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Disposable Receptor-Based Optical Sensor for Nitrate

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A new optical absorption-based disposable sensor for nitrate is described. The nitrate-sensitive element is a bicyclic cyclophane receptor next to a suitable pH-sensitive lipophilic dye immobilized in a plasticized polymeric membrane. The rigid amide-based receptor with C_3 symmetry controls the anion selectivity pattern of the optical element. The optical selectivity coefficients obtained for nitrate over a variety of naturally occurring anions in natural waters meet the requirements for the determination of nitrate in waters. The disposable sensor responds to nitrate rapidly—the typical response time is 5 min—and reversibly over a wide dynamic range (26 μM –63 mM) with sensor-to-sensor reproducibility (relative standard deviation, RSD, 3.68%, as $\log \alpha_{\text{NO}_3^-}$, at the medium level of the range and RSD 1.39% for repeated measurements with the same sensor). The performance of the optical disposable sensor was tested for the analysis of nitrate in different types of natural waters (river, well, spring), validating results against a reference procedure. The proposed method is quick, inexpensive, selective, and sensitive and uses only conventional instrumentation.

Anion recognition is a growing area of particular interest in supramolecular chemistry because of the challenge it brings when compared with cations (size, form, geometry, interactions, pH dependence, solvent effects) as well as its importance in environmental monitoring, medicinal diagnosis, and biological analysis.^{1,2}

Nitrate is an anion with a basic and weakly coordinating character, so selective recognition of nitrate requires both size and shape complementarity in the host. Different synthetic receptors have been studied for nitrate recognition mainly through hydrogen bond detaching. Here, the binding groups are arranged in a convergent and rigid manner using receptors with C_3 symmetry to match the functionality of the nitrate, as with the amide-based receptor prepared by Anslyn³ or the thiourea-based receptor by Herges.⁴ In other cases, both hydrogen bond-donating amides and an electrostatic contribution from the metal center is

used for nitrate recognition, such as with platinum(II) nicotinamide.⁵ Interaction via hydrogen bonding with protonated amines serves to complex two nitrates, such as with the C_3 symmetry bicyclic macrocycle obtained from condensation between tren and dicarboxaldehydes.⁶ Electrostatic interaction has been used also for nitrate recognition using quaternary ammonium hosts^{7–10} or cationic complexes such as Ni(II)–phenanthroline complex,¹¹ although selectivity is influenced by hydrophobicity according to the Hofmeister series,¹² which results in strong interference from anions of similar hydration energy, such as nitrate and chloride. Metal or Lewis acid coordination are other types of noncovalent interaction used to complex the anionic guest, such as the highly charged Pt complex, which encapsulates nitrate through Lewis acidic Pt atoms,¹³ or macrotricyclic Pd(II) metallocage, which in this case encapsulates nitrate through Pd atoms.¹⁴

The analytical use of these nitrate receptors is very limited. One citation is a spectrophotometric competition assay proposed for nitrate based on the displacement of an anionic dye, such as methyl red and resorufin, complexed with the receptor upon the addition of nitrate;¹⁵ however, the use of $\text{CH}_3\text{OH}/\text{CH}_2\text{Cl}_2$ (1:1) as the solvent prevents its use in real samples.

In any application of the above receptors to analysis the important role played by solvent effects in controlling anion binding, strength and selectivity must be considered.¹⁶ For any application to real-life samples, which will be mostly aqueous, the competition between the neutral receptor and solvated anions in homogeneous solutions can lead to no reaction or to a small

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displacement. The use of membrane-based schemes makes it possible to sense anions in aqueous solutions.

Nitrate ion is ubiquitous in environmental, food, industrial, and physiological systems, but the common sources of contamination in water are runoff from fertilizer use, leaching from septic tanks, sewage, and erosion of natural deposits.¹⁷ Nitrate can cause several sanitary problems¹⁸ among which the most serious include infant methemoglobinemia (or blue baby syndrome), especially in babies still on a milk diet, and the formation of nitrosamines upon reaction with secondary amines in food products, which is possibly related to the appearance of stomach cancer. These problems have been widely recognized, and as a consequence, statutory frameworks aimed at controlling their level within the wider environment, especially in water, have been established: one MCL of 10 mg/L as nitrogen in the United States¹⁹ and 50 mg/L as nitrate in the European Community countries.^{20,21}

Different analytical strategies have been employed to facilitate the detection, determination, and monitoring of nitrate²² mostly in water. These include disposable sensors, miniaturized self-contained analytical devices with the necessary reagents incorporated in the element in a dry format, which respond to the presence or concentration of an analyte, usually through a chemical or biochemical reaction. Electrochemical disposable sensors include capillary-fill device-type amperometric sensors,²³ based on the reduction of nitrate on a freshly prepared copper electrode from copper sulfate and a potassium hydrogen sulfate screen printed on the upper plate within the device.²⁴ Two types of potentiometric disposable sensors are described for nitrate: ion-selective electrodes prepared using screen-printing technology^{9,10} and potentiometric devices prepared by depositing the ion-sensitive polymer on a field-effect transistor gate insulator of ref 25. Tridodecylmethylammonium nitrate is the usual receptor, and here the selectivity follows the Hofmeister pattern.

Visual sensors for nitrate—test strips—are based on reduction to nitrite and the formation of a colored azo dye with the reagents adsorbed²⁶ or chemically immobilized on cellulosic supports.²⁷ Techniques used for qualitative or semiquantitative concentration determination are as follows: comparing the strip with a standardized color chart²⁸ or measuring the length or diameter of the colored zone of a polymer-sealed test strip.²⁷ Nitrate test strips in very different formats, mono- or multianalyte, are supplied by different companies such as Merck, Industrial Test Systems, Macherey-Nagel, Orion AQUAFast, Hach, Agri-Screen, CHEMetrics, and EnviroEquip among others for different applications such as water, soils, sap, plant tissues, forages, wines, fruit juices,

and whey. Optical disposable sensors for nitrate are based on the same reactions as visual ones, using measurement of an optical property, usually diffuse reflectance with free-standing instruments²⁷ or hand-held dedicated devices, as is the case with the pocket-sized digital Nitrachek reflectometer²⁹ and the Reflectoquant system from Merck.³⁰

The use of nitrate receptors or ionophores can improve conventional test strips for nitrate in different ways: (1) by not requiring reduction to nitrate or the formation of colored dyes, since in addition to the toxicity associated with nitrates, the reagents present in the commercialized reagent strips contain amines that are toxic in nature; (2) by increasing the dynamic range usually present in test strips for nitrate; (3) by easily determining nitrate concentration, while the majority of commercial strips only offer semiquantitative information; (4) by being reversible both because of the possibility of reuse that they offer and because of their advantage in error reduction.

The development of a highly selective optical disposable sensor procedure for nitrate based on a C₃ symmetry amide-based receptor with enhanced analytical characteristics over earlier disposable sensors is described.³¹

EXPERIMENTAL SECTION

Reagents. The chemicals used were of analytical reagent grade, and all aqueous solutions were prepared using reverse-osmosis-type quality water produced by a Milli-RO 12 plus Milli-Q purification system (Millipore, Bedford, MA).

Stock solutions (1 M) were prepared in water from potassium salts of nitrate, iodide, bromide (Merck, Darmstadt, Germany), sulfate (Panreac, Barcelona, Spain), and chloride (Sigma-Aldrich Química S.A., Madrid, Spain) and from sodium salts of perchlorate (Panreac), thiocyanate, nitrite, hydrogen carbonate, and acetate (Sigma-Aldrich). A 0.02 M concentration of a pH 6.0 buffer solution was prepared from sodium dihydrogen phosphate and sodium monohydrogen phosphate (Sigma-Aldrich).

For preparing the nitrate-sensitive films, high molecular weight poly(vinyl chloride) (PVC), tributyl phosphate (TBP), tris-(2-ethylhexyl) phosphate (TEHP), dioctyl phthalate (DOP), bis-(2-ethylhexyl) sebacate (DOS), 2-nitrophenyl octyl ether (NPOE), and tetrahydrofuran (THF) were purchased from Sigma-Aldrich. The chromoionophore *N,N*-diethyl-5-(octadecanoylimino)-5-benzotriazin-2-amine was synthesized, purified, and characterized by us according to ref 32. Sheets of Mylar-type polyester (Goodfellow, Cambridge, U.K.) were used as support.

Ionophore Synthesis. The synthesis of ionophore 2,16,18,32,45,47-hexaethyl-5,13,21,29,34,42,44,46,48-nonaazaheptacyclo-[15.15.11.1.3.31.1.7.11.1.15.19.1.23.27.1.36.40]-octatetraconta-1,3(45),7(48),8,10,15(47),16,18,23(46),26,31,36,37,39-pentadecaene-6,12,22,28,35,41-hexone was made according to the two-step procedure described by Anslyn et al.^{15,33} but using an alternative way to synthesize the intermediate 1,3,5-tris(aminomethyl)-2,4,6-triethylbenzene (Figure 1). The nucleophilic displacement of tris-

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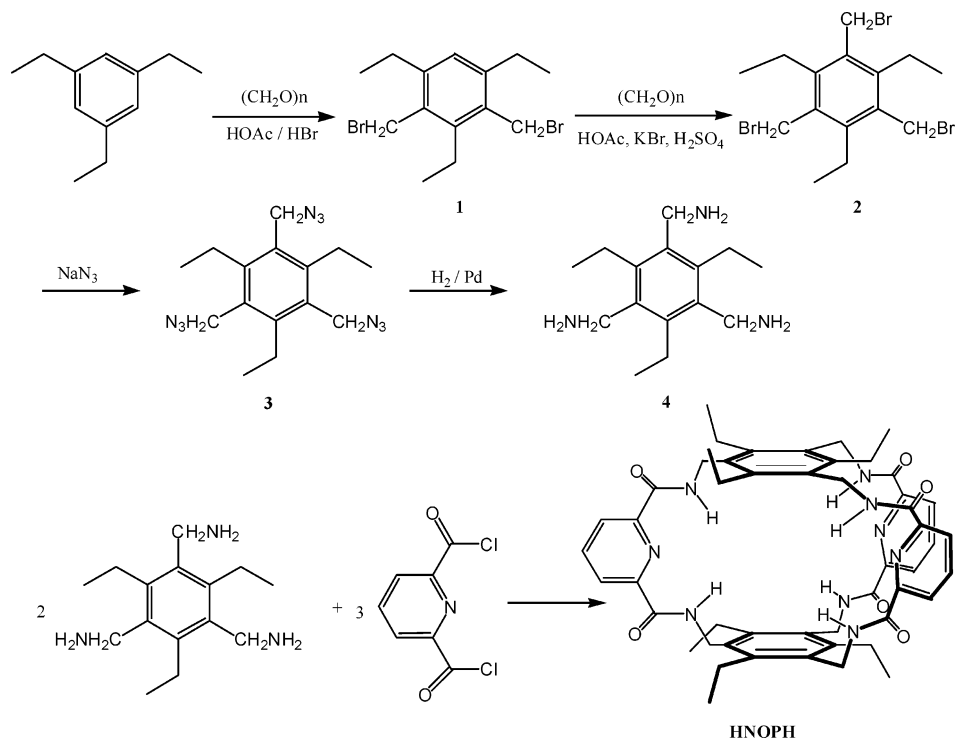


Figure 1. Synthesis of HNOPH ionophore.

(bromomethyl) derivative **2** with sodium azide to form the triazide derivative **3** was carried out using a hydro alcoholic mixture (ethanol/water 9:1) at reflux temperature. The transformation of triazide into triamine derivative **4** was not done with triphenylphosphine as described by Anslyn et al. but by means of reduction with hydrogen–Pd–C. Characterization of the intermediate and synthesized ionophore was made by ^1H NMR, ^{13}C NMR, IR, and HRMS (FAB +), obtaining data identical to that reported.

Preparation of Disposable Membranes and Measurement Setup. The membranes were produced on a polyester substrate using a spin-coating technique. Mixtures for the preparation of nitrate-sensitive membranes were made from a batch of 29.20 mg (32.2 wt %) of PVC, 58.80 mg (65.0 wt %) of TBP, 1.50 mg (1.7 wt %) of HNOPH, and 1.00 mg (1.1 wt %) of *N,N*-diethyl-5-(octadecanoylimino)-5*H*-benzo[*a*]phenoxazine-9-amine dissolved in 1 mL of freshly distilled THF. The disposable sensors were cast by placing 15 μL of the cocktail on a 14 mm \times 40 mm \times 0.5 mm thick polyester sheet using a homemade spin-coater³⁴ rotating at 180 rpm. After spinning for 10 s, the membrane was removed from the spin-coater and dried in a dryer with saturated THF atmosphere for 10 min at room temperature. The sensing area of the disposable sensor is a transparent and red 6-mm-o.d. circular film with a calculated thickness of $\sim 4.5\ \mu\text{m}$ and a concentration of ionophore and chromoionophore in a dry thin film of $1.71\ \text{mmol}\cdot\text{kg}^{-1}$ for both chemicals. It is also possible to prepare the disposable sensors in cellulosic or in clothlike supports by a two-bath process for buffer and cocktail with the advantages of simplicity and response time.

The response of the disposable sensors was evaluated by adding 8 mL of test solution to a polyethylene plastic tube together

with 2 mL of pH 6.0 buffer solution. The disposable sensor was then introduced for 5 min into the tube without shaking. After reaching equilibrium, the membrane was pulled out of the solution, wiped to remove any solution droplets, and its absorbance was measured at 660 nm in a Hewlett-Packard diode array spectrophotometer (model 8453; Nortwalk, CT) provided with a 44-mm-high, 12-mm-wide homemade membrane cell holder made of a matte black painted iron block.³⁵ To correct for the background absorbance, the measurements were made against a Mylar polyester strip. All measurements were carried out at room temperature. The membranes were not conditioned before use. The maximum and minimum absorbance values, corresponding to the fully protonated (A_{H^+}) and deprotonated (A_{C}) forms of the chromoionophore necessary for calculation of α values, were measured by conditioning the disposable sensors in $10^{-2}\ \text{M}$ HNO_3 and $2 \times 10^{-2}\ \text{M}$ NaOH , respectively. Activities were calculated according to the two-parameter Debye–Hückel formalism.³⁶ The coextraction constants $K_{\text{e}1}$, $K_{\text{e}2}$, and $K_{\text{e}3}$ were calculated according to ref 37 using values in the maximum slope zone of the experimental response functions for nitrate, which provided more information and less error, with disposable sensors prepared with ($K_{\text{e}1}$ and $K_{\text{e}3}$) and without ionophore ($K_{\text{e}2}$). The selectivity coefficients $K_{\text{NO}_3^-}^{\text{opt}}$ were determined with separate solutions of the interfering ions (SSM).^{38,39} Software programs used for the treatment of the data and fitting of experimental data to theoretical response function were as follows: Statgraphics software package

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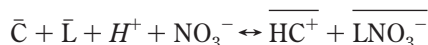
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RESULTS AND DISCUSSION

The sensing mechanism used in this disposable sensor relies on a coextraction process between a bulk membrane of plasticized PVC and the aqueous problem containing the analyte.⁴⁰ Although reversibility is not strictly necessary for a disposable sensor, this system provides advantages in terms of reversibility and also creates an ample measuring range and a short response time. In this mechanism, the selective binding of nitrate by the ionophore L is accompanied by protonation of the chromoionophore C to maintain the electroneutrality of the membrane, which generates the analytical signal. In this case, the use of lipophilic ionic additives is not required for extraction considering the neutral character of both the ionophore and basic form of chromoionophore, as is observed experimentally. The following coextraction equilibrium holds, if it is assumed that LNO_3^- and HC^+ are the species produced:



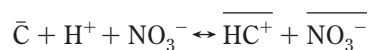
in which barred species are in membrane phase, while all other chemicals are in aqueous solution.

The description of the disposable sensor response characteristics can be accomplished through the protonation degree α ($[\text{C}]/[\text{C}_\text{C}]$) measured by the absorbance of the protonated form of chromoionophore, which is the measurable species in membrane phase, as a normalized absorbance.⁴⁰ The α value is related to the coextraction constant K_{e1} , the analytical concentrations of ionophore C_L and chromoionophore C_C , and the activities of NO_3^- and H^+ in the aqueous sample through the response function:

$$K_{\text{e1}} = \frac{(1 - \alpha)^2}{\alpha \left(\frac{\text{C}_\text{L}}{\text{C}_\text{C}} - (1 - \alpha) \right) a_{\text{NO}_3^-} a_{\text{H}^+}} \quad (1)$$

Accordingly, adjusting the pH with an appropriate buffer makes it possible to optically determine nitrate activity in solution.

In the absence of ionophore in the membrane, an extraction of nitrate to the sensing membrane is observed due to another coextraction process, nitrate and proton, due to the presence of the basic chromoionophore in membrane according to



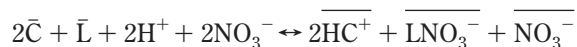
Analogously, the process can be characterized by a coextraction constant K_{e2} according to

$$K_{\text{e2}} = \frac{1 - \alpha}{\alpha a_{\text{H}^+} a_{\text{NO}_3^-}} \quad (2)$$

The selected ionophore HNOPH is a rigid bicyclic cyclophane¹⁶ proposed by Anslyn's group and obtained by condensation of 1,3,5-tris(aminomethyl)-2,4,6-triethylbenzene with 3 equiv of 2,6-pyridinedicarbonyl dichloride that produces a C_3 symmetric host with six amide groups converging in the center of a binding pocket.¹⁵ The trigonal prismatic array of this ionophore is able to coordinate to the π -electron system of planar nitrate within the cavity of the box forming an 1:1 complex.³

N,N-Diethyl-5-(octadecanoylimino)-5*H*-benzo[*a*]phenoxazine-9-amine was used as chromoionophore, taking into consideration its acidity characteristics and the pH requirements of the whole process. To select the plasticizer, we conducted a preliminary study with different plasticizers (TBP, TEHP, NPOE, DOS, DOP), monitoring the response signal over NO_3^- , Cl^- , and SO_4^{2-} , reversibility, and response time. DOP and DOS produced a small and nonreversible response (12 and 5% of the highest signal) and very similar for the three anions studied. On the contrary, NPOE is more selective, giving a higher response (75%), which is reversible but very slow. Phosphate esters gave high signals (TBP 100%, TEHP 83%), which were also selective and reversible, with less response time, but in this case, the signals for TBP were highest and fastest, and TBP was thus used as plasticizer for all subsequent experiments.

As the coextraction scheme indicates, the response of the disposable sensor depends on proton activity. An increase in proton activity displaces quantitatively the response to lower nitrate activity (Figure 2A) but also increases the coextraction of nitrate in membranes without ionophore (Figure 2B) in good agreement with both theoretical models. Moreover, at low pH values, the response of the membranes containing ionophore did not shift quantitatively with pH as indicated by the theory, which states that the product of nitrate and hydrogen ion activity in solution must produce a single theoretical response curve. At pH values lower than 5.0, the response function shifted to the right of the theoretical response function, which implies that the α values are lower than predicted by the theory, so, the fraction of protonated chromoionophore is higher than expected and with a higher slope. An additional coextraction process without ionophore might be taking place at proton activities from 10^{-5} and higher. This mixed model in membranes containing ionophore takes into consideration the coextraction of nitrate and proton, but not all the nitrate must be complexed with ionophore, according to



a process characterized by a coextraction constant K_{e3} :

$$K_{\text{e3}} = \frac{(1 - \alpha)^3}{\alpha^2 \left(\frac{\text{C}_\text{L}}{\text{C}_\text{C}} - (1 - \alpha) \right) a_{\text{NO}_3^-}^2 a_{\text{H}^+}^2} \quad (3)$$

Indeed, as shown in Figure 2, the response curves for $\text{pH} \leq 5$ fit to this mixed model, which accounts for the intermediate slope observed between that of the models with and without ionophore.

At a pH value from 6 on, the coextraction model given by eq 1 works well and the sensing system shows enough sensitivity to determine nitrate in water together with minimization of the nitrate

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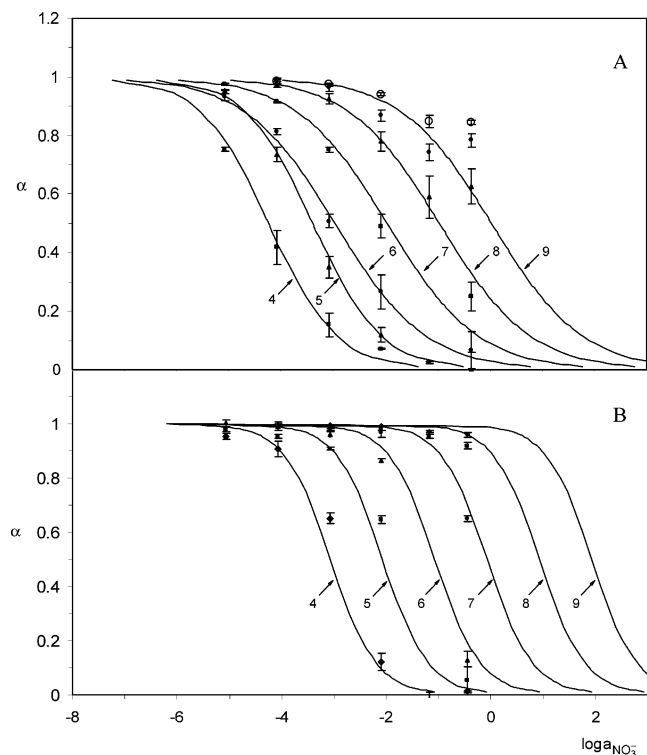


Figure 2. Effect of pH on disposable sensor response at the following pHs: (4) 4.0, (5) 5.0, (6) 6.0, (7) 7.0; (8), and (9) 9.0. (A) with ionophore; (B) without ionophore. Open circles in the top figure are tetramethylammonium nitrate response at pH 9.

coextraction without ionophore (no answer is observed for nitrate concentration lesser than 10^{-2} M) in agreement with the coextraction constants calculated: K_{e1} , 9.06 ± 0.04 ; K_{e2} , 6.90 ± 0.08 . Additionally, the slope for disposable sensors containing ionophore is half of that without ionophore as the models predicted, which shifts, in this case, the dynamic range to lower nitrate concentrations. For pH higher than 7, the response to nitrate decreases due to the low proton concentration, and the remainder of the experiments were thus performed at pH 6.0.

From the horizontal distance of the two response curves at half-protonation, obtained at pH 6.0 for membranes with and without ionophore, it is possible to estimate the complex formation constant of nitrate complex with HNOPH within the solvent polymeric membrane phase, using the procedure proposed by Bakker et al.⁴¹ We accept the assumptions indicated there and suppose that complexation with ionophore, forming a 1:1 complex, is the prevalent process at this pH. The value found ($\log \beta_{\text{LNO}_3^-} = 3.8 \pm 0.1$), although not very high, is higher than that reported for the same complex in bulk solution with solvents of increasing polarity. Namely, a value of 2.48 has been reported using ^1H NMR titration in $\text{CD}_2\text{Cl}_2/\text{CD}_3\text{CN}$ (25:75 v/v) as solvent³ (dielectric constant estimated, 31; dielectric constant of plasticizer TBP, 8), as is usual considering the different free energies of solvation of nitrate in each solvent.

At high pH values and high nitrate concentrations, there is a strong deviation from the theoretical behavior predicted for the membrane with the ionophore model (Figure 2A), with high

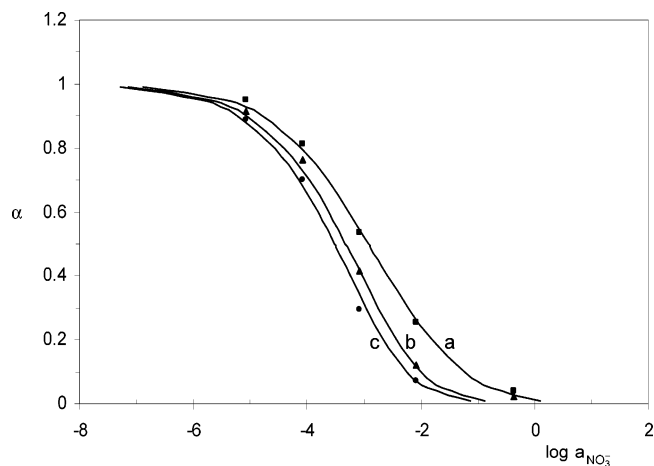


Figure 3. Displacement of response function with C_L/C_C ratio. (a) 1:1; (b) 2:1; (c) 3:1.

experimental α values observed and, thus, low protonation of basic chromoionophore. This could be explained considering that given that the proton activity at basic pH is low, the feasibility of chromoionophore protonation needed to counteract nitrate complexation in the membrane is also low, and therefore, the counterion present in solution, potassium in this case, is extracted. This behavior makes nitrate extraction possible, but creates a cation interference. Such behavior is not very common with bulk optodes but has been observed in some instances, such as by Ceresa et al. in a sensor for chloride based on [9]mercuracarborand-3 ionophore.⁴² Experiments performed with nitrate standard solution, in which the nitrate counterion potassium has been replaced by the more lipophilic tetramethylammonium, show a response curve even more shifted and confirm the cation interference (see Figure 2).

The scheme used for the development of a disposable sensor for nitrate does not need lipophilic anions whose ratio against ionophore greatly influences selectivity.⁴³ For this reason, the influence of ionophore on the membrane was tested as an ionophore/chromoionophore (C_L/C_C) ratio, maintaining an adequate quantity of the chromoionophore to minimize photometric error in the measurement of the absorbance. An increase in the C_L/C_C ratio implies, as predicted by the above-proposed model, a displacement of response function to the left and a slight increase in slope (Figure 3). For this reason, although the selectivity coefficient increases slightly with the C_L/C_C ratio (Figure 4), a 1:1 ratio was selected because of the lower response function slope, which shows a more extensive dynamic range to nitrate.

The percentage of plasticizer in the membrane is shown to have very little influence on selectivity. Consequently, and to optimize the amount of plasticizer, the response time and physical properties of the disposable sensor were studied. The response time to reach the equilibrium ($t_{95\%}$) was measured with different membranes for an intermediate nitrate concentration 10^{-4} M obtaining the following: 12 min for 50% TBP, 7.0 min for 60%, 5 min for 65%, 6.5 for 70%, and 7 min for 80%. With amounts higher than 70%, the physical properties are worse because the membrane is less homogeneous and the drying time increases. The value of 65% for TBP was chosen as a compromise.

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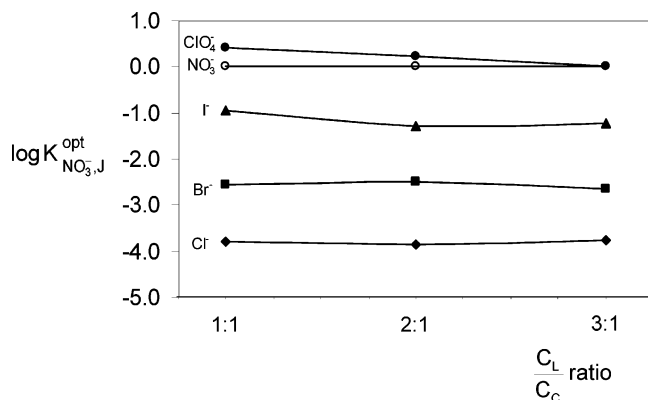


Figure 4. Selectivity (SSM method) variation of disposable sensor as a function of C_L/C_C ratio. (a) perchlorate; (b) nitrate; (c) iodide; (d) bromide, (e) chloride.

To calculate the selectivity coefficients for the disposable sensor described, we obtained the response curves for different anions using both membranes with and without ionophore, in all cases at pH 6.0, adjusted with $\text{NaH}_2\text{PO}_4/\text{Na}_2\text{HPO}_4$ 0.02 M buffer solution. The results obtained using membranes without ionophore fit the theoretical curve described by eq 2, but this is not the case for most of the anions using membranes with ionophore, since the slope of the curves is systematically higher than predicted by eq 1 but lower than indicated by eq 2. We assume that the complex formation constant for most of the anions is very small, as could be inferred from the position of the primary anion curve with respect to the interfering curves and the low value for nitrate constant discussed above. Consequently, the equilibrium due to complex formation could not be displaced very much and simple coextraction of anions governed by lipophilicity could take place simultaneously. The use of an equation such as eq 3 corresponding to the above mixed model makes it possible to fit experimental α values for most of the interferences, except for chloride and nitrite (Figure 5). In this last case, there is a strong deviation of the model at high nitrite concentrations.

The optical anion selectivity measured by $K_{\text{NO}_3-\text{J}}^{\text{opt}}$ of the disposable sensor described at pH 6.0, and measured by the SSM method, follows the Hofmeister series for membranes that do not contain ionophore, $\text{ClO}_4^- > \text{SCN}^- > \text{I}^- > \text{NO}_3^- > \text{Br}^- > \text{HCO}_3^- > \text{Cl}^- \approx \text{SO}_4^{2-} \approx \text{NO}_2^- > \text{CH}_3\text{COO}^-$, with a change in the usual order for $\text{Cl}^- > \text{HCO}_3^- > \text{CH}_3\text{COO}^- > \text{SO}_4^{2-}$. In the presence of ionophore, the displacement of the reaction is higher, an idea supported by the increase in absorbance due to the protonated form of the chromoionophore (see Figure 5). Additionally, the selectivity sequence changes to $\text{ClO}_4^- \approx \text{SCN}^- > \text{NO}_3^- > \text{I}^- > \text{Br}^- \approx \text{NO}_2^- > \text{Cl}^- > \text{HCO}_3^- > \text{SO}_4^{2-} \approx \text{CH}_3\text{COO}^-$, increasing the selectivity against nitrate, due to the selective interaction between the cyclophane and nitrate, which is consistent with the data previously reported in the bibliography.^{3,15} Table 1 offers $K_{\text{NO}_3-\text{J}}^{\text{opt}}$ values found. The poor selectivity observed with acetate is particularly striking in view of the acetate selectivity found for this same ionophore in $\text{CD}_2\text{Cl}_2/\text{CD}_3\text{CN}$ (25:75 v/v) mixtures as solvent with association constants of 770 and 300 M^{-1} for acetate and nitrate, respectively. However, other H-bonding ionophores containing calix[4]arene amide moieties show a similar pattern, thus, 370 and 200 M^{-1} for the same ions in acetone- d_6 .⁴⁴ In other cases, the association constant of H-bonding ionophores with

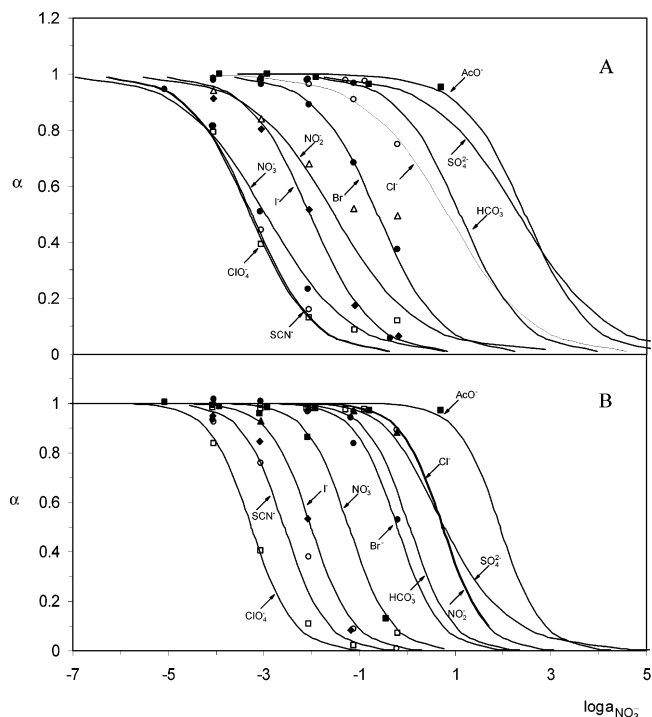


Figure 5. Selectivity of disposable sensor system at pH 6.0. (A) Membranes with ionophore. Solid lines are theoretical response curves according to eq 1 for nitrate, nitrite, and chloride and according to eq 3 for the rest of the anions. (B) Membranes without ionophore. Solid lines are theoretical response curves according to eq 2.

Table 1. Selectivity Coefficient $K_{\text{NO}_3-\text{J}}^{\text{opt}}$ for HNOPH-Based Optical Disposable Sensor in Comparison with Membranes without Ionophore (SSM Method)

anion	with ionophore	without ionophore
SCN^-	0.3 ± 0.08^a	1.4 ± 0.07
ClO_4^-	0.3 ± 0.13	2.04 ± 0.02
NO_3^-	0	0
I^-	-0.97 ± 0.01	0.78 ± 0.02
NO_2^-	-2.4 ± 0.13	-2.0 ± 0.12
Br^-	-2.3 ± 0.13	-0.98 ± 0.05
Cl^-	-3.8 ± 0.12	-1.96 ± 0.01
HCO_3^-	-4.0 ± 0.12	-1.26 ± 0.01
AcO^-	-5.4 ± 0.13	-3.5 ± 0.02
SO_4^{2-}	-5.3 ± 0.13	-1.99 ± 0.02

^a \pm standard deviation of three replicates.

nitrate is higher than that of acetate; this is the case with the Pt(II) nicotinamide complex ionophore (K_{a1} , 230 M^{-1} ; K_{a2} , 491 M^{-1} for acetate in $\text{CD}_3\text{CN}/\text{DMSO}-d_6$, 3 + 1 v/v; K_{a1} , 562 M^{-1} ; K_{a2} , 132 M^{-1} for nitrate in CD_3CN)⁵ although the difference is much greater with our case.

The response of the disposable sensor to nitrate activities between 2.59×10^{-1} and 8.37×10^{-7} M in activities (0.5 and 10^{-6} M in concentrations) at pH 6.0 is shown in Figure 6 along with the theoretical response function using eq 1 for the stoichiometric ratio of ionophore/nitrate, $p = 1$ and 2. The effect of the stoichiometry on the theoretical response curve for this case is small, hindering a clear distinction between different stoichio-

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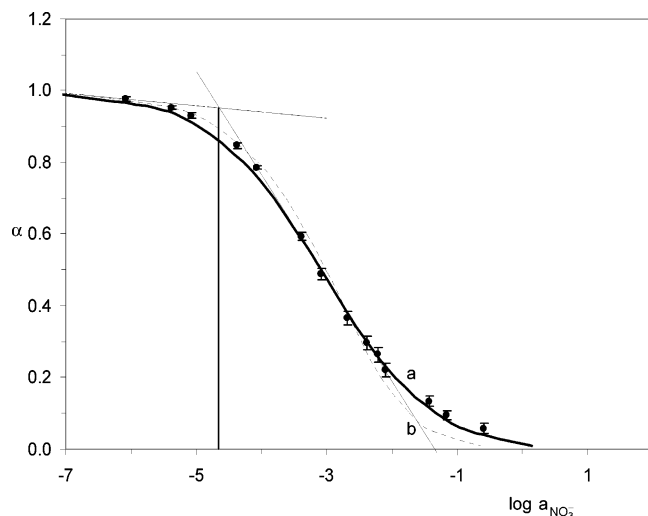


Figure 6. Calibration plot for free nitrate activity. Solid lines are theoretical curve for 1:1 stoichiometry (L/M) and dashed line for 2:1 stoichiometry.

metries in membrane. We assume, according to studies of Anslyn et al.,³ that a 1:1 complex occurs in the membrane. The fit by least squares of the experimental points, in the linear maximum slope zone (8 different concentration levels and 9 replicates of each one) of the response curve for nitrate to the theoretical model indicated by eq 1, makes it possible for us to calculate for $\log K_{e1}$ the value 9.06 ± 0.04 .

ANALYTICAL CHARACTERIZATION AND APPLICATIONS

The measuring range of a disposable sensor based on bulk optode membranes that exhibits a sigmoidal shape response function has been defined in different ways: (i) by the linear relationship in the middle of the response function in such a way that the slope does not deviate more than 1 or 0.1% from its maximum value³⁴ or that the slope does not decrease less than a factor of 2 compared with its maximum value;⁴⁰ (ii) taking into account the nonlinear behavior of the activities between the upper and lower limits of the measuring range,^{34,40} which depends on the definition of the detection limit.^{34,39,45} In this case, the linear relationship in the middle of the sigmoidal response function defined by means a lack-of-fit test is used as the measuring range and the intersection of the linear calibration function and a linear function adjusted in the minimal slope zone (background) as the detection limit.

To characterize analytical behavior of the disposable sensor, two series of standards are used, one in the maximum slope zone (8 standards, 9 replicates each one), between 2.20×10^{-5} and 8.09×10^{-3} in activities (2.62×10^{-5} and 1.00×10^{-2} M in concentration), and another in the minimum slope zone of lower activity (3 standards, 9 replicates each one), between 8.37×10^{-7} and 2.20×10^{-5} M in activities (1.00×10^{-6} and 2.62×10^{-5} M in concentration). The linearity of both series was tested by applying the lack-of-fit test,⁴⁶ and the linear functions obtained were, respectively, $\alpha = -0.3868 - 0.2875 \log a_{\text{NO}_3^-}$ and $\alpha = 0.8724 - 0.0172 \log a_{\text{NO}_3^-}$. The detection limit obtained by the interception

Table 2. Analytical Figures of Merit

parameter	value
intercept	-0.387 (0.010)
slope	-0.288 (0.003)
probability level % (lack-of-fit test)	5.6
linear range (mg/L)	1.63–3886
detection limit (mg/L)	1.63
RSD (%) ^a intrasensor	
1.0×10^{-4} M	1.68
2.5×10^{-3} M	1.39
1.0×10^{-2} M	2.77
RSD (%) ^a sensor-to-sensor	
1.0×10^{-4} M	0.79
2.5×10^{-3} M	3.68
1.0×10^{-2} M	6.25
anal. param. (nm)	absorbance, 660
response time (min)	5
cost (euro/strip)	0.04
lifetime (weeks)	6
reusability (weeks)	3

^a RSD for $\log(a_{\text{NO}_3^-})$.

of both functions is 0.022 mM in activities (0.026 mM in concentration) (Figure 6). The upper limit of the measuring range was obtained from the intercept of the linear calibration function with the axis of abscise; the value obtained is 45.15 mM in activities (62.68 mM in concentration). Thus, the measuring range for nitrate determination is between 22 μ M and 45.15 mM in activities (26 μ M and 62.68 mM in concentration or 1.63 and 3886 mg/L).

The precision (RSD) using the same disposable sensor was calculated at three activity levels of nitrate, namely, 1.0×10^{-4} , 2.5×10^{-3} , and 1.0×10^{-2} M and 10 replicates of each one, and has values of 1.68, 1.39, and 2.77% for $\log a_{\text{NO}_3^-}$. The sensor-to-sensor repeatability, working at the same three activity levels of NO_3^- and 10 replicates of each one, has values of 0.79, 3.68, and 6.25% for $\log a_{\text{NO}_3^-}$. The disposable sensor described here works in a reversible way, meaning reuse is possible after conditioning with 0.02 M NaOH. The reusability, tested with a series of membranes checked once a day at a nitrate concentration in the middle of the range and preserved dry and protected from light, has a mean of 3 weeks. The lifetime of disposable sensors, checked in a similar way and preserved from light and excessive humidity, is at least one and a half months. Table 2 shows these and other analytical parameters.

The analytical performance of the disposable sensor described here is better than that of other electrochemical and optical disposable sensors described to date. For instance, the amperometric capillary-fill devices work between 10^{-3} and 10^{-5} M²³ while the potentiometric disposable sensors work from 10^{-2} to 2.5×10^{-5} M¹⁰ and 10^{-2} – 10^{-5} M⁹ and 10^{-2} – 2.6×10^{-4} M.²⁵ Marketed disposable optical sensors range between 4.8×10^{-5} and 3.6×10^{-3} M (Reflectoquant³⁰) and 8.1×10^{-5} – 8.1×10^{-3} M (Nitrachek²⁹).

The values of $K_{\text{NO}_3^-}^{\text{opt}}$ obtained using membranes with the optimum composition reflect that the described disposable sensor is in the order of 6.3×10^3 times more selective for nitrate than for chloride, 1.0×10^4 times more than for hydrogen carbonate, and 2.0×10^5 times more than for sulfate. Although the concept of required selectivity coefficients³⁸ is not clearly suitable for a

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Table 3. Determination of Nitrate in Different Types of Water Using Voltamperometry as a Reference Method

matrix	disposable procedure ^a (mg/L)	s	reference procedure ^a (mg/L)	s	P value (%)
Monachil River water (Monachil, Granada, Spain)	3.6	0.24	3.3	0.45	29.17
Genil River water (Granada, Spain)	3.1	0.25	2.8	0.24	31.62
spring water (Granada, Spain)	27.3	0.63	29	2.0	20.42
spring water (Granada, Spain)	4.1	0.33	4.37	0.02	21.10
spring water (Haza Grande, Granada, Spain)	54	2.24	54	4.1	90.65
spring water (Zujaira, Granada, Spain)	62	2.78	61	3.57	59.95
spring water (La Peza, Granada, Spain)	2.92	1.49	2.7	17.26	39.00
well water (Illora, Granada, Spain)	20.4	0.29	23	1.71	14.30
cistern water (Illora, Granada, Spain)	91.8	5.26	87.9	0.94	27.20
well water (Valderrubio, Granada, Spain)	201	33.5	200	12.1	96.09

^a Three replicate samples in both cases

contaminant species such as nitrate, whose level changes depending on circumstances, it was applied as an example to underground waters in the area of Granada, Spain, using the high and low mean values of those anions⁴⁷ and a maximum tolerable error of 5% in the determination of nitrate. The selectivity values obtained for the disposable sensor fulfill the selectivity requirements ($K_{\text{NO}_3^-/j^{\text{opt}}}$ req: -3.5 (Cl^-); -3.4 (SO_4^{2-}); -3.7 (HCO_3^-)) for a nitrate assay in waters from the selected area (447 mg/L Cl^- ; 2,109 mg/L SO_4^{2-} ; 583 mg/L HCO_3^- ; 5 mg/L NO_3^-).

To test the usefulness of the proposed disposable sensor, it was applied to waters of diverse provenance (spring, mineral, tap) from the area of Granada, Spain, and with different nitrate content. The test method was validated by comparison with a voltamperometric method based on a catalytic wave of U(VI),⁴⁸ and the results obtained by both methods were satisfactory (*P* value) as shown in Table 3.

CONCLUSION

An optical disposable sensor has been developed using a host–guest recognition system based on an artificial C_3 symmetry amide-based ionophore for nitrate. The multiple H-bonding interactions permit a fine-tuned setting between free energies of nitrate–ionophore interaction and hydration, implemented in a transparent membrane that works by coextraction offering an easy-to-measure analytical signal.

We succeeded in preparing a very simple disposable optical sensor, usually more complex than electrochemical sensors for a

weakly coordinating character anion like nitrate. The selectivity pattern found meets most of the selectivity requirements for nitrate analysis in natural waters considering that nitrate more than a natural constituent is a contaminant with very different possible levels.

The described disposable sensor makes it possible to monitor nitrate in situ for most practical situations. Examples include water quality control to maintain the nitrate level within legal regulations or petiole sap testing in plants for nitrate to determine fertilization needs, with advantages in reducing contamination, losses and changes related to sample collection, and cost reduction. It is environmentally friendly and has an estimated lifetime of at least one and a half months when protected from light in normal conditions.

Drawbacks are related to response time, which in the developed format is roughly 5 min due to diffusion within the polymeric membrane, but could be reduced by using porous supports. Precision could be improved with mass production techniques, which could also cut costs. The use of different mass production techniques and the development of small, hand-held, and battery-operated instruments that will make decentralized analyses possible are now under investigation.

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