

New PVC Nitrate-Selective Electrode: Application to Vegetables and Mineral Waters

P. KONG THOO LIN,[†] A. N. ARAUJO,[‡] M. C. B. S. M. MONTENEGRO,^{*,‡} AND
 R. PÉREZ-OLMOS[§]

School of Life Sciences, The Robert Gordon University, St. Andrews Street,
 Aberdeen AB25 1HG, United Kingdom, Requite/Laboratório de Química-Física, Universidade do
 Porto, Faculdade de Farmácia, Rua Anibal Cunha 164, 4050 Porto, Portugal, and Departamento de
 Química Analítica, Universidad del País Vasco, EUITI, Plaza de la Casilla, 3, 48012 Bilbao, España

The paper refers the analytical potentialities of the oxa-azamacrocycles as potentiometric ionophores for the construction of electrodes selective to nitrate. Afterward, the membrane selective to nitrate is designed and optimized using a [3.3.3]oxazane as an ionophore. The membrane was prepared using dibutylphthalate as a solvent mediator, tetraoctylammonium as a lipophilic membrane additive, and poly(vinyl chloride (PVC) as a polymeric matrix, applied directly onto a conductive graphite/epoxy resin support. The electrodes presented a slope of 60 ± 0.1 mV log⁻¹, a low limit of linear response of 4.2×10^{-6} mol L⁻¹, a useful lifetime of 1 year, and improved selectivity characteristics when compared with other nitrate electrodes. The good working characteristics of this electrode, constructed without inner-reference solution, made possible its application to the determination of nitrate in different types of vegetables and bottled mineral waters without the use of a conditioning solution. The application of a significant *F* test proved that the results obtained were similar to those attained by application of the brucine spectrophotometric method adopted as a reference technique. Linear regression analysis also showed good agreement between the results obtained by the proposed method and the reference one.

KEYWORDS: Oxa-azamacrocycles; ionophore; nitrate-selective electrode; vegetables; water

INTRODUCTION

Agronomists usually express concern that large quantities of ammoniacal-based fertilizers, previously oxidized, will result in toxic nitrate levels in forage and vegetable crops. Because the plants often cannot utilize all of the nitrogen applied to the fields, some is left in the soil that can leach into water. Moreover, nitrates have been used as additives in meat products to prevent botulism (1) and slow lipid oxidation and thus the production of unpleasant smells. The dairy industry has also used nitrate as a food additive to prevent the swelling of cheese caused by gases released by some microorganisms. On the other hand, nitrates are also present in some industrial products, such as carbon black, used for cutting fluids, explosives, or electroplating baths.

The most frequent toxicological risk of ingested nitrate arises from its reduction to nitrite. The latter oxidizes Fe^{II} of hemoglobin to Fe^{III}, thus resulting in the metahemoglobin being unable to form a bond with oxygen (2). Sucklings are more vulnerable to metahemoglobinaemia because their stomach

acidity values allow the existence of microorganisms containing nitroreductase enzymes. Subsequent formation of nitrites would react with specific amines to give nitrosamines, which had been proved to be highly carcinogenic (3, 4). Hence, there has been much effort in the development of analytical procedures employing different instrumental techniques for the determination of nitrate in waters, vegetables, and foodstuff (5). Many laboratories are interested in an analytical methodology capable of overcoming the inconveniences of the UV spectrophotometric methods but avoiding the high costs involved in the chromatographic systems. Considering the well-established advantages of potentiometry as an electroanalytical technique, it is not surprising that since the 1960's many different nitrate ion-selective electrodes (ISEs) have been developed to achieve more expeditious procedures with application in different matrixes. To improve the performance of the electrodes, several chemical compounds have been proposed as ionophores, mainly quaternary ammonium or phosphonium salts and metal complexes of phenanthroline. Different construction techniques have been used in the their development such as conventional configurations, microelectrodes, ion-sensitive field-effect transistors, or flow-through and tubular detectors for flow analysis. Most of them have been reviewed and are summarized in the Supporting Information of this paper. However, two major

* To whom correspondence should be addressed. Telephone: +351 02 208 7132. Fax: +351 02 200 3977. E-mail: mcbranco@ff.up.pt.

[†] The Robert Gordon University.

[‡] Universidade do Porto.

[§] Universidad del País Vasco.

drawbacks are encountered during their application: (a) the difficulty to determine nitrate at low concentrations because most of the electrodes available presented detection limits ranging between 5×10^{-5} and 1×10^{-4} mol L⁻¹; and (b) the interference of several anions, e.g., chloride, nitrite, and hydrogencarbonate, whose presence in the different types of samples analyzed is highly probable and evidenced by their potentiometric selectivity coefficients. To avoid these problems, different strategy methodologies such as the standard addition method and the use of ion-exchange resins or interference-eliminating solutions have been developed accordingly. Some authors proposed the formulation of conditioning solutions to eliminate interferences and regulate the pH and ionic strength of the sample solutions. Some of these conditioning solutions were characterized by the use of silver salts with a relative toxicity but expensive preparation (6), and others were based on lead salts, which were much cheaper and yet much more toxic (7).

Studies with neutral- or charged-carrier complexes used as ionophores for potentiometric sensors have shown that functional poly(vinyl chloride (PVC) matrix membrane ion-selective electrodes may be made for many ions (8). Because azamacrocycles (cyclams) are important chelating agents for metals and organic cations, anions, and neutral molecules (9), it was decided to study, for the first time, the analytical potentialities of this type of chemical compound as ionophores for inorganic anions. In this work, a new oxa-azamacrocycle, named [3.3.3.3]-oxazane, previously synthesized (10), was used as ionophore in the design of electrodes selective to nitrate ions. The working characteristics of the constructed electrodes are evaluated and compared with those previously described in the literature. Their analytical usefulness is assessed by the determination of nitrate in vegetables and bottled mineral waters without using a conditioning solution, even at low nitrate concentrations.

EXPERIMENTAL SECTION

Apparatus and Materials. The potentials were measured with a Crison, 2002 digital potentiometer (± 0.10 mV sensitivity). Electrodes switchers of the same brand were coupled to the potentiometer. An Orion 90-02-00 double junction reference electrode was used with a 0.033 mol L⁻¹ potassium sulfate solution as an outer reference solution. For pH determinations, an Orion 91-02-00 combined glass electrode was used.

During the evaluation of the working characteristics of all of the units constructed, measurements were carried out in double-walled cells at 25.0 ± 0.2 °C by means of a thermostated water bath Selecta, Tectron 3473 100.

A Perkin-Elmer Lambda 3B UV-vis spectrophotometer was used for the samples of nitrate determined by the brucine spectrophotometric method.

Reagents and Solutions. Throughout all of the experimental work, analytical-grade reagents were used without further purification. The solutions were prepared with distilled and deionized water, with conductivity less than 0.1 μ S cm⁻¹.

The 0.10 mol L⁻¹ nitrate stock solution was prepared by dissolving potassium nitrate, previously oven-dried at 110 °C for 24 h in distilled water. When necessary, the working solutions used in the calibration curves were prepared by rigorous dilution of the corresponding stock solution.

A 0.033 mol L⁻¹ solution prepared from solid potassium sulfate was used as an ionic strength adjuster (ISA) solution, during the evaluation of the electrode working characteristics.

Construction of the ISE. The ionophore [3.3.3.3]oxazane macrocycle system (Figure 1) was synthesized according to our previously published method (10). Briefly, an alkylation reaction between α,ω -bis[(2-mesitylsulfonyl)aminoxy]propane and 3-bromopropanol gave the corresponding bisalkanol, which was condensed with another

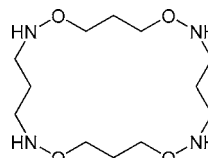


Figure 1. Heteromacrocycle[3.3.3.3]oxazane.

molecule of α,ω -bis[(2-mesitylsulfonyl)aminoxy]propane under Mitsunobu conditions to give protected macrocycle. The latter was deprotected with 30% HBr/HOAc to give the macroheterocycle as the tetrabromide salt, which was converted into its free base with methanolic KOH (66% yield).

For membrane electrode preparation, a sensor solution was obtained by dissolving 0.0453 g of the ionophore in 0.1517 g of dibutylphthalate (DBP) as a mediator solvent. An amount of about 0.0020 g of tetraoctylammonium chloride (TOAC) as a lipophilic membrane additive was added to the previously prepared solution. The sensor system was immobilized in 0.15 g of PVC previously dissolved in 6 cm³ of tetrahydrofuran (THF). Conventionally shaped electrodes were prepared in accordance to the technique described elsewhere (11) by applying dropwise the membrane solution on a graphite conductive support until obtaining a film with a thickness of about 3 mm. The constructed electrodes were conditioned by soaking in 10⁻¹ mol L⁻¹ potassium nitrate solution and when not in use were stored in a solution with a concentration of 10⁻⁴ mol L⁻¹.

General Procedures: Potentiometric Determination of Nitrate in Vegetables. To accomplish nitrate determinations in real samples, an accurately weighed sample of about 1 g of previously homogenized and fully dried (65 °C) vegetable was extracted in 100 mL of deionized water during 30 min at room temperature. The extract was filtered over a 100 mL volumetric flask and was made up to volume with deionized water.

For the potentiometric determination, an aliquot of 50.0 mL of the extracted sample was pipetted directly into a 100 mL beaker, 5 mL of ISA solution was added, and the nitrate-selective and reference electrodes were immersed. The nitrate concentration was determined by direct potentiometry.

The brucine spectrophotometric method, with slight modifications, was adopted as a reference method because this method resulted to be adequate in the determination of nitrate in carbon black (12), tobacco (13), and meat products (14). While the extracted samples of vegetables are colored, an error in the determination of the absorbance can arise. To circumvent this problem, active carbon was added to the sample extracts before the analysis. The samples were filtered through an acid-washed filter paper to avoid contamination by trace nitrate contained in filter papers (15).

Potentiometric Determination of Nitrate in Bottled Mineral Waters. A volume of 100 mL of the water sample was transferred into a beaker, and its pH was adjusted to a value of 3.5 by means of a diluted sulfuric acid solution. The sample was boiled during 10 min and after cooling was transferred to a 100 mL volumetric flask and was made up to volume with deionized water. A volume of 50.0 mL was pipetted into a beaker, and after adding 5 mL of the same ISA solution and immersing the electrodes, the nitrate concentration was determined as in the previous procedure.

The brucine spectrophotometric method was also used as a reference method (16). For water samples, whose nitrate content was lower than 1.0 mg L⁻¹, a cell with a path length of 10 cm was used.

RESULTS AND DISCUSSION

Because the potentiometric response of the nitrate-selective electrodes based on mobile carriers depends on the concentration of the ionophore and also on the amount of the lipophilic cationic additive incorporated within the membrane, several membrane compositions were studied varying their proportions. The best response in terms of slope, reproducibility, and lower limit of detection was obtained for the membrane with a composition of 42.98% (w/w) PVC, 12.98% (w/w)

Table 1. General Working Characteristics of the Nitrate ISE

Characteristics		
lower limit		4.2×10^{-6} ;
of linear response		$(8.2 \times 10^{-7}$,
(mol L ⁻¹)		without ISA)
practical limit		3.4×10^{-6} ;
of detection		$(6.9 \times 10^{-7}$,
(mol L ⁻¹)		without ISA)
slope (mV log ⁻¹ C)		60 ± 0.1 ;
		$(54.8 \pm 1.2,$
		without ISA)
response stability		± 0.3
(mV day ⁻¹)		
response time (s)		
10^{-5} – 10^{-4} mol L ⁻¹		12
10^{-4} – 10^{-3} mol L ⁻¹		11
10^{-3} – 10^{-2} mol L ⁻¹		10
working pH range		
10^{-4} mol L ⁻¹		2.5–7.0
10^{-3} mol L ⁻¹		2.0–8.5
10^{-2} mol L ⁻¹		2.0–12.0
lifetime (months)		12

Potentiometric Selectivity Coefficients [$\log(K_{\text{NO}_3^-}^{\text{pot},X})$]			
X	10^{-4} mol L ⁻¹	10^{-3} mol L ⁻¹	10^{-2} mol L ⁻¹
SO ₄ ²⁻	-4.5	-5.3	-5.9
F ⁻	-2.5	-3.3	-4.3
Cl ⁻	-2.1	-2.5	-2.7
Br ⁻	-1.2	-1.3	-1.3
I ⁻	-1.4	-0.2	+1.6
HCO ₃ ⁻	-0.8	-1.5	-2.2
Ac ⁻	-1.0	-1.8	-2.0
NO ₂ ⁻	-1.8	-1.9	-1.8
PO ₄ H ₂ ⁻	-1.3	-2.2	-3.1
PO ₄ H ²⁻	-2.8	-3.2	-3.4
ClO ₄ ⁻	+1.8	+1.9	+1.9
IO ₄ ⁻	+1.8	+1.9	+1.9
BF ₄ ⁻	+1.1	+1.2	+1.2
SCN ⁻	+1.5	+1.7	+1.8

[3.3.3.3]oxazane, 43.47% (w/w) DBP, and 0.57% (w/w) TOAC. Membranes prepared with DBP instead of *o*-nitrophenyloctyl ether as a mediator solvent give rise to electrodes with increased analytical response ranges and higher slope values. Membranes prepared with an anionic lipophilic additive such as tetraphenylborate did not give any answer toward nitrate, which lead to confirm a neutral mechanism of response for this kind of ionophore. Therefore, a cationic additive as TOAC was used in the posterior membrane preparations. This membrane was, therefore, used to evaluate the working characteristics of the nitrate electrode, which are summarized in **Table 1**.

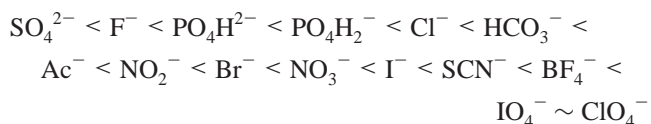
The electrode response characteristics were evaluated on the basis of repeated calibration curves obtained by measuring the potential values of a series of nitrate solutions with concentrations between 10^{-7} and 10^{-1} mol L⁻¹, covering the linear and nonlinear response range. The experiments were performed in solutions with or without the ionic strength being adjusted with ISA solution. The lower limit of the linear response, the practical limit of detection, the slope, and the reproducibility of the potential values of the electrodes were established according to the International Union of Pure and Applied Chemistry (IUPAC) convention (17). **Table 1** shows the values obtained for the general operating characteristics of nitrate electrode under the aforementioned conditions. The data presented correspond to the average of six values obtained in two determinations with each of three electrodes. When the results were compared with those commercially available and the majority of the “home-made” electrodes, an improvement in the lower limit of the linear range (at least one-half of an order of magnitude) was

obtained. The limit of detection obtained results in a similarity to the best values recently reported in the literature by Le Goff et al. (18) or by Zuther and Camman (19), who used sensor membranes based on *N,N,N*-triallyl leucine betaine hydrochloride or modified quaternary ammonium salts, respectively.

The response time was determined by spiking a dilute solution (10^{-5} , 10^{-4} , and 10^{-3} mol L⁻¹) with a more concentrated one to obtain a 10 times more concentrated solution, and recording the time required for a stable potential (± 0.1 mV). The records obtained showed a short response time, inferior to 12 s, and high stability of the potentials, which is important for direct potentiometric determinations. The electrodes showed an initial drift of potential, and the response stability appeared after the nitrate ISE had been conditioned for 3 days in 10^{-1} mol L⁻¹ solution of the primary ion. Theoretically, in the all-solid-state PVC membrane electrodes without internal reference solution, there is no well-defined internal reference potential system (20). In practice, however, these electrodes provide reproducible potentials. The establishment of a constant electrode potential requires a period to stabilize the internal reference potential in the graphite conductive epoxy-PVC boundary, by means of the O₂/H₂O coupled as previously suggested (21, 22).

The extent of the proton and hydroxide interferences was evaluated by measuring, at different pH values, the potentials of the electrodes in solutions of constant concentration of the primary ion (10^{-4} , 10^{-3} , and 10^{-2} mol L⁻¹). The pH was adjusted with dilute sodium hydroxide and sulfuric acid solutions. The Reilly diagrams obtained evidenced pH operational ranges between 2.0 and 12.0 for the largest concentration of nitrate solution tested; however, for lower concentrations, the pH operational range diminishes, despite that this range covers the pH of the majority of the matrix in which nitrate is habitually determined. Hence, the adjustment of the pH of the samples before analysis will be only necessary in some applications at very low nitrate concentrations in basic media.

The potentiometric selectivity coefficients were determined for three different concentration levels of the primary ion together with the same concentration of an anion interfering (10^{-4} , 10^{-3} , and 10^{-2} mol L⁻¹), without ionic strength adjustment, following the separated solution method previously reported (17). From the results obtained (**Table 1**), it is possible to state that the selectivity pattern of this new nitrate-selective electrode for several interfering anions is as follows:



As can be seen, the electrode exhibited roughly the same selectivity sequence as that of the Hofmeister lyotropic series characterized by the solubility properties of anions in organic membrane moieties. This behavior is similar to the majority of the nitrate-selective electrodes previously reported, mainly those based on ion exchangers, with the exception of the electrodes that show evidence of some kind of chemical recognition of the nitrate by the ionophore, such as suggested by Hutchins and Basas (23).

Unfortunately, the comparison of the results obtained with those collected from the manufacturers and other authors (Tables 2 and 3 of the Supporting Information) is difficult because of the variation of determination procedures or experimental conditions adopted. However, it can be confirmed that the new electrode exhibits a relatively low interfering effect of chloride because the concentration of this ion can be 500 times that of

Table 2. Comparative Study of the Precisions and Accuracies Attained in the Determination of Nitrate in Different Types of Vegetables and Bottled Mineral Waters (BW) by Simultaneous Application of the Proposed Potentiometric (ISE) and Reference Methods (UV–Vis)

sample	UV–vis method			ISE method			RE ^d	F
	X ^a	CV ^b	R ^c	X ^a	CV ^b	R ^c		
artichoke	3.97 ± 0.15	3.8	99.7	4.05 ± 0.16	3.9	100.6	+1.9	0.88
broad bean	1.86 ± 0.04	2.1	98.0	1.89 ± 0.02	1.1	100.3	+1.6	4.00
cabbage	2.16 ± 0.05	2.3	101.3	2.22 ± 0.03	1.3	100.0	+2.4	2.78
carrot	0.74 ± 0.02	2.7	101.8	0.76 ± 0.02	2.6	101.4	+2.4	1.00
celery	15.17 ± 0.17	1.1	101.7	15.02 ± 0.13	0.9	101.6	-1.0	1.71
cucumber	1.69 ± 0.04	2.4	101.3	1.65 ± 0.03	1.8	101.3	-2.4	1.77
endive	0.65 ± 0.01	1.5	102.4	0.66 ± 0.01	1.5	98.6	+1.5	1.00
lettuce	5.78 ± 0.09	1.5	101.7	5.62 ± 0.06	1.1	101.7	-2.8	2.25
parsley	12.13 ± 0.15	1.2	98.9	11.90 ± 0.11	0.9	101.1	-1.9	1.86
pea	6.09 ± 0.12	2.0	99.2	6.39 ± 0.07	1.1	101.1	+4.6	2.94
radish	13.18 ± 0.14	1.1	98.6	12.98 ± 0.12	0.9	101.4	-1.5	1.36
spinach	8.07 ± 0.12	1.5	101.6	7.93 ± 0.10	1.3	101.6	-1.6	1.44
BW1	0.32 ± 0.01	3.1	98.0	0.31 ± 0.01	3.2	98.6	-3.2	1.00
BW2	3.89 ± 0.10	2.5	100.9	3.86 ± 0.08	2.1	100.2	-0.8	1.56
BW3	1.62 ± 0.06	3.5	102.3	1.58 ± 0.05	3.2	99.7	-2.5	1.44
BW4	2.77 ± 0.09	3.2	101.1	2.88 ± 0.10	3.5	101.9	+4.0	1.23
BW5	1.16 ± 0.04	3.4	102.0	1.13 ± 0.04	3.5	99.8	-2.6	1.00
BW6	1.20 ± 0.05	4.2	98.9	1.25 ± 0.03	2.4	100.7	+4.2	2.78
BW7	0.42 ± 0.02	4.8	98.8	0.44 ± 0.02	4.5	99.0	+4.8	1.00
BW8	2.05 ± 0.06	2.9	100.5	2.01 ± 0.04	2.0	98.9	-1.9	2.25
BW9	1.80 ± 0.07	3.9	99.2	1.81 ± 0.04	2.2	100.4	+0.5	3.06
BW10	2.06 ± 0.05	2.4	99.7	2.08 ± 0.03	1.4	99.9	+1.0	2.78
BW11	0.51 ± 0.03	5.9	103.1	0.50 ± 0.02	4.0	102.0	-2.0	2.25
BW12	0.39 ± 0.02	5.1	102.9	0.41 ± 0.01	2.4	101.7	+5.1	4.00

^a Mean nitrate concentration and standard deviation (g/kg⁻¹) for vegetable samples and (mg L⁻¹) for water samples. ^b Coefficient of variation (%). ^c Mean percentage of spike recovery (%). ^d Relative error of the potentiometric method versus the reference method (%). The critical F-value, considering a 95% confidence level and 10 degrees of freedom, for a two-tailed test is 3.72.

the nitrate concentration in the sample. The worst selectivity coefficients were toward perchlorate, periodate, tetrafluoroborate, iodide, and thiocyanate ions, which also are the main interference of the majority of the nitrate-selective electrodes previously constructed.

The variation in the working characteristics with time was used as a criterion for evaluating the lifetime of the electrodes. The units when in regular use were stored in a 10⁻⁴ mol L⁻¹ potassium nitrate solution. All of them had a lifetime generally greater than 12 months, which is longer than the durability of electrodes whose membranes are based on mobile carrier sensors but constructed using an internal reference solution. This is probably due to the absence of just a single contact surface between the membrane and solution, instead of two as in conventional electrodes; that is, the area through which leaching occurs is halved. Moreover in the conventional assembly there is a net hydrodynamic pressure, favoring the loss of the sensor to the external solution (24).

Analytical Applications. To evaluate the analytical usefulness of the constructed electrodes, it was decided to devise simple and quick procedures for determining the nitrate concentration in different types of vegetables and mineral waters. Vegetable samples were stirred in deionized water at room temperature after different extraction times followed by the nitrate analysis using the brucine method. From the plots obtained, it was concluded that 30 min was adequate for complete nitrate extraction. When the approximate ionic composition and the pH of the vegetable-extracted samples, the corresponding Reilly diagrams, and the selectivity coefficients obtained for the electrodes were taken into account, only the addition of an ISA solution was carried out. In the case of water samples, adjusting their pH at a value of 3.5 and boiling for 10 min were sufficient to avoid the interference of hydrogencarbonate. Because nitrate solution is a culture medium for bacteria

and algae, boric acid could be added to prevent biological growth if samples need to be stored.

Hence, 12 different types of vegetable samples were extracted and 12 bottled mineral waters commercialized in France, Portugal, and Spain were treated. The resulting solutions were simultaneously analyzed by the spectrophotometric method and by direct potentiometry. In each case, the precisions were evaluated by their application to 11 samples of the same product and were expressed in terms of their standard deviations and coefficients of variation. Standard nitrate additions were used to evaluate the accuracies of the methods, and the average percentages of spike recovery, for three different additions, were calculated. The data obtained (Table 2) confirm that the potentiometric methods developed for vegetable and water analysis had good precision and accuracy, because the mean coefficients of variation and the average percentages of spike recovery were 1.5 and 100.9% for vegetables and 2.9 and 100.2% for bottled mineral waters, respectively. To test whether the potentiometric and reference methods differ in their precision, a significance (two-tailed) *F* test was carried out. The calculated *F* values, using ISE, were less than the critical *F* value for the majority of the vegetables and water samples analyzed. Hence, there is no significant difference between the two standard deviations at the 95% confidence level (25). The limits of detection of the proposed methods were obtained as recommended by the Analytical Methods Committee (26) and were found to be 0.05 g kg⁻¹ for vegetables and 0.25 mg L⁻¹ for bottled mineral waters.

Finally, 32 samples of different types of vegetables and 22 bottled mineral waters were simultaneously analyzed by the potentiometric and reference methods. From the results obtained, it can be concluded that the nitrate content in vegetables ranged, with a large distribution, from 0.5 to 17.2 g kg⁻¹, with an average value of 5.3 g kg⁻¹, and the concentrations ranged from

0.21 to 8.5 mg L⁻¹, with an average value of 1.8 mg L⁻¹, for water samples. The percentages of difference obtained between the potentiometric and reference methods ranged from -3.3 to +4.6, with an average value of -0.4, for vegetable samples, and from -0.4 to +5.8, with an average value of -2.4, for water samples. The linear regression analysis of the results obtained showed the following equations:

for vegetables

$$C_{UV-vis} = (0.999 \pm 0.004)C_{ISE} + (0.014 \pm 0.029)$$

$$r = 0.9997 \quad t = 244.89$$

for waters

$$C_{UV-vis} = (1.005 \pm 0.004)C_{ISE} - (0.025 \pm 0.036)$$

$$r = 0.9996 \quad t = 158.05$$

The confidence limits of the slope intercepts of the regression lines and the *t* values for the correlation coefficients at a 95% confidence level and *n* - 2 degrees of freedom were also determined. From these, the calculated slopes and intercepts do not significantly differ from the ideal values of 1 and 0, respectively. Thus, there is no evidence of systematic difference between the proposed methods and the reference one. From the correlation coefficients, the calculated *t* values were greater than the tabulated *t* values (*t* = 2.04 for *n* = 32 and *t* = 2.09 for *n* = 22), hence showing that a significant correlation exists between both methods (25).

Supporting Information Available: Four tables concerning the main features of previously developed nitrate-selective electrodes as well the corresponding references. This material is available free of charge via the Internet at <http://pubs.acs.org>.

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