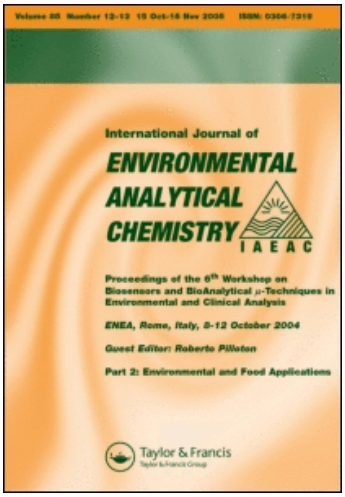

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Direct Determination of Nitrate and Nitrite in Soils by Use of a Hydrodynamic Injection Probe Based on Filtration-Dialysis Processes

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DIRECT DETERMINATION OF NITRATE AND NITRITE IN SOILS BY USE OF A HYDRODYNAMIC INJECTION PROBE BASED ON FILTRATION–DIALYSIS PROCESSES

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A flow system integrating a soil sample pretreatment unit and a flow manifold was developed for the sequential determination of nitrate and nitrite. The pretreatment unit comprises a probe where filtration and dialysis are performed in order to clean-up aqueous soil suspensions. The flow manifold is a straightforward configuration including a copperized cadmium minicolumn in a bifurcated channel for the sequential determination of nitrate and nitrite. The analytes were determined at the microgram per gram level in soils with relative standard deviations between ±3 and ±5%.

KEY WORDS: Soil sample, automated analysis, hydrodynamic injection probe, nitrate, nitrite.

INTRODUCTION

Unsegmented flow systems, particularly flow injection analysis (FIA), have proved to have a great potential for solving a wide variety of analytical problems.[1] A scan of the flow analysis literature reveals that these systems offer major advantages for the analysis of liquid samples, pretreatment of which is normally quite simple. However, one of the greatest challenges of today's Analytical Chemistry is the development of simpler, automated procedures for implementation of the preliminary operations of the analytical process.[2] The difficulties involved in performing such operations are even greater when solid or gaseous samples are to be processed.

Only a few applications involving the analysis of solid samples directly introduced in automatic flow systems have so far been reported. The first two were developed by Bergamin *et al.*, for the for the determination of aluminium[3] and molybdenum[4] in steels; they carried out a brief electrolysis in a sample cylinder as anode and brought into contact with an acid stream that dissolved the electrode partially, thus providing a dissolved sample plug that was subsequently analysed in an FIA manifold. Ultrasonic irradiation as a physical agent has also been used in flow manifolds for the direct determination of iron in plant materials and available boron in soils.[5] McLeod developed a micro-distillation unit as a module for coupling to continuous-flow analysers, where ammonium and nitrate can be determined in digest extracts[6] and soils.[7] Recently, a flow-through gas-diffusion probe was developed for the direct determination of ammonium in solid samples[8].

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In this work, a hydrodynamic injection probe combining filtration and dialysis was used for clean-up of soil samples previously suspended in an aqueous solution. Sample extracts are then driven to a flow manifold where nitrate and nitrite are determined spectrophotometrically in a sequential manner. These two analytes were previously determined in liquid samples by FIA using different approaches (e.g., references 9 – 12), but never directly in aqueous solid (soil) extracts.

EXPERIMENTAL

Apparatus

A Pye-Unican SP6-500 spectrophotometer equipped with a 10-mm light-path Hellma 178.12 QS flow-cell (18 µl inner volume) was used. A Servograph REC 80 recorder furnished with a REA 112 high-sensitivity unit (Radiometer, Copenhagen) with a range of 50 mv cm^{-1} and a chart speed of 10 min cm^{-1} was also used. The filtration/dialysis membrane probe was developed in our laboratory, and tested with different types of cellulose membranes from Millipore, pore size 0.22 µm (GSWP), 0.45 µm (HAWP) and 1.2 µm (RAWP). A Gilson minipuls-3 peristaltic pump, a Rheodyne 5011 six-way switching valve and a Rheodyne 5401 four-way injection valve were also used. Teflon tubing of 0.5 mm i.d. was used to construct the manifold unless otherwise stated.

Reagents

All reagents were of analytical grade and distilled water was used throughout.

The reagent solution was prepared daily by dissolving 4.0 g of sulphanilamide (Merck) and 0.1 g of N-(1-naphthyl)-ethylenediammonium dichloride (Merck) in 100 ml of 5% (V/V) hydrochloric acid. The carrier/aceptor and extractant/donor solutions were prepared by dissolving 20 g of ammonium chloride and 2.0 g of EDTA (disodium salt) in 1 l of distilled water. The pH of this solution was adjusted to 8.5 with concentrated ammonia.

Preparation of the coppered cadmiun reduction column. – A glass wool plug is inserted as far as the bottom of a 110 x 1.5 mm PTFE tube that is then filled with water; then sufficient 0.8 – 1.0 mm grain-size cadmium granules are added to obtain a 10-cm long column. Next, the column is washed by pumping a 5.0 M hydrochloric acid solution (5 ml), and coated with copper by pumping 10 ml of a solution containing 0.1% (w/v) copper sulphate in 0.1 M EDTA. The column thus obtained provides a reduction efficiency of ca. 96 – 97% for 0.1–1.0 $\mu g ml^{-1}$ nitrate. This Cu-Cd column can be reactivated by pumping a few milliliters of hydrochloric acid followed by a $CuSO_4$-EDTA solution through the column containing the reductant.

Standard solutions – A stock nitrate solution was prepared by dissolving 0.7216 g of dried potassium nitrate in distilled water and diluting to 1000 ml. A stock nitrite solution was made by dissolving 0.6072 g of potassium nitrite distilled water and diluting to 1000 ml. Both stock solutions were supplied with a few drops of chloroform and kept in a refrigerator. Working standard solutions were prepared by appropriate dilution of the stocks.

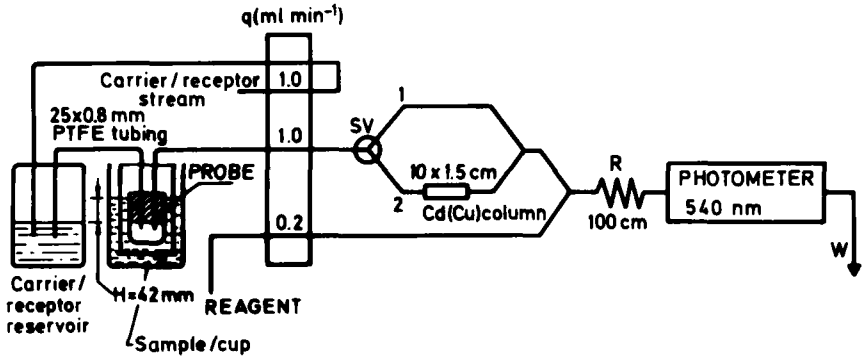

Figure 1 Manifold used for the direct determination of nitrite and nitrate in soil samples(for details see text).

Manifold and procedures

The manifold used for the direct determination of nitrate and nitrite in soils is shown in Figure 1. It included a filtration-dialysis membrane probe acting as a hydrodynamic injection valve for FIA (Figure 2). The probe was assembled from a 40 mm long x 5 mm i.d. glass tube and two lengths of 0.8 mm i.d. Teflon tubing serving as the inlet and outlet for the probe chamber (volume ca. 60 µl).

The experimental procedure involves passing the analytes across the membrane into the probe chamber. By keeping an appropriate pressure difference between the two sides of the membrane, the trapping of the analytes in the probe chamber is facilitated. Such a pressure difference can be established by keeping the liquid level in the acceptor reservoir lower than that of the donor in the sample cup (this level difference should be

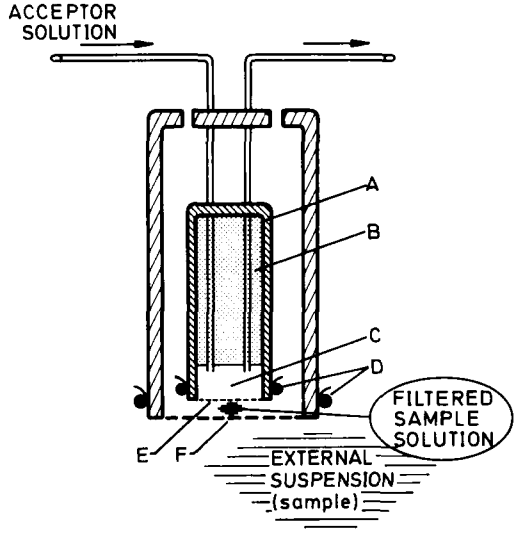

Figure 2 Scheme of the hydrodynamic injection probe used for the direct treatment of soil sample: A = glass tube; B = Teflon support; C = internal solution; D = o–ring; E = cellulose membrane; F = filter paper.

kept constant throughout measurements). A simple arrangement such as that shown in Figure 1 can readly be used for this purpose. The operational procedure consists of three steps: (a) the acceptor solution is continuously pumped through the probe chamber at a constant flow-rate with the probe outside the sample solution in order to obtain a stable baseline (penetration of air across membrane is avoided by its compact structure); (b) the pump is stopped and the probe immersed in the sample solution over a preset period (3 min), in order to allow the probe to trap enough analytes; and (c) the probe is taken out of the sample solution, rinsed with distilled water and dried with a fine filter paper. Then, the sample plug trapped in the probe chamber is aspirated and mixed with the reagent steam (Greiss-Ilosvay method). The coloured product formed is transfered to the flow-cell, where its absorbance is measured at 540 nm by means of a spectrophotometer. Nitrate and nitrite can thus be determined sequentially by selecting channel 1 or 2 (Figure 1) via the switching valve.

RESULTS AND DISCUSSION

The effects of chemical variables including the concentration of reagents and pH of the carrier and sample solution, as well as flow variables, were similar to those observed in normal FIA and independent of the sampler system used. Consequently, such variables were optimized by placing an injection valve between the pump and switching valve, and directly injecting a preset volume (80 µl) of 0.1, 0.2 and 0.5 $\mu g ml^{-1}$ NO_2^--N standard solutions.

The optimum values for the flow system are given in the schematic diagram of the manifold (Figure 1). The carrier-to-reagent flow-rate ratio was set at 5:1 in order to minimize dilution of the sample plug. A 100-cm long reaction coil and an overall flow-rate of 1.2 ml min^{-1} (corresponding to a residence time of 20 s) were found to result in the highest sensitivity. The effect of the concentration of the reagents including sulphanilamide, N-(1-naphthyl)-ethylenediammonium and hydrochloric acid, were individually studied. The results showed concentrations of N-(1-naphthyl)-ethylenediammonium in the range of 0.05–0.5% (v/v) and hydrochloric acid in the range 4–10% (v/v) to have a slight influence on the peak height, which , however increased almost proportionally to the concentration of sulphanilamide from 0.2 – 4% (w/v). A more concentrated solution could be used to lower the detection limit somewhat, but the linear range of the calibration curve would also be shortened as a result. Therefore, a reagent solution consistent of 4% sulphanilamide, 0.1% N-(1-naphthyl)-ethylenediammounium and 5%(v/v) HCl was finally chosen to obtain the best possible results.

The influence of temperature during reaction development in the flow system was studied by immersing the reaction coil in a thermostated water bath. The results showed sensitivity to increase by ca. 24% on increasing the temperature from 25°C to 45°C; higher temperatures, however, resulted in a virtually constant peak height for 0.1, 0.2 and 0.5 $\mu g ml^{-1}$ NO_3^--N. Nevertheless, room temperature (ca. 25°C) was finally adopted in order to avoid bubble formation inside the flow system and simplify the procedure.

For nitrate determination, the coppered cadmium reducing column was incorporated into the manifold. Preliminary experiments revealed the optimun pH range for reducing nitrate to nitrite to be 6.5 – 9.0. Because ammonium did not interfere at all, a NH_4Cl-NH_3 buffer of pH 8.5 was used as acceptor/carrier when the probe was introduced in the sampler unit. The effect of the ammonium chloride concentration was further studied over the range of 0.2–5%, where NH_4Cl was found to have no effect on the trapping of

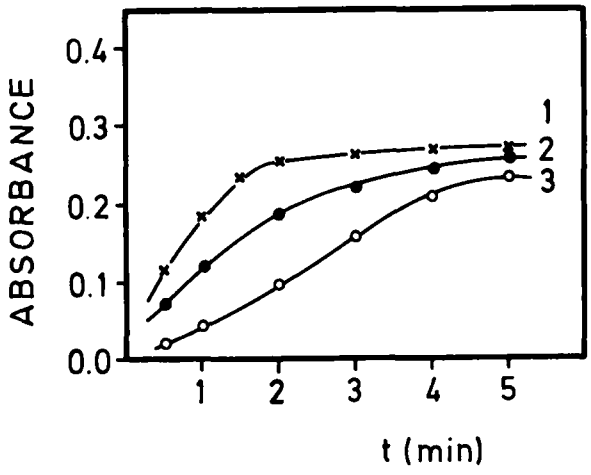

Figure 3 Effect of the trapping time on the determination of nitrite by the use of membrane probes with different membrane pore sizes: (1) 1.2 μm; (2) 0.45 μm; and (3) 0.22 μm. $[NO_2^-] = 0.3\ \mu g ml^{-1}$.

NO_2^- and NO_3^- in the probe chamber. A 2% NH_4Cl solution in NH_3 was thus used as the buffer (pH 8.5) in order the acceptor solution would have a reasonably high buffering capacity so as to overcome any pH change arising from insertion of the real sample. A 0.2% EDTA was added to the solution in order to mask Cd^{2+} ions formed in the column, as well as other ions introduced in the flow system by the sample. The conditions for extraction of nitrite and nitrate from the soil samples were relatively non-critical. A solution of the same components and pH as the carrier/acceptor was used as extractant. The solution provided a somewhat lower blank signal relative to distilled water as extractant/donor stream.

Optimization of the functioning of the sampling unit

The main variables affecting the sampling procedure were the membrane pore size and the liquid level difference between the carrier reservior and sample cup. These variables were optimized by using a nitrite standard containing 0.5 $\mu g ml^{-1}$. As can be seen in Figure 3, for a given period of time, the larger the pore size of membrane was, the more analytes crossed it. However, when real samples were used, a 1.2-μm pore size was found to allow a relatively highly turbid filtrate solution to reach the probe chamber that was unsuitable for photometric detection. A 0.45-μm membrane pore size gave the best results, since smaller pore sizes provided poorer detection limit and required longer trapping times. Therefore, a 0.45 μm membrane was used for further experiments and sample analyses.

Other experiments showed the peak height to increase dramatially with increasing pressure difference across the membrane. Such a difference can be expressed as the liquid level difference between the carrier reservoir and sample cup solution. As can be seen in Figure 4, the higher liquid difference was, the more analytes penetrated the probe chamber, but also the greater was the tendency of the membrane pores to become clogged by suspended particles in the solution and hence the shorter was the membrane life-time. Therefore, a liquid level difference of ca. 42 mm was used throughout.

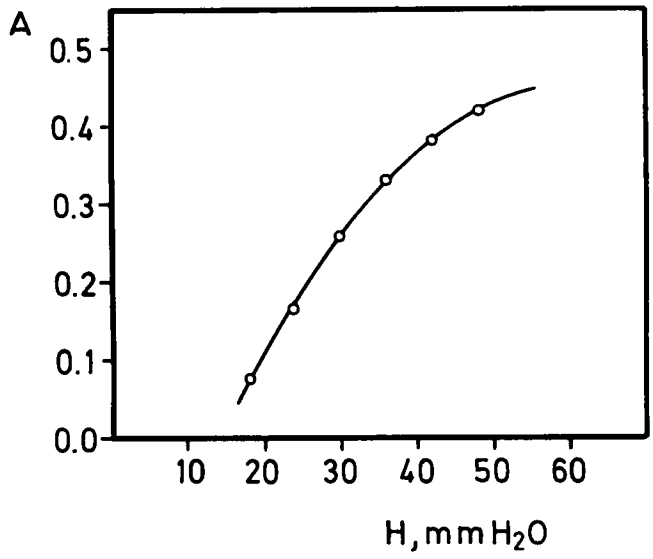

Figure 4 Effect of the presure difference between the two sides of the probe membrane on the determination of 0.5 μgml^{-1} of nitrite (membrane pore size = 0.45 μm; traping time = 3 min).

Calibration

Under the working conditions given above, the peak height was found to be linearly related to the concentration from 0.01 to 0.75 μgml^{-1} NO_2^- and 0.02 to 0.60 μgml^{-1} NO_3^- for a trapping time of 3 min and a 0.45-μm membrane pore size. The corresponding regression equations were as follows:

Absorbance (A) = $0.675[NO_2^-] + 0.024$ ($r = 0.9988$, $n = 6$)
Absorbance (A) = $0.667[NO_3^-] + 0.019$ ($r = 0.9981$, $n = 6$)
where concentrations are expressed in $\mu g\ ml^{-1}$.

The relative standard deviation for 0.25 μgml^{-1} of nitrite and nitrate were ±2.6 and ±3.6, respectively ($P = 0.05$ and $n = 11$). Higher concentrations of the analytes could be accommodated by shorting the trapping time, whereas longer trapping duration could be used to lower the detection limit and raise the sensitivity (by a small factor, though).

Each overall determination took 4 min, so the throughput was ca. 15 samples/h. The performace of the proposed method was tested by applying it to various synthetic samples which prepared by mixing known amounts of nitrite and nitrate in different ratios. Table 1 shows the results obtained.

Determination of nitrite and nitrate in aqueous suspensions

The design of the probe shown in Figure 2 prevented direct contact between the aqueous sample suspensions and the cellulose membrane. In fact, the determination of nitrate and nitrite in soil samples by making an aqueous suspension and immersing the "internal

Table 1 Determination of nitrite and nitrate in synthetic solid samples.

Composition ($\mu g\ g^{-1}$)		*Results found ($\mu g\ g^{-1}$)($n = 3$)*	
Nitrite	*Nitrate*	*Nitrite*	*Nitrate*
0.10	0.10	0.098 ± 0.003	0.103 ± 0.005
0.30	0.30	0.294 ± 0.007	0.314 ± 0.014
0.10	0.30	0.102 ± 0.003	0.328 ± 0.009
0.10	0.50	0.110 ± 0.005	0.493 ± 0.013
0.30	0.10	0.319 ± 0.014	0.107 ± 0.006
0.50	0.10	0.517 ± 0.018	0.110 ± 0.007

probe" (without the external glass tube for the previous filtration) directly in the suspension provided scarcely reproducible results and shortened the membrane life-time through deposition of the solid on its surface, which was thus soon clogged and required frequent replacement. The external paper filter-capped cell proved to be an effective means for preventing most of the particles from reaching the cellulose filtration/dialysis membrane.

Study of interferences

A study of interferences with the determination of 0.2 μgml^{-1} NO_2^- and NO_3^- was carried out. The species tested were those commonly found in different types of soil extracts. The results obtained are shown in Table 2. The determination of nitrate was found to be

Table 2 Influences of species commonly present in soil samples on the determination of nitrite and nitrate by the proposed method (0.2 $\mu g\ g^{-1}$ of both nitrite and nitrate)

Species	*Maximum tolerated foreign species/analyte ratio*	
	Nitrite	*Nitrate*
Na^+	$> 10^4$	$> 10^4$
K^+	$> 10^4$	$> 10^4$
NH_4^+	10^2	50
Ca^{2+}	10^4	10^4
Mg^{2+}	10^4	10^4
Mn^{2+}	8×10^3	10^4
Zn^{2+}	3×10^3	10^4
Fe^{3+}	10^2	5
Cu^{2+}	2×10^3	10^2
Cl^-	$> 10^4$	$> 10^4$
SO_4^{2-}	10^4	10^4
SO_4^{3-}	2×10^3	5×10^2

Table 3 Determination of nitrite and nitrate in soil samples by using the proposed method and a reference method (see reference 13)(*)

Sample	*Proposed method ($\mu g\ g^{-1}$)*		*Proposed method ($\mu g\ g^{-1}$)*	
	Nitrite	*Nitrate*	*Nitrite*	*Nitrate*
S_1	0.98 ± 0.03	460 ± 21	0.95 ± 0.04	468 ± 6
S_2	0.58 ± 0.03	16.0 ± 0.8	0.65 ± 0.02	16.0 ± 1.0
S_3	0.32 ± 0.05	24.0 ± 1.1	0.38 ± 0.03	23.0 ± 1.4
S_4	0.74 ± 0.04	5.0 ± 0.3	0.70 ± 0.03	6.0 ± 0.5

(*) Average of three determinations

more severely interfered because some species diminish the reduction efficiency of nitrate in the reducing column. As a rule, such species usually occur in soil extracts at concentrations below the tolerated limit, however. In addition, some metal cations contained in the samples that precipitate as hydroxides at pH 8.5 can be masked by the EDTA added to both the donor and the acceptor stream.

Application to soil samples

The analytical potential of the proposed method was tested with the determination of nitrite and nitrate in soil samples. A 2% NH_4Cl-0.2% EDTA solution of pH 8.5 was used as acceptor/carrier and extractant. An amount of ca. 1.0 g of air-dried powdered soil sample was weighed and transferred to each sample cup; 10 ml of extractant solution was then added and the mixture was stirred for 5 min. Then, the probe was sucessively immersed in each sample cup, and the above described procedure for the standard test solution was followed. The results obtained for four different soil samples are listed in Table 3. They were consistent with those obtained by the normal FIA method, where analytes in soil are extracted by shaking and centrifugation, and the solution colour is bleached by using aluminium hydroxide[13]. This procedure is longer than the proposed procedure, it involves more intensive sample manipulation increases and hence is more prone to error.

CONCLUSIONS

The proposed method based on fitration/dialysis, allows the analytes in soil suspensions to be trapped on-line, transferred to the flow system through a membrane probe and then determined directly. The method is much simpler than its conventional manual counterpart. Its sensitivity and determination limits are similar to those of usual FIA methods for liquid samples. It can be applied to other analytes and types of samples since both the filtration supporting material and the dialysis membrane can be replaced by other types of materials suited to the analytes to be determined or the type of sample to be analysed.

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