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ANALYSIS FOR AQUEOUS NITRATES AND NITRITES AND GASEOUS OXIDES OF NITROGEN BY ELECTRON CAPTURE GAS CHROMATOGRAPHY

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SUMMARY

A highly sensitive gas chromatographic method is presented for the determination of aqueous nitrates. By suitable chemical pretreatment, this method is also applicable to the analysis of aqueous nitrites and gaseous oxides of nitrogen. The method has been applied to the analysis of drinking water, urine, and nitric acid. The technique involves the conversion of the nitrate ion to nitrobenzene with subsequent analysis by electron capture gas chromatography. The detection limit for nitrobenzene is *ca.* 10^{-12} g, making possible the analysis of environmentally significant concentrations of aqueous nitrates and nitrites and gaseous oxides of nitrogen.
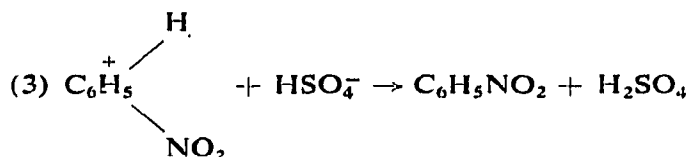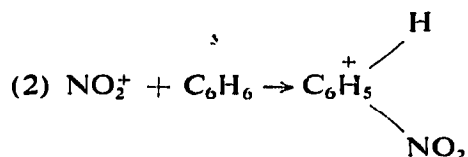
INTRODUCTION

Nitrogen oxide gases and aqueous soluble nitrites and nitrates are all of primary environmental concern because of their toxicity and reactivity as well as the synergistic effects they exhibit with other compounds. Several methods of analysis have been developed and are presently being evaluated by the Environmental Protection Agency[1-4].

In an earlier preliminary report[5] we described a gas chromatographic (GC) method of analysis which showed promise for gaseous oxides of nitrogen (nitrogen dioxide and nitric oxide) as well as aqueous soluble nitrate, nitrite, and nitric acid. Glover and Hoffsommer[6] have published a note on the analysis of aqueous standard potassium nitrate and potassium nitrite.

The analyses are based on the nitration of an aromatic compound, *e.g.*, benzene, which —in the presence of concentrated sulfuric acid— forms nitrobenzene. The reagents used in the Saltzman and Hocheiser methods form relatively unstable diazo·

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dyes, and analyses must be performed soon after absorption. In contrast, nitrobenzene is relatively stable provided it is separated from the acid layer. Therefore, field samples can be collected and treated to form nitrobenzene and then shipped to an analytical laboratory for immediate analysis or stored if necessary until the analysis can be performed. The commonly accepted mechanism of nitration with a mixture of nitric and sulfuric acids involves the following sequence of reactions:

(1) $HONO_2 + 2H_2SO_4 \rightleftharpoons H_3O^+ + 2HSO_4^- + NO_2^+$

(2) $NO_2^+ + C_6H_6 \rightarrow C_6H_5 \overset{+}{\underset{NO_2}{\overset{H}{<}}}$

(3) $C_6H_5 \overset{+}{\underset{NO_2}{\overset{H}{<}}} + HSO_4^- \rightarrow C_6H_5NO_2 + H_2SO_4$

The resulting nitrobenzene can be detected with great sensitivity by the electron capture detector (ECD), and determinations can be made with excellent selectivity even at very low concentrations.

EXPERIMENTAL

Reagents

   Fisher Scientific (Pittsburgh, Pa., U.S.A.) and Allied Chemical (Morristown, N.J., U.S.A.) A.C.S. reagent grade sulfuric acid with nominal analysis specifications of 0.00005 % and 0.00002 % nitrate, respectively, were used. It was necessary to further treat the sulfuric acid with benzene to remove additional nitrate for ultratrace analysis. Furthermore, we observed that different lots of sulfuric acid varied considerably in nitrate impurity content. For analysis of low levels of nitrate, it is desirable to check the blank levels in the available bottles of sulfuric acid and select the one with the lowest impurity level. This is not necessary when measuring higher concentrations of nitrate. One should attempt to keep exposure of the sulfuric acid to the atmosphere minimal to prevent contamination from atmospheric NO_x. Mallinckrodt (St. Louis, Mo., U.S.A.) Nanograde benzene and 0.1 *M* hydrogen peroxide, prepared by dilution of 30 % Fisher A.C.S. Certified reagent grade hydrogen peroxide, were used. In experiments at low concentration levels at which high blanks cannot be tolerated, the benzene was purified by fractional distillation to remove traces of nitrobenzene and other electron capturing impurities. Nitrobenzene (Chem Services, West Chester, Pa., U.S.A.) and National Bureau of Standards nitrogen dioxide (Standard Reference Material 1629) were employed.

   Additional reagents for the analysis of nitrate, nitrite, and nitric acid samples are potassium nitrate (Fisher, Certified reagent grade), potassium nitrite (Matheson, Coleman & Bell, East Rutherford, N.J., U.S.A.; Reagent Grade), and nitric acid (Mallinckrodt, A.C.S. reagent grade).

Standard solutions

Nitrobenzene standard solutions were made by dissolving weighed amounts of nitrobenzene in benzene to produce the following concentrations: 2.50×10^{-7}, 5.00×10^{-7}, 1.00×10^{-6}, 5.00×10^{-6}, and 1.00×10^{-5} g/ml. Standard aqueous solutions were prepared with triply distilled and deionized water at the following concentrations: potassium nitrate, 2.50×10^{-6}, 5.00×10^{-6}, 1.00×10^{-5}, and 2.50×10^{-5} g/ml; potassium nitrite, 1.00×10^{-5} and 5.00×10^{-5} g/ml; nitric acid, 5.00×10^{-6} and 2.5×10^{-5} g/ml.

Chromatographic conditions

The gas chromatographs used were Hewlett-Packard Models 402, 810 and 5713, equipped with ECDs (^{63}Ni or titanium tritide). The chromatographic columns were 1 m $\times$ 3 mm I.D. glass or PTFE packed with 3.8 % Union Carbide W98 silicone on 80–100 mesh Diatoport S. Isothermal column and injection port temperatures in various experiments ranged from 100 to 120°. The argon–methane (90:10) carrier gas flow-rate was 60 ml/min.

Analytical procedure for aqueous nitrate and nitrite

A 1-ml aliquot of the sample is added to 10.00 ml of benzene in a 125-ml erlenmeyer flask with a 24/40 standard taper ground-glass neck. For nitrite ion analyses, 1 ml of 0.1 M hydrogen peroxide is added to convert the nitrite to nitrate. A 10-ml volume of concentrated sulfuric acid is added. The reaction flask is sealed with a ground-glass stopper, shaken briefly by hand, and the pressure is relieved by slightly loosening the stopper. The stopper is replaced and secured, and the flask is placed on a wrist action shaker for 10 min. The benzene layer is decanted and a 1-ml aliquot is injected into the gas chromatograph for analysis. The peak height of the nitrobenzene produced from the sample is compared with that of standard nitrobenzene solutions which have been subjected to the identical analysis procedure (see Calibration procedures).

Standard. gas permeation train

Experiments were also performed with the goal of adapting this analytical method to the determination of oxides of nitrogen in air. Fig. 1 is an illustration of the standard gas permeation train used to produce known concentrations of nitrogen dioxide to study the efficiency of conversion of nitrogen dioxide to nitrobenzene. An individually calibrated nitrogen dioxide permeation device (NBS Standard Reference Material 1629) was used to provide a constant source of nitrogen dioxide. This device was.specifically designed for the standardization of apparatus and procedures used in air pollution and related chemical analyses. The certified permeation rate at 30° was $1.31 \pm 0.05 \mu$g of nitrogen dioxide per minute. The flow-rate of dry nitrogen diluent (Airco prepurified) was adjusted to produce nitrogen dioxide concentrations ranging from 0.1 to 10 ppm (v/v).

Nitrogen oxide gas absorption and reaction system

Fig. 1 also illustrates the absorption and reaction vessel (K) for nitrogen oxide gases. The vessel is a standard three-necked (24/40 taper ground-glass) 1-l flask, the volume of which has been accurately determined. Necks a and c are each fitted with

a ground-glass adapter and PTFE.stopcock leading to a section of 1/4-in. O.D. glass tubing. The center port b is fitted with an adapter reduced to 1/4-in. straight glass tubing, which is sealed with a small silicone rubber stopper. Reagents are added to the system by removing this stopper.

The absorption reaction vessel is carefully cleaned and dried, then purged with dry nitrogen via necks a and c while heating with a heat gun. The reaction vessel is then attached to the permeation train and the calibrated gas is passed through the vessel until purging is complete. Entrance and exit stopcocks are simultaneously closed. The gas is then analyzed according to the described procedure, and the concentration of nitrobenzene generated is compared with the theoretical value based on the calculated output of the permeation device.
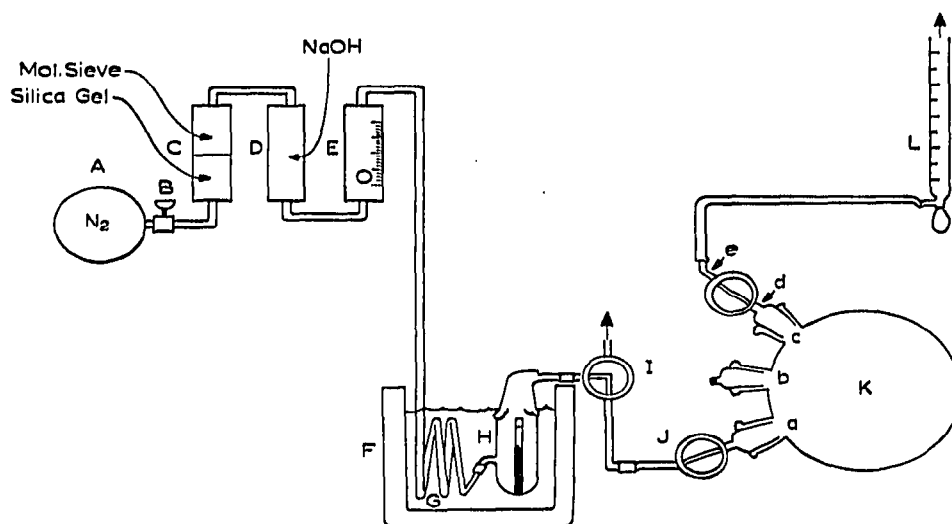

Fig. 1. Schematic drawing of the standard gas permeation train, and the absorption and reaction vessel.

Analytical procedure for nitrogen dioxide

The calibrated reaction flask (Fig. 1) is cleaned, purged with dry nitrogen via necks a and c and heated to remove adsorbed gases and water. Clamps are attached to secure the ground glass fitting in each neck. The vessel is attached to the permeation train and purged to obtain a standard sample. (After calibration, unknown gas samples can be substituted at this point in the procedure.) The small silicone stopper is removed from the opening in neck b and 25 ml of the absorbing solution used in EPA method 7 (ref. 4) is quickly added. (The absorbing solution is prepared by adding 2.8 ml of concentrated sulfuric acid to 1 l of water; the mixture is stirred and 6 ml of 3 % hydrogen peroxide is added.) The silicone rubber stopper is replaced and the flask is briefly shaken by hand, then placed on a wrist action shaker for 1 h. The absorbing solution is transferred to a 30-ml sample vial and an appropriate aliquot (2–10 ml) is taken by pipette and placed in a 125-ml erlenmeyer flask (with a 24/40 ground glass stopper) containing 10.00 ml of benzene. This absorbing solution aliquot is subjected to the procedure for aqueous nitrate described above starting with the addition of sulfuric acid. The amount of concentrated sulfuric acid added should be five

times the volume of the absorbing solution aliquot in order to insure that the final acidity is sufficiently high to cause quantitative conversion to nitrobenzene*.

RESULTS AND DISCUSSION

The high sensitivity and selectivity of GC–ECD make it an attractive method for determining very low concentrations of compounds which are susceptible to the method. The non-volatility of nitrates and nitrites, the high reactivity of nitrogen di oxide, and the low ECD sensitivity of nitrogen oxide prohibit the use of this method for the direct analysis of these species at low concentrations. However, the conversion of these species to a compound which is stable, volatile, and sensitive to electron capture detection could provide the basis for a valuable analytical method.

Such a procedure offers several important possibilities: (i) The conversion of highly reactive species such as nitrogen dioxide to a more stable compound which can be stored and/or shipped before analysis. (ii) The ability to do many different species (NO_2^-, NO_3^-, NO_2, NO, etc.) using the same instrumentation (i.e., GC–ECD) and, with minor modifications, the same analytical procedures. In addition, other environmentally significant compounds such as peroxyacyl nitrates can also be determined using the same instrumentation. (iii) The recognized ECD sensitivity promises the capability of measuring typical environmental levels of these compounds. Furthermore, since the lower limit of detectability is ca. 10^{-12} g, only very small biological samples are required for analysis. Consequently, we have investigated a method for converting these species to nitrobenzene, which is subsequently measured by GC–ECD.
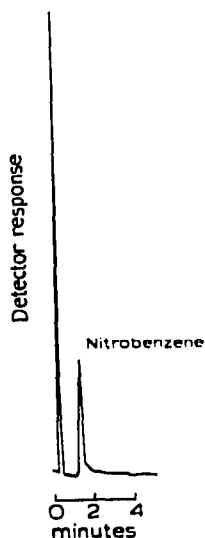

Fig. 2. Chromatogram of a standard solution of nitrobenzene in benzene. Sample, 1 μl of a solution containing 5.0 × 10^{-7} g nitrobenzene per milliliter of benzene.

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* *Caution*: Excessive heat may be generated when large volumes of sulfuric acid are used. Adequate safety precautions should be taken and sample loss must be prevented.

Analysis for aqueous nitrates and nitrites

Experiments were designed to evaluate the reactivity of benzene with nitrate and of the resulting nitrobenzene in the analytical procedure. Factors studied included efficiency of reaction, solubility of nitrobenzene in benzene, solvent extraction efficiencies, and the efficiency of oxidation of nitrite-containing species. Calibration studies were then made in preparation for the analysis of unknown samples of nitrate in urine and tap water.

Gas chromatographic properties of nitrobenzene

Nitrobenzene has been found to be very sensitive to electron capture detection by other investigators[7]. The detection limit was determined in our study to be *ca.* 10^{-12} g for a 1-μl sample using the conditions previously described. Fig. 2 is a chromatogram of a standard solution of nitrobenzene in benzene. The peak was produced by the injection of 1 μl of benzene containing 5.0×10^{-7} g of nitrobenzene per milliliter.

The chromatograms are remarkably free from interfering peaks (see Figs. 2 and 3). At low concentration levels, small peaks sometimes appear at retention times considerably longer than that of nitrobenzene. These peaks only occur in samples which have been treated with sulfuric acid. The major impurity was tentatively identified by nuclear magnetic resonance as benzenesulfonic acid. These peaks can be minimized or eliminated by periodic special cleaning of the syringe needle. Normally, the syringe is rinsed with benzene after each injection. Periodically it is rinsed with the following sequence of solvents: benzene, acetone, water, acetone, benzene.

Solubility studies

When benzene and sulfuric acid are shaken together with nitrobenzene, the nitrobenzene becomes distributed between the two layers and serious errors can result if this is not taken into account. For example, 25% of the nitrobenzene is lost in the acid layer when 10 ml of a solution containing 2.5×10^{-7} g nitrobenzene per milliliter of benzene was shaken with 10 ml of conc. sulfuric acid plus 1 ml of water. In this experiment usual sample reaction conditions were duplicated. The same percentage was lost in the acid layer when the initial concentration was 1.0×10^{-5} g/ml and at intermediate concentrations as well. Consequently, it is necessary to correct for this loss in the calibration procedure.

Calibration procedures

Three methods of calibration were investigated. If a calibration curve determined from standard solutions of nitrobenzene in benzene is used directly, errors result due to the partial solubility of nitrobenzene in the aqueous phase of the reaction mixture. However, a calibration curve determined with nitrobenzene in benzene standards which have undergone the same reaction procedure as unknowns, including the incorporation of a blank (1 ml of distilled, deionized water), accurately reflects the same reaction parameters as the samples experience. In this manner the consistent loss of nitrobenzene to aqueous solubility (25%) and the increase in signal due to reagent and glassware blanks are automatically corrected. For most determinations, this second method of calibration appears to be most suitable.

In the third method, sequential external standards can be used if the

TABLE I

EFFICIENCIES OF CONVERSION TO NITROBENZENE

Compound	Concentration (g/ml)	Average conversion* (%)	Mean**
KNO_3	2.5×10^{-6}	87.6 ± 8.8	
	5.0×10^{-6}	90.4 ± 9.2	
	1.0×10^{-5}	86.7 ± 3.7	90.3 ± 7.9
	2.5×10^{-5}	96.4 ± 6.8	
KNO_2	1.0×10^{-5}	103.4 ± 3.9	
	5.0×10^{-5}	97.3 ± 1.3	100.4 ± 4.2
HNO_3	0.5×10^{-5}	97.0 ± 4.6	
	2.5×10^{-5}	102.9 ± 4.4	99.9 ± 5.2

* Mean of five reactions. Standard deviation of 25 chromatographic determinations (five aqueous sample aliquots, five injections from the reaction of each).
** Calculated using all % conversion values (i.e., five values for each concentration).

detector is less stable than desired, e.g., when detector sensitivity is lowered by successive injections of samples which contaminate the detector cell. The procedure involves bracketing the nitrobenzene peak of the unknown samples with slightly higher and lower concentrations of standard nitrobenzene in a repetitive sequence, and determining the concentration from a two-point calibration. For this third calibration method, additional corrections for reagent and glassware blanks, extraction efficiency and reaction efficiency must be made. A fourth method of calibration is still being evaluated. Standard solutions of potassium nitrate in water are subjected to the same treatment as unknowns and the results are compared. Glover and Hoffsommer[6] used yet another method of calibration, viz., internal calibration with added nitrotoluene. However, this method has the disadvantage that losses of nitrobenzene to the acid layer are harder to assess.

Efficiency of reactions

The efficiencies of conversion of nitrate, nitrite and nitric acid to nitrobenzene were determined by the analyses of 1- ml aliquots of standard KNO_3, KNO_2, and HNO_3 solutions. The analyses were performed by the previously described procedures for aqueous nitrates and nitrites. The results of this study are contained in Table I. Mean efficiencies of conversion are 90.3% for KNO_3, 99.9% for HNO_3, and 100.4% for KNO_2.

By adding the reagents in the concentrations and order described in Experimental, it is possible to take advantage of the heat generated upon mixing to effect quantitative conversion without the addition of external heat.

Analysis for nitrate in urine and drinking water

An evaluation of the procedure for nitrate determination was made on two samples: human urine and drinking water from a city water supply. Five samples of each type were analyzed. The samples were taken over a period of one month, i.e., each value in Table II represents the nitrate level on a different day. It was found that the nitrate levels were relatively constant over a 1-month period, both in the urine

TABLE II

ANALYSIS FOR NITRATE IN DRINKING WATER AND URINE

	Concentration NO₃⁻ (ppm)	Standard deviation*
Drinking water	1.29	±0.04
	1.20	±0.09
	1.29	±0.14
	1.15	±0.08
	0.88	±0.07
Human urine	33.3	±4.5
	37.8	±2.1
	37.3	±2.4
	30.2	±2.8
	27.6	±1.4

* Standard deviation of five replicate chromatographic injections of the same sample.

from a single individual and in the Dayton city water supply. The mean concentration of nitrate in the water supply was 1.16 ppm. The relative standard deviation over the 1-month period was 13.8%. The mean concentration of nitrate in the urine of an adult male donor was 33.2 ppm, with a relative standard deviation over 1 month of 13.6%. Fig. 3 is a chromatogram of nitrobenzene formed from the reaction of the nitrate in urine.
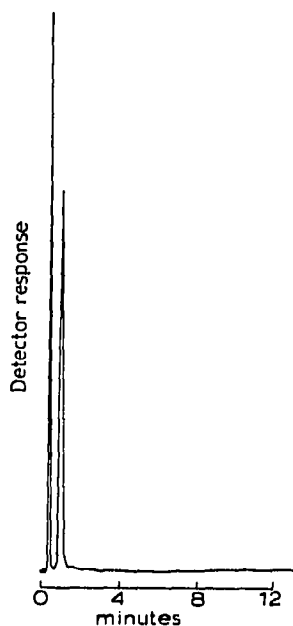

Fig. 3. Chromatogram of the products of the reaction of nitrate in urine with benzene. A 1-ml sample of urine containing 33.4 ppm of nitrate was subjected to analysis.

Studies of the conversion of nitrogen dioxide to nitrobenzene

The highly reactive nature of nitrogen dioxide causes major problems both in sampling and analysis. For this reason, in the generally accepted methods[1,2,4] nitrogen dioxide is converted into other species before analysis. In the phenoldisulfonic acid method (EPA Method 7)[4], an absorbing solution consisting of dilute sulfuric acid and hydrogen peroxide is employed to convert oxides of nitrogen to aqueous nitrate. We reasoned that a similar approach could be used to convert nitrogen dioxide to nitrate ion in aqueous solution with subsequent conversion to nitrobenzene and analysis by the GC-ECD procedure described above.

In order to investigate this possibility, we have conducted preliminary experiments on samples of nitrogen dioxide in nitrogen. The samples were prepared with known concentrations of nitrogen dioxide using the permeation train described in Experimental. Determinations of nitrogen dioxide concentrations were made on eight samples calculated from permeation data to contain 3–5 ppm nitrogen dioxide. Recoveries were 98, 100, 97, 100, 99, 100, 103, and 98 %, respectively. There is more than adequate sensitivity to make measurements at these levels, although the method must be regarded as tentative until more experiments are performed. The method is undergoing additional study and revision to reduce blank problems.

We envision several possible advantages accruing from the perfection of this method. In comparison with the phenoldisulfonic acid method, it is faster and potentially applicable over a wider concentration range. In qualitative experiments, it was possible to detect ambient levels of oxides of nitrogen in air samples from a metropolitan area. It could also afford an alternative means of calibration for other instrumentation. The instrumentation is widely available, is not highly specialized, and, consequently, can be used for the analysis of other sample types. Finally, the GC-ECD method appears to be highly specific. Even samples as complex as auto exhaust produce chromatograms with no evidence of interfering peaks.

ACKNOWLEDGEMENTS

REFERENCES

1 Environmental Protection Agency, *Fed. Regist.*, 36, No. 228 (Nov. 25, 1971) 22384–22397.
2 B. E. Saltzman, *Anal. Chem.*, 26 (1954) 1949.
3 R. E. Baumgardner, T. A. Clark and R. K. Stevens, *Environ. Sci. Technol.*, 9 (1975) 67.
4 Environmental Protection Agency, *Fed. Regist.*, 36, No. 247 (Dec. 23, 1971) 24891–24893.
5 W. D. Ross, *8th Quarterly Progress Report*, AF Contract F33615-72-C-1304, May 15, 1974.
6 D. J. Glover and J. C. Hoffsommer, *J. Chromatogr.*, 94 (1974) 334.
7 D. Hoffman and G. Rothkamp, *Anal. Chem.*, 42 (1970) 1643.