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Note

Rapid simultaneous determination of nitrate and nitrite by high-performance liquid chromatography using ultraviolet detection

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A number of methods for the combined determination of nitrate and nitrite have been described. Usually a derivatization step is necessary and nitrate is found from the difference between the level of nitrite measured after cadmium reduction of nitrate and that determined directly. The AutoAnalyzer method for routine analysis of large numbers of samples is based on this technique and up to 15 samples per hour can be analysed in this way. The limits of detection are 10–100 μg/l as nitrogen, depending on the conditions used. The sample volumes required are of the order of 1 ml. Highly sensitive gas chromatographic methods, requiring amounts of sample of only a few microlitres, for determining nitrate and nitrite exist[1,2], with a limit of detection of 1 μg/l as nitrogen. A rather complicated derivatization procedure is necessary, however. The possibility of direct measurement of nitrate and nitrite based on ultraviolet adsorption at 200–210 nm has been described by Armstrong[3]. The method suffers, however, from the necessity of tedious and often unreliable background correction. Methods for the simultaneous determination of nitrate and nitrite by high-performance liquid chromatography (HPLC) have been reviewed by Davenport and Johnson[4]. They also reported the use of an electrochemical detector and obtained a limit of detection of 100 μg/l as nitrogen and a linear range between 0.7 and 14 mg/l. Separation was accomplished on a polymer-based anion exchanger.

As far as can be gathered from the available literature, in the HPLC of nitrite and nitrate no use has been made of the detectability of these anions at a wavelength of about 200 nm.

A method is given here in which nitrate and nitrite are separated on a cellulose anion exchanger in about 10 min and detected in a spectrophotometric flow-through cell at 210 nm. No derivatization is required. The detection limit is 1–5 μg/l as nitrogen with a sample volume of 100–200 μl. The linear range extends up to 20 mg/l as nitrogen.

The cellulose anion exchanger was found to be more efficient and less subject to deterioration than polymer-based anion exchangers and pellicular-type anion exchangers when complicated samples such as sewage sludge, animal slurry and soil extracts are used.

METHOD

A stainless-steel column (SS 316) of length 30 cm and I.D. 0.3 cm was filled

with a mixture of Kieselguhr (BDH, Poole, Great Britain) and Ecteola ET 41 cellulose anion exchanger (Whatman; W. & R. Balston, Maidstone, Great Britain), following the procedure of Van der Wal and Huber[5]. The eluent used was 0.03 *M* potassium sulphate solution and 0.01 *M* Tris buffer at pH 7 in water. Simple filling of the column by tapping in small amounts of a mixture of Kieselguhr (sieved fraction with particle size range 5–10 μm) and Ecteola ET 41 was found to produce columns giving sufficient resolution of nitrate and nitrite. A ratio of Kieselguhr to Ecteola of 1:2 (by weight) was used. The column was operated at a pressure of 40 bar. The eluent was monitored with a Cecil CE-212 spectrophotometric flow-through detector at a wavelength of 210 nm. The temperature of the column was ambient. Samples were injected with the aid of a sample injection valve (Valvo C20), using a sample loop of 60 μl.

RESULTS

Examples of chromatograms of tap water and of sludge solution resulting from aeration of anaerobically digested sewage sludge are shown in Fig. 1. The sludge was centrifuged at 40,000 *g* for 1 h, then the supernatant was diluted 1:100 with distilled water and injected into the column. Tap water was injected undiluted. Without dilution, samples with concentrations of up to 20 mg/l each (as nitrogen) of nitrite and nitrate could be injected. A chromatogram of a mixture containing 10 μg/l each (as nitrogen) of nitrite and nitrate is shown in Fig. 2.

Under the conditions used, the contribution of the column to dispersion of nitrite and nitrate is about 200 μl (expressed as the standard deviation of the output

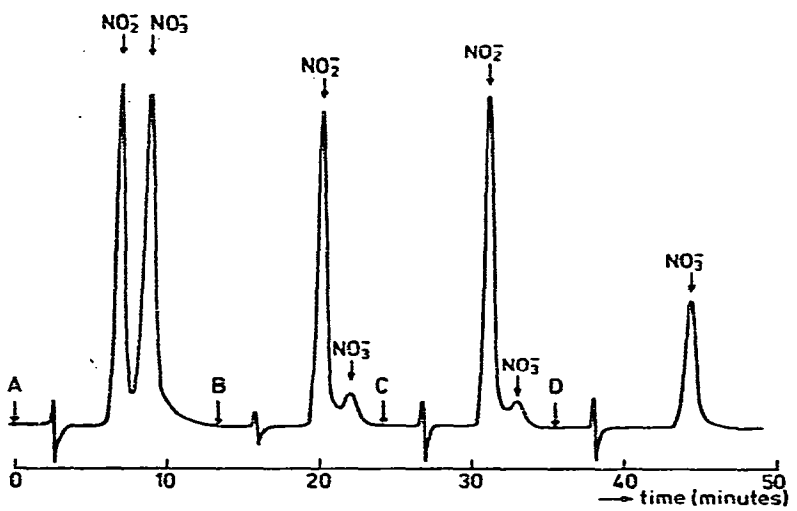

Fig. 1. Chromatograms resulting from the injection of samples containing nitrate and nitrite into a mixed-bed column containing Kieselguhr and Ecteola ET 41 cellulose anion exchanger. Eluent: 0.03 *M* K_2SO_4, 0.01 *M* Tris buffer (pH 7). A, Standard mixture containing 1.4 mg/l as nitrogen of both NO_2^- and NO_3^- (attenuation 0.1); B, sewage sludge sample, diluted 1:100, containing 2.7 mg/l as nitrogen of NO_2^-, and 0.35 mg/l as nitrogen of NO_3^- (attenuation 0.2); C, sewage sludge sample, diluted 1:100, containing 1.4 mg/l as nitrogen of NO_2^- and 0.1 mg/l as nitrogen of NO_3^- (attenuation 0.1); D, tap water containing 0.45 mg/l as nitrogen of NO_3^- (attenuation 0.1).

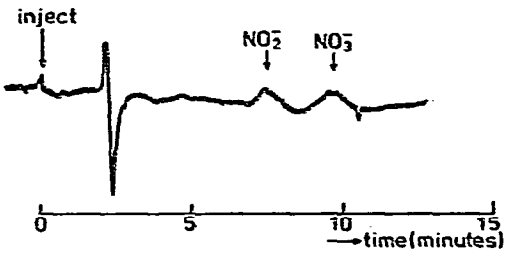

Fig. 2. Chromatogram of a sample containing 0.01 mg/l as nitrogen of both NO_2^- and NO_3^-. Attenuation factor, 0.01; other conditions as in Fig. 1.

function measured in the detector). The volume of injection is 60 μl, so that, without too great a loss of resolution, the volume of injection can be increased to 200 μl. This should increase the detection limit by a factor 3, as the detected concentration is proportional to the injected volume, provided that the contribution of the injected volume to the dispersion in the column is small. Alternatively, of course, the column performance can be improved, making it possible to obtain the same limit of detection with a smaller injected volume and with better resolution. From the results shown in Fig. 2 and the foregoing discussion, a limit of detection of about 1–5 μg/l as nitrogen for both nitrite and nitrate can be expected.

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