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Note

High-performance liquid chromatographic measurement of nitrite in aqueous solution by *in situ* formation of methyl nitrite

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Nitrite is found in a wide variety of natural systems. It also has found extensive use as a food preservative and corrosion inhibitor, and for the prevention of bacterial growth in coolant formulations for metal cutting. Methods for its measurement range from the classic colorimetric spectrophotometric approach to the more esoteric instrumental techniques. These are well summarized in a recent publication¹. Using a unique combination of eluents and columns^{2,3}, the use of high-performance liquid chromatographic (HPLC) columns has been applied to the separation of inorganic anions, including the nitrite ion.

In the course of analyzing glycol-based antifreeze for nitrite, it was observed that the retention time for this additive on a reversed-phase column using an acidic methanol-water eluent was much longer than would be expected if the species, nitrous acid, was being retained by ion suppression. A study was undertaken to explain this apparent anomaly.

EXPERIMENTAL

Chemicals

Inorganic chemicals used were reagent grade, available from J. T. Baker (Phillipsburg, NJ, U.S.A.). Methanol and isopropanol were distilled-in-glass grade from Burdick and Jackson Labs. (Muskegon, MI, U.S.A.). The alkyl nitrites were obtained from Aldrich (Milwaukee, WI, U.S.A.) and used without further purification. Water used to prepare eluents and samples was deionized and passed through a Milli-Q® (Millipore, Bedford, MA, U.S.A.) water purification system.

HPLC conditions

The liquid chromatograph consisted of a Model 6000 pump (Waters Assoc.), a Rheodyne Model 7120 injection valve equipped with a 20- μ l loop, an LC-55 (Perkin-Elmer) variable-wavelength detector, and a Hewlett-Packard 3380A computing integrator. For high-sensitivity determinations, the Kratos Spectroflow 773 variable detector was used in conjunction with a Sargent Model SRG recorder.

For examination of the component in real time as it elutes from the column, a Chromatix CMX-50 HPLC detector (Milton-Roy, Riviera Beach, FL, U.S.A.) was used.

A 4.6 mm × 25 cm Zorbax® ODS (DuPont Instruments, Wilmington, DE, U.S.A.) reversed-phase column was used for all separations. The mobile phase was methanol-water (40:60) with 0.005 *N* sulfuric acid relative to the entire mobile phase. Also, different concentrations of methanol were used. For special studies, ethanol and isopropanol were substituted. Flow-rate was 1.5 ml/min (2800 p.s.i.g.). Detection was made by UV at 214 nm and detector sensitivity generally 0.08 a.u.f.s., unless otherwise indicated. Separations were made at room temperature, nominally 25°C.

Standard solutions

A 1000-ppm nitrite stock solution was prepared by dissolving 0.151 g of sodium nitrite in 100 ml of deionized water. A 200- μ l aliquot was diluted to 10 ml to give a 20 μ l/ml solution.

Alkyl nitrite solutions were prepared by dissolving 50–70 mg in respective one-liter volumes of water giving 50–70 ppm solutions.

RESULTS AND DISCUSSION

When an aqueous solution of nitrite is injected onto a reversed-phase high-performance liquid chromatography column having an acidified methanol-water mobile phase, the chromatogram shown in Fig. 1 results. The component in the chromatogram was suspected to be methyl nitrite.

Various experiments were undertaken to verify this. When acetonitrile was substituted for methanol, or acid was not added to the methanol-water phase, the peak appeared at the column void volume.

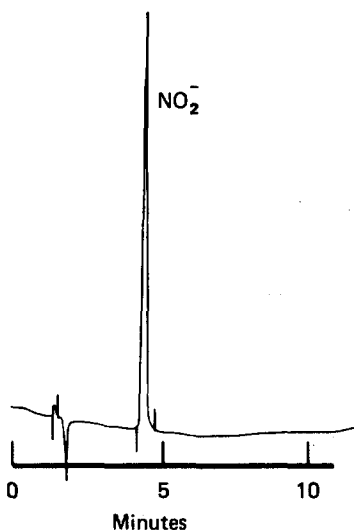


Fig. 1. HPLC chromatogram of 20 μ l of 20 μ g/ml aqueous nitrite according to the conditions described in the Experimental section.

Acidification with acetic acid (2%, v/v) gave the same chromatogram as that obtained for the methanol-water (40:60)-0.005 *N* sulfuric acid mobile phase. However, the presence of acetic acid, a strong UV absorber at 214 nm precludes the measurement at an optimum wavelength.

Examination of a 25 $\mu\text{l/ml}$ nitrite solution in mobile phase by UV spectrophotometry gives the spectrum shown in Fig. 2A. It is contrasted with the same concentration in methanol-water which was not acidified (Fig. 2B).

Using the Chromatix CMX-50 HPLC detector to record the UV spectrum of the component in real time as it elutes from the column, a spectrum identical to that in Fig. 2A was obtained.

Further evidence for methyl nitrite existence was obtained by infrared spectroscopy. Sodium nitrite was dissolved in methanol, acidified with sulfuric acid and

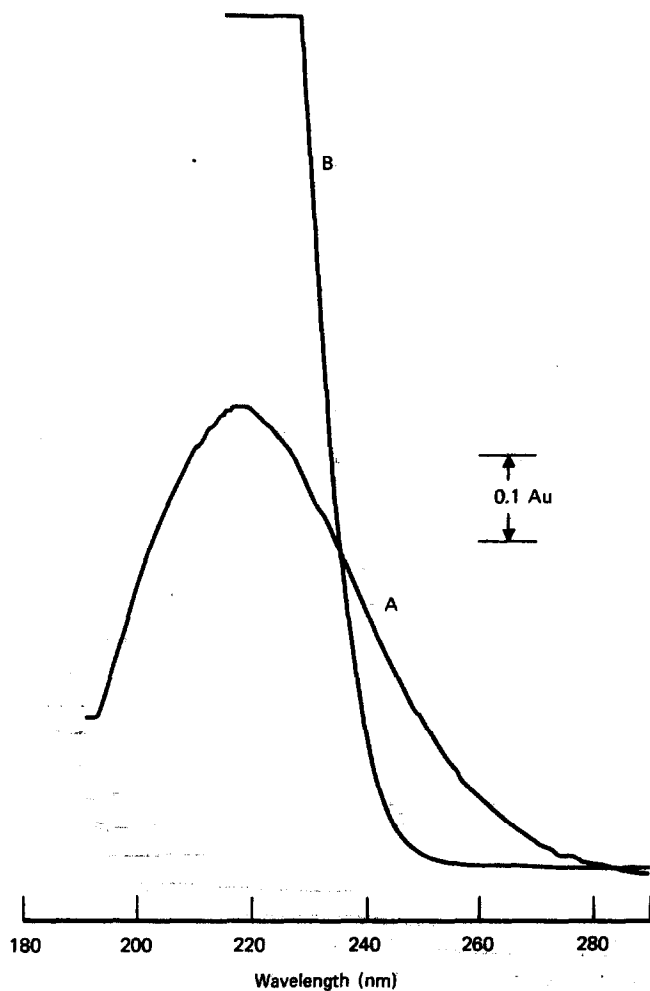


Fig. 2. UV spectrum of (A) 25 $\mu\text{g/ml}$ nitrite in methanol-water (1:1)-0.005 *N* sulfuric acid and (B) 25 $\mu\text{g/ml}$ nitrite in methanol-water (1:1) vs. methanol-water (1:1) in a 1-cm cell.

TABLE I

EFFECT OF METHYL NITRITE RETENTION IN VARYING ACIDIFIED METHANOL-WATER COMPOSITIONS

| Mobile phase composition (%) | | | t_R |
|------------------------------|-----------|-------------------|-------|
| Methanol (%) | Water (%) | Sulfuric acid (N) | |
| 80 | 20 | 0.005 | 2.22 |
| 60 | 40 | 0.005 | 3.04 |
| 40 | 60 | 0.005 | 4.36 |
| 20 | 80 | 0.005 | 5.68 |
| 10 | 90 | 0.005 | 6.22 |
| 5 | 95 | 0.005 | 6.52 |
| 0 | 100 | 0.005 | 2.20 |

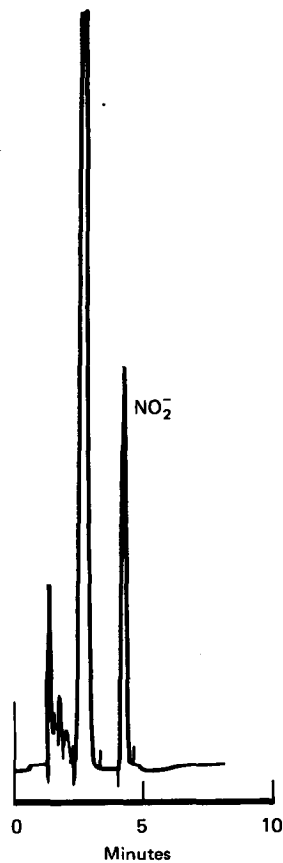
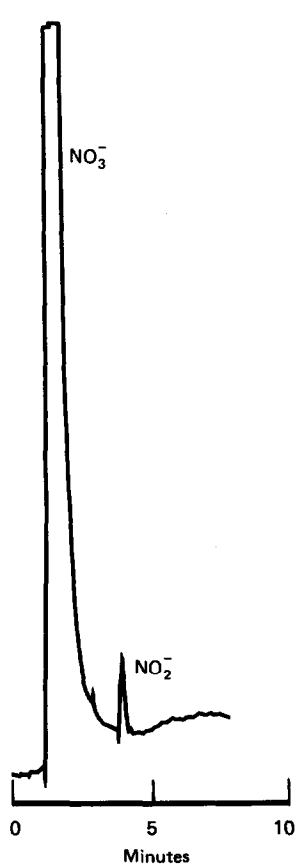


Fig. 3. HPLC chromatogram of a 20- μ l injection of a solution containing 1000 μ g/ml NO_3^- and 1 μ g/ml NO_2^- according to the conditions described in the Experimental section.

Fig. 4. HPLC chromatogram of 20 μ l 0.2 g technical 2,2-dichloropropionic acid in 100 ml water containing 2% sodium nitrite relative to the solute according to the conditions described in the Experimental section.

examined by infrared. Alkyl nitrites always exist in a *cis-trans* configuration, and the N=O stretch frequency for the trans isomer always occurs at a higher frequency than the *cis* isomer. In acidified methanol solution, the N=O stretch mode for the trans isomer occurs at 1640 cm^{-1} and for the *cis* isomer, occurs at 1601 cm^{-1} .

When a $20\text{ }\mu\text{g/ml}$ aqueous nitrite solution was injected into acidified methanol-water mobile phase, varying in methanol concentration from 80 to 0%, the retention time of the component, as expected, increased (Table I). Even at a methanol concentration of 5%, excellent peak symmetry was obtained. However, at 0.005 *N* sulfuric acid composition (absence of methanol), the peak emerged just slightly after the column void volume. This peak is undoubtedly nitrous acid.

For the determination of nitrite in different sample matrices, the choice of the methanol-water composition will depend on the retention time for other components in the sample. As shown in Table I, a wide range of methanol concentrations can be used. This will allow the determination of weakly or strongly retained components in the same run.

When alkyl nitrites were dissolved in water and injected under the conditions described in this work, other than some disturbance at the column void volume, a peak was observed at the identical retention time as that obtained from aqueous nitrite injection. With the exception of a small response by *sec*-butyl nitrite, the other nitrites gave 30–80% of the expected response if the corresponding nitrite had been theoretically converted to methyl nitrite. Alkyl nitrites studied include isopentyl, *n*-

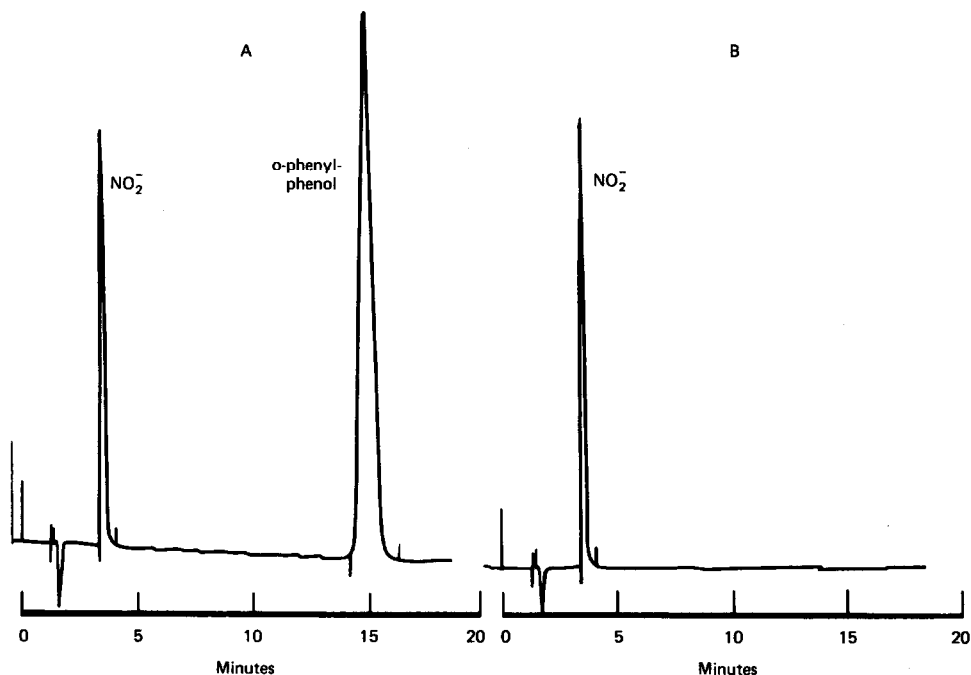


Fig. 5. HPLC chromatogram of (A) $20\text{ }\mu\text{l}$ of 2.45 g per 100 ml water of a hospital germicidal spray on the Zorbax ODS column, methanol-water (50:50)–0.005 *N* sulfuric acid, 1.5 ml/min, 214 nm, 0.8 a.u.f.s.; (B) same as (A), but put through a Waters Assoc. μ ep-Pak.

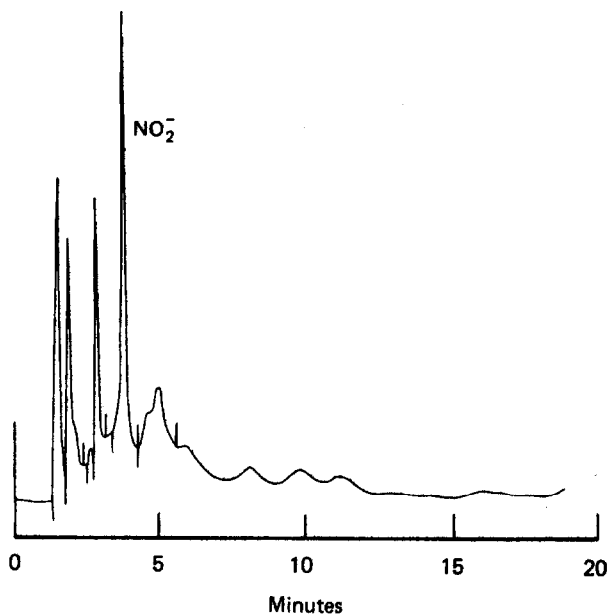


Fig. 6. Determination of nitrite in a metal cutting fluid according to the conditions described in the Experimental section.

propyl, isopropyl and the four isomers of butyl nitrite. Infrared examination of these nitrites indicated that they were not sufficiently pure to make quantitative studies. Since this was not the main subject of this work, no attempt was made to purify or obtain greater purity materials.

In order to determine if the methyl nitrite formation from injection of alkyl nitrites results from transesterification or proceeds through a hydrolysis route to give nitrite, 50–70 ppm aqueous solutions of alkyl nitrites were analyzed for nitrite concentrations by ion-exchange chromatography⁴. Surprisingly, for all the samples, nitrite levels of the same order of magnitude were obtained when compared with the results on the reversed-phase column. Therefore, it was concluded that the alkyl nitrites hydrolyzed to nitrite as soon as they came in contact with the water and subsequently formed methyl nitrite in the acidified methanol–water eluent.

Ion-exchange analysis of aqueous alkyl nitrites that had been prepared ten days earlier showed that the nitrite formed by hydrolysis had extensively oxidized to nitrate.

Figs. 3–6 illustrate nitrite determination in various sample matrices. Fig. 3 shows the separation of nitrite in the presence of a thousand-fold concentration of nitrate. Nitrite is sometimes used as a corrosion inhibitor in agricultural spraying equipment. Fig. 4 gives the determination of nitrite in technical 2,2-dichloropropionic acid. In Fig. 5A, a germicidal aerosol is analyzed. If one wishes to remove more lipophilic components prior to injection, the solution can be passed through a C₁₈ Waters Assoc. Sep-Pak®. Fig. 5B shows the results of this sample treatment. In Fig. 6, nitrite is measured in a coolant formulation used in the metal cutting industry.

Peak height and area measurements for nitrite were found to be linear over a

concentration range of 1 to 1000 ppm. Repeated injection of a 20- μ l aliquot of the 2,2-dichloropropionic acid herbicide gave a relative standard deviation of 0.33%.

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