°. Reagent grade pentamethylbenzene was obtained from Eastman-Kodak (Rochester, N.Y., U.S.A.) and practical grade phenylmercuric acetate from Matheson, Coleman, and Bell (East Rutherford, N.J., U.S.A.). Potassium nitrate and nitrite, silver sulfate, sodium citrate, and urea were Analytical Reagent Chemicals from Mallinckrodt.

Other organic liquids were obtained from commercial sources and were distilled at least once prior to use. All other reagents were used without further purification.

Microanalysis procedure for nitrate and nitrite

The general procedure for microanalysis of nitrate and nitrite in aqueous media is as follows. For the determination of NO_3^-, a one-dram vial with a polyethylene stopper (Kimble No. 60975-L) was used as a reaction vessel. A 0.20-ml aliquot of aqueous sample was introduced into the vial, followed by 1.0 ml of benzene. The reaction was catalyzed by addition of 1.0 ml of concentrated sulfuric acid. The vial was then shaken for 10 min. The benzene layer was removed immediately from the reaction vial with a Pasteur pipette, placed in a separate vial, and analyzed by GC–ECD for the nitrobenzene concentration generated. Either 1- or 2-μl aliquots were injected. If the benzene is allowed to stand in contact with the acid for long periods, by-

products are formed and these appear in the chromatogram. Standard solutions of KNO_3 were treated in the same manner to generate a standard calibration plot relating nitrobenzene concentration to peak height. If higher precision is desired (approximately 4% relative standard deviation), 2,5-dimethylnitrobenzene can be added to the benzene prior to reaction as an internal standard (concentration 5×10^{-7} g/ml) to correct for differences in injection size, detector sensitivity, fluctuations, etc. When this internal standard was used, the peak heights of nitrobenzene were normalized to the peak heights of the standard before further data reduction.

Nitrite was determined by difference: a second identical sample was treated with an oxidant to convert NO_2^- to NO_3^-. Consequently, the concentration of nitrite plus nitrate is subsequently measured, so nitrite must be determined by difference. Specifically, the procedure used was to introduce 0.15 ml of the sample into the reaction vial, followed by addition of 0.05 ml of 0.1 *N* H_2O_2. The rest of the reaction procedure is the same as that for NO_3^-.

The general procedure outlined above, with appropriate modifications, was applied to the analysis of drop-sized biological samples. Analyses of drinking waters from several Colorado towns were also performed using the procedure above without modification. Because nitrate concentrations can be reduced by bacterial action, it is recommended that the drinking water samples be stabilized after collection by adding two drops of 1% phenylmercuric acetate per 100 ml of drinking water. As a demonstration of the efficacy of the GC–ECD method, identical drinking water samples were also analyzed for nitrate by standard ion-selective electrode procedures, described in the Orion instruction manual for the Model 93-07 nitrate ion electrode.

Saliva analysis

To analyze saliva for NO_3^-, 0.05 ml of 3×10^{-5} *M* phenylmercuric acetate was placed in a vial, which was then weighed. The subject expectorated directly into the vial and the vial was reweighed. The saliva was then diluted threefold with distilled water to obtain a more uniform sample and to lower the NO_3^- concentration to a range in which the ECD exhibits a more nearly linear response to the nitrobenzene generated. An aliquot (0.15 ml) of the saliva solution was then pipetted into the reaction vial, followed by 0.05 ml of saturated silver sulfate solution. The remainder of the procedure is identical to the general microtechnique described above. To analyze for NO_2^- plus NO_3^-, 0.05 ml of 0.1 *N* H_2O_2 was used in place of the silver sulfate above. By subtraction of the nitrate concentration determined without addition of H_2O_2, the nitrite concentration can be calculated.

Blood analysis

The following is a tentative method for the determination of nitrate in blood. A solution of 2% sodium citrate (0.05 ml) was placed in the bottom of a small test tube to prevent clotting of the blood. The test tube was then weighed. A drop of freshly drawn blood was placed directly into the test tube, and the test tube was reweighed. To the tube was added 0.1 ml of a 4% (w/v) $ZnSO_4$ solution followed by 0.5 g of solid Ag_2SO_4. The mixture was shaken well, and the tube was centrifuged for about 5 min. An aliquot (0.20 ml) of the supernatant liquid was transferred to the reaction vial and the general reaction procedure outlined above was followed.

Analysis for nitrate in airborne particulates

Particulate samples were collected on 20.3 × 25.4 cm glass fiber filter papers using a standard Hi-Vol air sampler. A cork borer (bore sizes Nos. 5–8) was used to cut out 0.90–1.72 cm^2 pieces of the glass fiber filter for NO_3^- analysis. This particulate "aliquot" was placed in a 125-ml glass-stoppered erlenmeyer flask, 2.0 ml of distilled water was added, followed by 10 ml of benzene and 10 ml of concentrated sulfuric acid and the flask was stoppered. The flask was shaken by hand one time (use eye protection), the stopper was carefully removed to relieve the pressure and then replaced. The flask was shaken for 10 min and the benzene layer was analyzed by chromatography by injection of 1- or 2-μl aliquots. For determination of nitrite plus nitrate, 2.0 ml of 0.1 *N* H_2O_2 should be used in place of the water above.

Use of organic reactants other than benzene

Other organic compounds such as toluene, *m*-xylene, *p*-xylene, anisole, 2,6-dimethylanisole, mesitylene, pentamethylbenzene, and 1,3,5-trimethoxybenzene (TMB) were also tested as possible reactants in preliminary experiments. Conditions were generally the same as those used with benzene, *i.e.*, 0.1–0.2 ml of sample was allowed to react with 1 ml of the organic compound and 1 ml of concentrated sulfuric acid (although in some cases less concentrated acid was used).

A suitable procedure for the determination of NO_3^- using TMB as a reactant has been developed. With this reactant a greater water:acid ratio can be used than with benzene; consequently, a larger aqueous sample can be taken, improving the effective sensitivity of the method. Equal volumes (0.5 ml) of aqueous sample and concentrated sulfuric acid were mixed in a 1-dram glass reaction vial and allowed to cool. TMB (0.1 g) was added and the vial held at 67° for 10 min in a bath. The vial was then shaken for 5 min while it cooled. One milliliter of benzene was added, the vial was shaken again for 5 min, and the benzene layer was drawn off with a pipette and analyzed by chromatography for the concentration of 1-nitro-2,4,6-trimethoxybenzene (NTMB). The identity of the NTMB peak was confirmed by comparison of retention times with a standard prepared according to Fukui *et al.*[8] and by gas chromatography–mass spectrometry. Under these conditions, none of the benzene used as an extractant is converted to nitrobenzene. Standard KNO_3 solutions treated in the same manner were used to generate a calibration plot.

RESULTS AND DISCUSSION

Microtechnique studies

The amount of benzene used for the microtechnique (1 ml) was chosen as being about the minimum amount that can be easily handled for separation and subsequent GC analysis. Although it must be carefully measured for quantitation, this amount is not a critical factor in the yield of nitrobenzene. The water:sulfuric acid ratio in the reaction is more important. Experiments in which the reaction yield was studied as a function of this ratio demonstrated the ratio 0.2 ml of water to 1.0 ml of sulfuric acid to be optimum. When volumes of water greater than 0.2 ml are used, the increased heat of mixing with the acid produces undesirable benzene pressure buildup inside the reaction vials. Although quantitative conversion of nitrate to nitrobenzene

can be obtained when water volumes up to 0.5 ml are used, it is necessary to secure the vial caps with tape to prevent them from being blown off.

It should be noted here that new, unused vials must be well cleaned prior to use. A suitable simple cleaning method is to put the vials through the same reaction procedure to be utilized later, substituting distilled water for the samples. The vials were then rinsed sequentially with hot water, distilled water, acetone, and distilled water, before introducing the samples to be analyzed.

The results of applying the microtechnique to the analysis of nitrate in several samples of drinking waters are summarized in Table I. In no case were detectable amounts of NO_2^- found. (The limit of detection for NO_2^- depends on the NO_3^- concentration, since NO_2^- is determined by difference. Generally a NO_2^- concentration which is 5% of the NO_3^- concentration can be detected.) In Table I the results are shown of a comparison study of the GC–ECD microtechnique *vs.* ion-selective electrode measurements (ISE). The close agreement (usually within 5%) of the totally independent methods used for the analysis of identical samples is an excellent demonstration of the efficacy of the methods. No significant interferences (such as high levels of chloride) were found in the drinking water samples. The EPA maximum allowable NO_3^- concentration in bottled drinking water is 45 ppm (w/v)[9].

TABLE I

COMPARISON OF ANALYSES OF DRINKING WATERS FOR NO_3^- CONCENTRATION BY GC–ECD AND ISE

Numerical designations refer to selected municipal water supplies in Crowley County, Colo., U.S.A. Unless otherwise specified, concentrations reported in this paper are expressed in ppm (w/w). Abbreviations: GC–ECD = electron capture gas chromatography; ISE = ion-selective electrode measurement.

Site	*Concentration NO_3^- (ppm)*	
	GC–ECD	*ISE*
Boulder, Colo.	0.40	—
TW0011	44	48
TW0012	42	46
TW0013	51	52
TW0014	16	16
TW0015	35	36
TW0016	12	13

The analysis of saliva and blood is complicated by the presence of known interferences, primarily chloride and thiocyanate ions, as well as possible interferences arising from the presence of peptides, proteins, and other nitrogen-containing compounds. Incidentally, high levels of chloride also interfere seriously with measurement of nitrate by ISE. Human saliva has been found to contain as much as 280 ppm (w/v) SCN^- and 630 ppm Cl^-, while blood serum may have as much as 3800 ppm Cl^-[10,11]. Both Cl^- and SCN^- can be removed by precipitation with Ag^+ prior to addition of the sulfuric acid. Analyses of saliva without this prior Ag^+ treatment gave NO_3^- values 15–30% lower than values obtained otherwise. Addition of quantities of Ag^+ in excess of that specified in Experimental did not affect the final result. Fig. 1

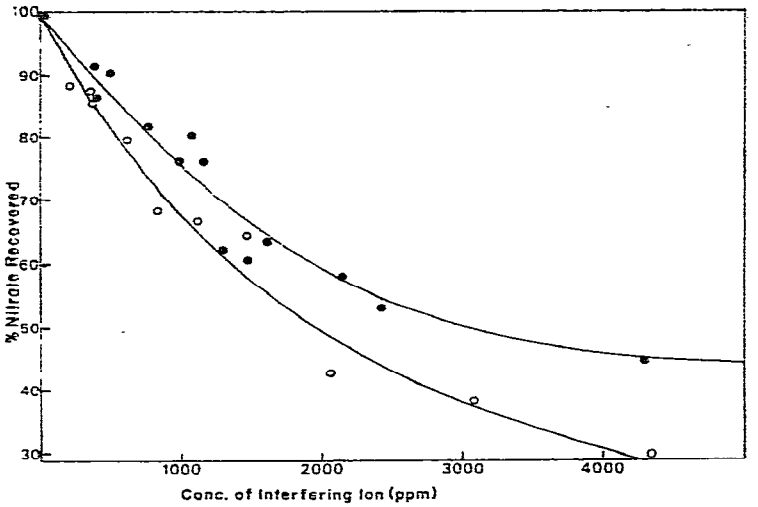

Fig. 1. Effect of interfering ions on the measurement of nitrate at the 2-ppm concentration level. The open circles are for the thiocyanate ion, the closed circles for the chloride ion.

is a plot of interfering ion concentration *vs.* NO_3^- recovery; this shows that appreciable concentrations of Cl^- and SCN^- will cause interference. The fact that this interference is irregular in nature precludes the use of a numerical correction.

Hydrogen peroxide has been demonstrated to inhibit the action of these interferences. Table II shows the results of treating solutions of known NO_3^- concentrations and known Cl^- concentrations with H_2O_2. Addition of H_2O_2 also prevents interference by SCN^-. For this reason the Ag^+ treatment is unnecessary in NO_2^- determinations. The fact that H_2O_2 alone or in conjunction with Cl^- does not produce a positive interference is also shown by the data in Table II obtained when no nitrate was present.

Other possible interferences were tested at the 1% (w/v) level in a 2-ppm standard solution of nitrate and found to produce less than 10% error in the value determined. These were: $ZnSO_4$, KF, Na_2CO_3, and sodium citrate.

The following were found not to interfere when present in quantities sufficient

TABLE II

EFFECT OF ADDITION OF HYDROGEN PEROXIDE IN REDUCING INTERFERENCE BY CHLORIDE

Cl^- concentration (ppm)	*H_2O_2 concentration (N)*	*NO_3^- concentration (ppm)*	
		Expected	*Found*
0	0	2.5	2.5
3000	0	2.5	1.2
3000	0.012	2.5	2.5
3000	0	0	0
0	0.012	0	0
3000	0.012	0	0

to produce saturated solutions: aspartic acid, glutamic acid, phenylalanine, and Ag_2SO_4. Tyrosine and tryptophan, when present at concentrations of 1000 ppm as free amino acids, appear to interfere with the nitrate determination.

In order to determine whether appreciable amounts of nitrobenzene are formed from nitrite in the absence of an oxidizing agent, the following test was made. A standard solution of KNO_2 containing 17.7 ppm of nitrite was subjected to the procedure for the analysis of nitrate. The benzene layer, which contained some nitrobenzene apparently resulting from nitrate impurities, was discarded and a fresh aliquot of benzene was added to the acidic nitrite solution. The solution was heated to simulate the heat arising from mixing the sulfuric acid with water, which would normally be generated, and the benzene layer was analyzed for nitrobenzene. No appreciable quantities of nitrobenzene were found; consequently, one may conclude that nitrite is not converted to nitrobenzene in the absence of an oxidant under the conditions specified in our method.

In the absence of a preservative, NO_3^- in saliva is rapidly reduced by microbial[2] and enzymatic activities[12]. Therefore, the saliva should be treated with a preservative immediately after collection. The NO_3^- concentrations in untreated samples were 90% lower after 2 h. Fig. 2a shows the chromatogram obtained from the analysis of a saliva sample. The absence of extraneous peaks is noteworthy. Table III shows some typical results. The GC–ECD method for saliva analysis is quite simple and was performed as a routine experiment by our undergraduate students.

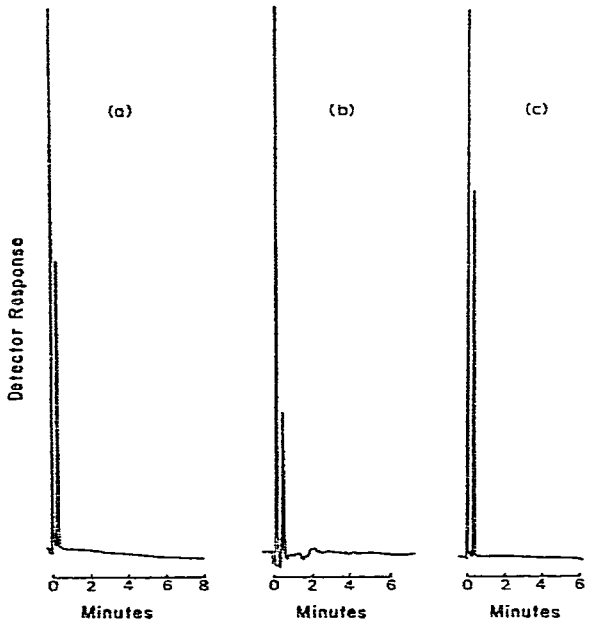

Fig. 2. (a) Chromatogram obtained from the analysis of 0.05 g of saliva containing 12 ppm NO_3^- (w/w). Attenuation, × 8. (b) Chromatogram obtained from the analysis of 0.1 ml of whole blood containing 1.8 ppm NO_3^- (w/w). Attenuation, × 4. (c) Chromatogram obtained from the analysis of particulate matter contained on a 1.45 cm^2 section of glass fiber filter. The suspended particulate nitrate concentration was found to be 0.8 $\mu g/m^3$. In each chromatogram the nitrobenzene generates the second peak.

TABLE III

TYPICAL NO_3^- AND NO_2^- CONCENTRATION LEVELS IN SALIVA AS DETERMINED BY GC–ECD

Subject	*NO_3^- concentration (ppm)*	*NO_2^- concentration (ppm)*
L. J.	15	<0.7
A. N.	12	4.6
R. S.	15	3.7
B. E.	12	12
P. L.	53	21
J. T.	46	5.0
S. S.	12	11
R. N.	7.9	1.5
J. C.	5.2	<0.2
C. H.	2.3	0.9
P. G.	12	6.0
A. R.	8.1	4.7
C. D.	16	9.5

Fig. 2b shows the chromatogram obtained from the analysis of a whole blood sample. The high chloride concentration in blood causes this matrix to be more difficult to analyze than drinking water. Also there is the possibility of other, as yet unidentified, interferences. The clean baseline shown in Fig. 2b suggests the utility of this method in the simple and rapid microanalysis of blood for nitrate concentration. These experiments demonstrate the feasibility of using this technique in the analysis of small whole blood samples obtained from finger pricks.

Airborne particulate analysis

The method used for the analysis of nitrates in airborne particulates is an extension and modification of the general method we previously reported[7]. The optimum amount of water used in the reaction was found in the present work to be 2 ml. The uniformity of collection on the 20 × 25 cm filter was checked by analyzing five different 1.45 cm^2 sections of a suspended particulate filter sample; results are shown in

TABLE IV

UNIFORMITY OF NO_3^- DISTRIBUTION ON A GLASS FIBER FILTER ON WHICH SUSPENDED PARTICULATES HAD BEEN COLLECTED

The size of the entire glass filter was 20 × 25 cm; individual samples taken for analysis from the filter measured 1.45 cm^2 (circular pieces 1.36 cm in diameter).

Position on filter analyzed	*NO_3^- concentration ($\mu g/m^3$ of air collected)*
Center	0.49
Front edge	0.43
Back edge	0.48
Front corner	0.48
Back corner	0.42
Mean	0.46
S.D.	± 0.03

TABLE V

TYPICAL NO_3^- CONCENTRATION LEVELS IN SUSPENDED PARTICULATE MATTER AS DETERMINED BY GC–ECD

Date	*Concentration of total suspended particulates in air ($\mu g/m^3$)**	*Concentration of particulate NO_3^- in air ($\mu g/m^3$)*	*% (w/w) NO_3^- in particulates*
3-26-76	22.7	0.81	3.6
3-27-76	32.6	1.19	3.6
3-28-76	10.5	0.43	4.1
4-01-76	67.4	1.35	2.0
4-12-76	82.0	2.10	2.2
4-26-76	79.0	1.14	1.4
4-27-76	16.8	0.88	5.2
5-05-76	32.7	0.82	2.5

* Sampling station located at the University of Colorado, Boulder, Colo., U.S.A.

Table IV. Table V shows some typical data for airborne particulates while Fig. 2c shows a representative chromatogram, and Fig. 3 shows preparation of the sections.

Values previously reported for total suspended particulates in the Denver metropolitan area are in the range of 11–283 $\mu g/m^3$. The arithmetic mean of total suspended particulates in Boulder in 1967 was 49 $\mu g/m^3$ while the downtown Denver mean was 105 $\mu g/m^3$. Nitrate levels in the suspended particulates in the Denver metropolitan area were 0.4–5.8 $\mu g/m^3$ with a mean of 1.7 $\mu g/m^3$ (ref. 13).

As a check for possible interferences in the particulate analysis an 11 × 3 cm section was cut from a sampled filter and extracted according to the established EPA procedure[13]. The extract was then analyzed for NO_3^- using both the general microtechnique presented here and the nitrate ISE. Values obtained were 0.99 and 0.97 $\mu g/m^3$, respectively. These values compare well with the value of 0.94 $\mu g/m^3$ obtained from a 0.90 cm^2 section of the same filter analyzed according to the particulate procedure presented above in Experimental. NO_x can also be determined by GC–ECD[7].

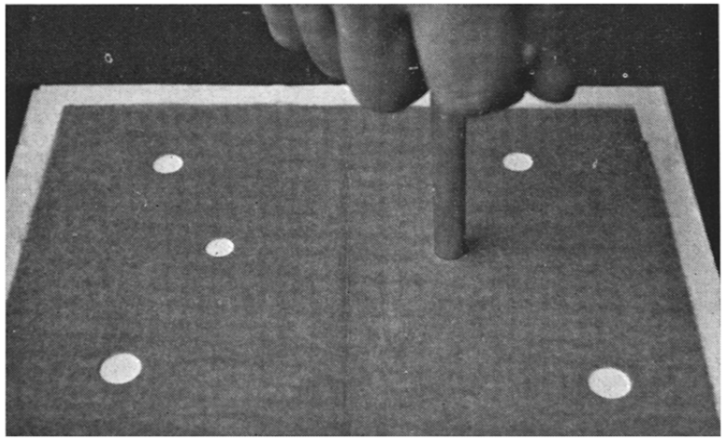

Fig. 3. Preparation of sections of glass fiber filters containing particulate samples from air prior to analysis for nitrate.

Use of trimethoxybenzene as a reactant

The advantages of benzene as a reactant are that chromatographically pure benzene is readily available and relatively inexpensive. Other reactants may be somewhat more reactive, but in general they are difficult to obtain in acceptable purity (and even difficult to purify), more expensive, and usually more susceptible to acid attack. Benzene, on the other hand, is toxic and is a volatile and flammable liquid. The volatility of benzene results in pressure buildup inside the reaction vessels due to the heat generated by the mixing of the aqueous nitrate sample and the acid, which causes one to take extra precautions.

One of the goals of this research was to find a reactant that would be more reactive than benzene so that more dilute final solutions of sulfuric acid would still be effective in catalyzing nitration. Of the other possible organic reactants considered, toluene, anisole, 2,6-dimethylanisole, *m*-xylene, *tert.*-butyl benzene, and cymene gave more than one reaction product, and no apparent advantage in reactivity was observed. Preliminary experiments with mesitylene, pentamethylbenzene, and TMB showed that the latter two compounds merited further investigation.

Of these two, pentamethylbenzene is more stable to heat and strong acid while TMB reacts better at lower acid concentrations. Both form mononitro derivatives which are easily chromatographed at 170° (see Experimental for other conditions). TMB has been more extensively studied to date, but both are under consideration as possible substitutes for benzene.

Figs. 4 and 5 are the results of the optimization of conditions in the TMB nitration reaction, using aqueous standard samples containing 2 ppm NO_3^-. The optimum acid concentration (after dilution with the aqueous NO_3^- sample) was found to be 50–60% (Fig. 4). Fig. 5 shows that the optimum reaction temperature is *ca.* 67°; at higher temperatures, undesirable pressure buildups occur in the vials. The narrow usable range of acid concentration displayed in Fig. 4 is striking; apparently, at higher

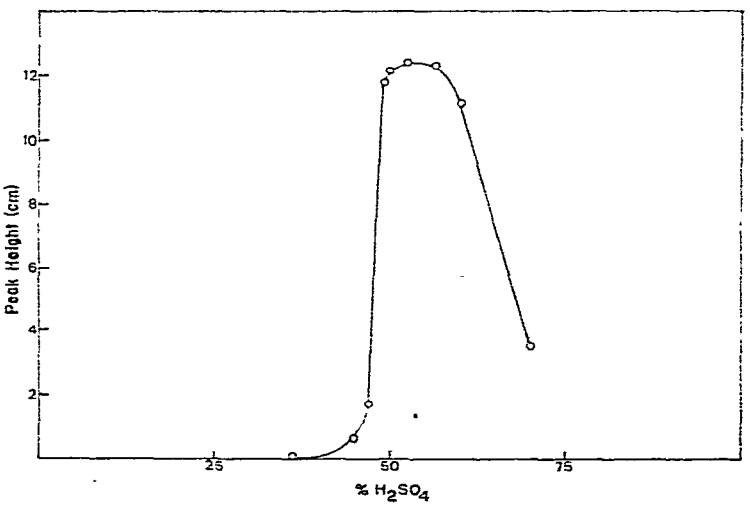

Fig. 4. Effect of the sulfuric acid concentration on the conversion of NO_3^- to NTMB. The NO_3^- concentration was 2 ppm.

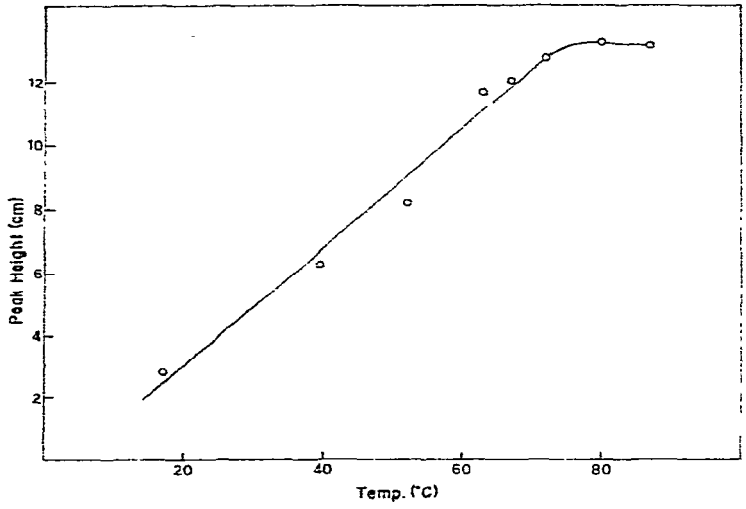

Fig. 5. Effect of the reaction temperature on the conversion of NO_3^- to NTMB. The NO_3^- concentration was 2 ppm. Each reaction was allowed to proceed for 10 min at the given temperature.

acid concentrations the yield is reduced by hydrolysis of TMB. The use of less than 0.1 g of TMB with 0.5 ml of aqueous sample and 0.5 ml of sulfuric acid results in incomplete reaction.

Table VI shows that the conversion efficiency of aqueous nitrate to NTMB is 81 $\pm$ 3%. The efficiency of the extraction of the TMB nitration product from the aqueous reaction mixture by shaking with benzene was found to be essentially quantitative (99%). One-milliliter aliquots of 1.06×10^{-6} g/ml and 2.57×10^{-7} g/ml of NTMB in benzene were each separately shaken with 1-ml portions of 50% sulfuric acid for 5 min, and in both cases 99% of the NTMB was determined to be still present in the benzene layer. Fig. 6 shows the chromatogram obtained using TMB as a reactant, with a 1-ppm NO_3^- sample.

The identity of the NTMB peak was confirmed by injection of a sample of independently synthesized NTMB and comparison of retention times. The last compound to be eluted in Fig. 6 is NTMB, while the second peak arises from much larger quantities of the reactant TMB, which has a lower ECD cross-section.

TABLE VI

EFFICIENCIES OF CONVERSION OF NO_3^- TO NTMB

NO_3^- concentration (ppm)*	*μg NTMB product*		*% Conversion*
	Theoretical	*Found*	
0.2	0.69	0.57	83
0.4	1.37	1.12	82
0.6	2.06	1.59	77

* The final concentration of sulfuric acid was 50% rather than 70% as in the experiments with benzene as reactant.

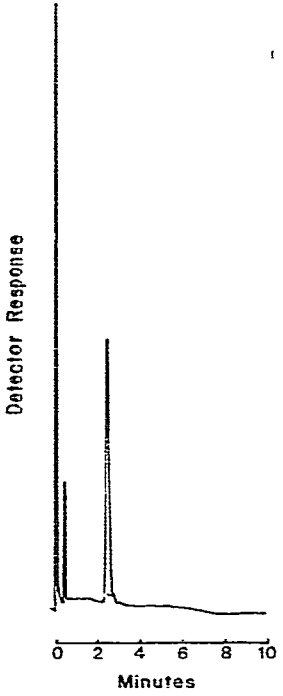

Fig. 6. Reaction products of NO_3^- with TMB. A 0.05-ml aliquot of 1 ppm NO_3^- was allowed to react.

CONCLUSION

A general microanalysis technique has been developed for the analysis of nitrate and nitrite in as little as one drop of sample, with a detection limit of 0.1 ppm NO_3^-. This technique, with slight modifications, has been applied successfully to the analysis of drinking water and biological fluids such as human saliva and whole blood, engendering the possibility of analyzing for nitrate and nitrite in blood samples obtained from small finger prick samples.

Suspended particulate samples collected from ambient air in Boulder, Colo. have been analyzed successfully for nitrates. The procedure presented here requires much less time than the EPA method, which involves an extraction step. Agreement between the EPA and our GC–ECD methods was found to be excellent.

TMB can be used as a suitable replacement for benzene as a reactant in the determination of trace levels of nitrate.

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