

Flow Injection Determination of Nitrate in Vegetables Using a Tubular Potentiometric Detector

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A flow injection system with potentiometric detection for the determination of nitrate in vegetables was developed. For this purpose a tubular nitrate ion-selective electrode with the ion-exchanger system [(4,7-diphenylphenanthroline)nickel(II) dissolved in *o*-nitrophenyl octyl ether] immobilized in a PVC membrane and without inner reference solution was prepared. The flow injection manifold incorporating the tubular electrode was used for the determination of nitrate in vegetables (lettuce, parsley, and spinach), using a mixture of lead acetate, lead oxide, and sodium acetate as nitrate extractant, ionic strength adjustor, and interference suppressor solution. The quality of the results obtained by the proposed methodology was assessed by comparing them with those provided by a conventional potentiometric methodology and also through recovery tests (recoveries from 94 to 104% were found). A sampling rate of 120 samples per hour was achieved with a relative standard deviation lower than 2%.

Keywords: *Flow injection analysis; nitrate; ion-selective electrodes; vegetables*

INTRODUCTION

In recent years, an increasing interest in the determination of nitrate levels in food products has been observed, essentially due to the potential reduction of nitrate to nitrite, which is known to cause adverse effects on human and animal health. In fact, nitrite in foodstuffs may react with secondary amines to form toxic and carcinogenic nitrosamine compounds. Fresh and processed vegetables have often been cited as the major sources of dietary nitrate intake, owing to their nitrate accumulation capacity (Maynard et al., 1976). For this reason, in the past few years efforts have been made to study nitrate accumulation in vegetables and the factors that might influence its occurrence. Actually, the amount of available nitrate in soil (which may be related to the amount of commercial fertilizers applied) appears to be a major factor determining the nitrate concentration level in vegetables (Maynard et al., 1976; Bosch et al., 1991).

The analytical methods currently used for the nitrate determination in plants, soils, and waters are usually based on molecular absorption spectrophotometric procedures involving a previous nitrate reduction to nitrite. Due to the morosity and dependence on the operator's skill in these analytical processes, alternative procedures for the nitrate determination in plants (Baker and Smith, 1969; Milham et al., 1970; Mitrakas et al., 1990), including vegetables (Consalter et al., 1992), using a commercial Orion ion-selective electrode, have been reported. In fact, potentiometry can be a much more straightforward method as only simple sample composition treatments, namely ionic strength adjustment, are necessary. Extract concentration adjustments are not needed due to the broad linear dependence range of potentiometric measurements with ion-selective electrodes. Therefore, it is an advantageous alternative to spectrophotometric methodologies, in which concentra-

tion adjustments are necessary to adjust sample composition to a narrow linear dependence range.

In this paper, a flow injection analysis (FIA) system for the automatic determination of nitrate in extracts of vegetables, using a tubular ion-selective electrode without inner reference solution, is described. The tubular electrode was constructed for this purpose as previously described (Alonso-Chamarro et al., 1993), being the PVC membrane composed of (4,7-diphenylphenanthroline)nickel(II) dissolved in *o*-nitrophenyl octyl ether. The option for using a tubular unit in the FIA manifold was determined by the problems that occur when conventional electrodes are intercalated in the flow systems (Alegret et al., 1987; Ferreira and Lima, 1993), especially electric noise, mechanical instability of the setups, and also the eventual formation of dead volumes in the modular devices designed to accommodate them. These difficulties can be minimized if tubular units with an inner diameter similar to that of the FIA tubes (Ferreira and Lima, 1993) are constructed. A FIA system incorporating the prepared tubular detector was devised and applied to the nitrate determination in some vegetables (lettuce, parsley, and spinach). As nitrate extractant, ionic strength adjustor, and interference suppressor solution, a mixture of lead acetate, lead oxide, and sodium acetate (Mitrakas et al., 1990) was used. Determinations by batch direct potentiometry with conventional electrodes based on the same sensor were also performed for comparison purposes.

MATERIALS AND METHODS

Reagents and Solutions. All chemicals used were of analytical reagent grade, and doubly deionized water (with a specific conductance of less than $0.1 \mu\text{S cm}^{-1}$) was used throughout.

As the nitrate extractant solution (ISA-Pb) and simultaneously ionic strength adjustor and interference suppressor,

a mixture of 1×10^{-2} M lead acetate, 5×10^{-3} M lead oxide, and 1×10^{-2} M sodium acetate (Mitrakas et al., 1990) was used.

Nitrate stock solution was prepared by dissolving previously dried (100 °C) potassium nitrate in the ISA-Pb solution. Nitrate standards were obtained by rigorous dilution of the stock solution in the ISA-Pb solution.

Apparatus and Electrodes. A Crison 2002 voltmeter (± 0.1 mV sensitivity) was used for potentiometric measurements. In the FIA determinations this voltmeter was coupled to a Kipp & Zonnen BD 111 chart recorder.

As reference electrodes for the batch determinations with conventional electrodes and for the FIA determinations with the tubular electrodes, AgCl/Ag Metrohm 6.0726.100 and Russel Model 90-0029 double-junction reference electrodes were used, respectively. The outer compartment of these reference electrodes was filled with the ISA-Pb ionic strength adjustor solution.

Construction of the Tubular Electrode. The sensor membrane was prepared as described by Alonso-Chamarro et al. (1993): 0.04 g of (4,7-diphenylphenanthroline)nickel(II) was dissolved in 0.36 g of *o*-nitrophenyl octyl ether, and subsequently 0.16 g of PVC dissolved in 6 mL of tetrahydrofuran was added. The mixture was shaken until complete dissolution and then used for the preparation of the electrodes.

Previously described procedures for the construction of the tubular electrode (Alegret et al., 1984, 1987) were used. In the flow module preparation, a perspex cylinder was filled with a conductive mixture of Araldite epoxy resin and graphite powder. After heat-curing, the conductive support was longitudinally drilled to obtain a channel where the membrane sensor solution could be deposited. The ion-exchanger sensor solution was added dropwise down the cylindrical conductive surface placed vertically. After solvent evaporation, the tube was turned upside down so that the next drop was applied to the other end of the tube. This operation was consecutively repeated to guarantee the formation of a coating as uniform as possible. This way, a flow channel of about 0.8 mm i.d. and 10 mm length was created. Once the application of the sensor membrane was completed, the electrode was left to dry at room temperature for at least 10 h. Finally, to allow the formation of the nitrate complex, the electrode was conditioned for at least 48 h in 0.1 M potassium nitrate (using this solution as carrier stream in the flow injection system). The same solution was used for storing the electrodes between determinations.

To carry out the determination of nitrate by a batch procedure, conventionally shaped electrodes without inner reference solution and the same sensor membrane were constructed in a similar way as described by Lima and Machado (1986).

Flow Injection System. The flow system used to evaluate the response characteristics of the tubular electrode and also to determine nitrate in vegetables is depicted in Figure 1.

A Gilson Miniplus 3 peristaltic pump was used for propelling the solutions. Samples (or standard solutions) were intercalated in the carrier stream with a Rheodyne Type 5020 six-port rotary injection valve. Omnifit Teflon tubing (0.8 mm i.d.) with Gilson end-fittings and connectors was used as manifold conduits. Auxiliary devices, namely stainless steel ground electrodes (10 mm long, 0.8 mm i.d.) and reference electrode support modules, constructed as reported by Alegret et al. (1987), were also used. The internal diameters of both metallic and Teflon tubes used for connections were identical, and so hydrodynamic characteristics of the flux were not altered.

Vegetable Extract Preparation. Approximately 100 mg of previously dried (80–100 °C) and powdered vegetable materials was mixed with 20 mL of ISA-Pb solution. This mixture was shaken for 5 min and then filtered (Mitrakas et al., 1990). Determinations were performed over this extract solution.

RESULTS AND DISCUSSION

Tubular Electrode Evaluation. The behavior of the tubular electrode was assessed in a low dispersion

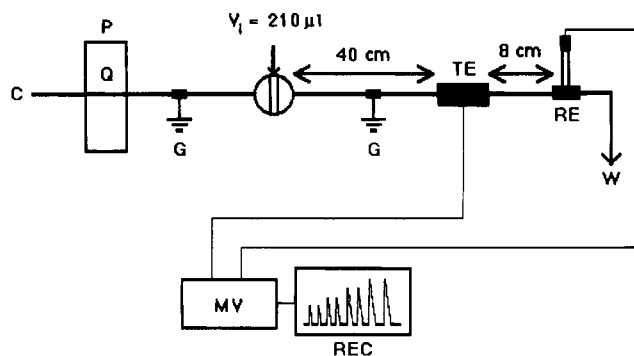


Figure 1. Low dispersion flow injection manifold used for the evaluation of the electrode characteristics and for the determination of nitrate in vegetables: P, peristaltic pump; V_i , injection volume; G, ground electrodes; TE, nitrate selective tubular electrode; RE, reference electrode; MV, millivoltmeter; REC, recorder; Q, flow rate = 6 mL min^{-1} ; W, waste; C, carrier solution, 10^{-6} M KNO_3 in ISA-Pb for the electrode evaluation and 2×10^{-5} M KNO_3 in ISA-Pb for the nitrate determination in vegetables.

single-channel flow injection manifold (Figure 1) with a dispersion coefficient of 1.5, to guarantee that the nitrate concentration in the sample plug reaching the detector is similar to that present at the moment of injection. The electrode's functioning characteristics were assessed using the ISA-Pb solution as carrier stream, in order to submit the electrodes to the same experimental conditions that would be subsequently used for the nitrate measurements in the sample extracts. A low nitrate concentration in the carrier was also used to guarantee a constant conditioning of the electrode surface, thus contributing to the baseline stabilization.

The lower limit of linear response and practical detection limit were established through the calibration curves in the nitrate concentration interval from 3.0×10^{-6} to 1.1×10^{-2} M. An average value of 5.6×10^{-5} M (3.5 mg L^{-1}) for the lower limit of linear response and 2.8×10^{-5} M (1.7 mg L^{-1}) for the practical detection limit were obtained. For the electrodes slope, an average value of -56 mV per concentration decade was found.

The reproducibility of the analytical signals was evaluated by repeated injections of solutions with nitrate concentration lying within the linear response range (between 4.0×10^{-4} and 4.0×10^{-2} M) throughout a working day. It was found that the potential drift is no more than 6 mV per day (relative standard deviations of potentials measured were lower than 5%). This drift implies that periodic calibration (every hour) should be carried out. As the sampling rate provided by this methodology is about 120 determinations per hour, performing one calibration per hour does not significantly affect the system performance.

These results show that the nitrate tubular electrode presents good functioning characteristics in this flow injection conditions and so could be used for the nitrate determination in vegetables.

Determination of Nitrate in Vegetables. Due to the wide linear dependence range typical of potentiometric detection, and also to the fact that the nitrate concentration levels in the analyzed extracts of vegetables (between 42.7 and 421 mg L^{-1}) are higher than the lower limit of linear response (3.5 mg L^{-1}) for the FIA assembly used, no concentration adjustments inside the flow system would be necessary. So, nitrate determinations in vegetables were carried out using the same

Table 1. Results Obtained in the Determination of Nitrate (mg g⁻¹ of Dried Vegetable) by a Conventional Procedure and by the Developed FIA System

sample	parsley			lettuce			spinach		
	conv proc (mg g ⁻¹)	FIA (mg g ⁻¹)	RD ^a (%)	conv proc (mg g ⁻¹)	FIA (mg g ⁻¹)	RD ^a (%)	conv proc (mg g ⁻¹)	FIA (mg g ⁻¹)	RD ^a (%)
1	35.7	33.7	-5.6	17.0	15.9	-6.5	84.3	79.6	-5.6
2	8.19	8.15	-0.47	75.3	70.3	-6.6	34.2	30.6	-10
3	10.2	9.53	-6.2	47.1	42.6	-9.6	54.7	50.3	-8.0
4	20.2	17.3	-14	27.7	27.0	-2.5	66.6	59.5	-11
5	16.2	15.0	-7.8	30.8	29.7	-3.6	46.7	46.7	0
6	28.5	28.0	-2.0	56.0	54.7	-2.3	43.1	43.1	0
7	13.1	13.1	0	25.7	24.5	-4.7	60.5	59.4	-1.8
8	17.5	17.1	-2.4	36.6	33.6	-8.2	43.1	44.7	3.7
9	21.2	21.2	0	52.4	48.4	-7.6	64.1	64.5	0.62
10	15.0	14.8	-1.4	64.8	63.3	-2.3	36.8	37.2	1.1

^a Relative deviations.

low dispersion manifold (Figure 1) described for the electrode's evaluation. Analyses on samples of lettuce, parsley, and spinach were performed. As carrier stream, the ISA-Pb solution, with a 2×10^{-5} M concentration of KNO₃ for stabilizing the baseline, was used. A larger nitrate concentration would increase the lower limit of linear response, thus not allowing the analysis of low nitrate levels.

The extracts were directly injected in the flow system, being the concentration calculated by interpolation in a previously established plot obtained with standards prepared with the same ISA-Pb concentration. For comparison purposes, determinations on the same extracts of vegetables were also carried out by a conventional procedure using potentiometric detection (Mitras et al., 1990). The results obtained are presented in Table 1.

Considering the 30 samples of vegetables, a regression analysis was established between the results obtained (expressed in milligrams of analyte per gram of dried vegetable) by the developed FIA methodology (C_f) and those provided by the conventional procedure (C_c); the equation $C_f = 0.3 + 0.949C_c$, with a correlation coefficient of 0.996, was obtained. Considering the regression parameters and the values of the relative deviations presented in Table 1, it can be noticed that there is a systematic trend for higher concentration values when using the conventional method.

These results could be due to matrix interferences in the potentiometric measurements. To assess the extension of these interferences, recovery tests were carried out for each analyzed extract, by both the conventional and the developed FIA methodologies. The results are presented in Table 2.

The FIA methodology provided recovery ratios with an average of 100% (standard deviation of 3%), while an average of 111% (standard deviation of 4%) was obtained using the conventional procedure. These results show that electrode interferences are less severe in FIA measurements due to the lower contact time of the sample solution with the membrane surface. Additionally, the tubular electrode sensing membrane is washed and conditioned by the carrier solution between consecutive sample injections.

In fact, only when the concentration of the extracting solution (whose composition aims to minimize the extension of the interferences) was increased 20 times was it possible to achieve recovery ratios for the conventional procedure similar to those obtained by the FIA methodology.

The reproducibility of the flow injection system was assessed from repeated injections of sample extracts

Table 2. Recovery of Nitrate Added to Extracts of Vegetables Using the Conventional Procedure and the Developed FIA System

sample	FIA			conv proc		
	concn found (mg L ⁻¹)	additn (mg L ⁻¹)	recov (%)	concn found (mg L ⁻¹)	additn (mg L ⁻¹)	recov (%)
parsley						
1	176	125	100	185	125	107
2	42.7	125	100	42.9	125	105
3	48.8	125	104	52.0	125	115
4	93.3	125	95.2	109	125	121
5	75.3	125	100	81.7	125	117
6	144	131	104	147	131	113
7	73.0	65.4	103	73.0	65.4	111
8	98.6	88.7	98.5	101	88.7	115
9	117	88.7	103	117	88.7	117
10	79.4	131	103	80.5	131	112
lettuce						
1	90.5	327	102	96.6	327	114
2	383	327	98.5	410	327	111
3	239	327	103	264	327	112
4	160	131	101	164	131	108
5	185	131	101	192	131	110
6	313	131	96.8	320	131	112
7	146	131	96.2	153	131	108
8	189	261	96.1	206	261	109
9	266	222	98.0	288	222	110
10	340	327	100	348	327	106
spinach						
1	421	313	98.1	446	313	107
2	171	261	98.7	191	261	111
3	270	327	99.2	294	327	107
4	307	327	93.8	344	327	106
5	250	231	104	250	231	112
6	225	327	98.2	225	327	112
7	303	327	100	314	327	111
8	231	222	102	223	222	108
9	328	327	99.4	326	327	108
10	208	222	102	206	222	108

with different nitrate concentrations, the relative standard deviation being lower than 2%.

The FIA methodology developed enables the determination of nitrate in extracts of vegetables with a sampling rate of 120 per hour.

Therefore, the FIA methodology is an alternative for the determination of nitrate in vegetables and possibly in other food products for which evaluation is currently performed by colorimetric procedures as it enables good quality results with a high sampling frequency and low sample consumption.

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