Available online at www.sciencedirect.com
SCIENCE DIRECT
Talanta 69 (2006) 450–455
Talanta
www.elsevier.com/locate/talanta

Conductometric nitrate biosensor based on methyl viologen/Nafion®/nitrate reductase interdigitated electrodes

Wang Xuejiang [a,b,c], Sergei V Dzyadevych [c,d], Jean-Marc Chovelon [b], Nicole Jaffrezic Renault [c,*], Chen Ling [a], Xia Siqing [a], Zhao Jianfu [a]

[a] *State Key Laboratory of Pollution Control and Resources Reuse, Tongji University, Shanghai 200092, China*
[b] *Laboratoire d'Application de la Chimie à l'Environnement, UMR CNRS 5634, Université Claude Bernard Lyon 1, 43 Boulevard du 11 Novemvre 1918, Villeurbanne Cedex 69622, France*
[c] *CEGELY, UMR/CNRS 5005, Ecole Centrale de Lyon, 69134 Ecully Cedex, France*
[d] *Laboratory of Biomolecular Electronics, Institute of molecular Biology and Genetics, 150, Zabolotnogo St., Kiev 03143, Ukraine*

Received 27 June 2005; received in revised form 27 June 2005; accepted 11 October 2005
Available online 28 November 2005

Abstract

A highly sensitive, fast and stable conductometric enzyme biosensor for determination of nitrate in water is reported for the first time. The biosensor electrodes were modified by methyl viologen mediator mixed with nitrate reductase (NR) from *Aspergillus niger* by cross-linking with glutaraldehyde in the presence of bovine serum albumin and Nafion® cation-exchange polymer. The process parameters for the fabrication of the enzyme electrode and various experimental variables such as pH, the enzyme loading and time of immobilization in glutaralaldehyde vapor were investigated with regard to their influence on sensitivity, limit of detection, dynamic range and operational and storage stability. The biosensor can reach 95% of steady-state conductance value in about 15 s. Linear calibration in the range of 0.02 and 0.25 mM with detection limits of 0.005 mM nitrate was obtained with a signal-to-noise ratio of 3. When stored in 5 mM phosphate buffer (pH 7.5) at 4 °C, the sensor showed good stability over 2 weeks.

Keywords: Conductimetric biosensor; Nitrate reductase; Methyl viologen; Nitrate; Interdigitated electrodes

1. Introduction

During the past two centuries, the human species has substantially altered the global nitrogen cycle, increasing both the availability and the mobility of nitrogen over large regions of Earth [1–3]. Consequently, in addition to natural sources, inorganic nitrogen can nowadays enter aquatic ecosystems via anthropogenic input such as animal farming, urban and agricultural runoff, industrial wastes and sewage effluents (including effluents from sewage treatment plants that are not performing tertiary treatments). As a result, concentrations of nitrate in ground and surface waters are increasing around the world, causing one of the most prevalent environmental problems on a worldwide scale. Water containing high concentrations of nitrate can create serious problems, such as eutrophication of rivers, deterioration of water quality and potential hazard to human health, because nitrate in the gastrointestinal tract can be reduced to nitrite ions. In addition, nitrate and nitrite have the potential to form N-nitrous compounds, which are potential carcinogens [4]. Another toxic affect of high concentrations of nitrites is the rare disease of methemoglobinema or "baby blue syndrome" [5]. Predominantly on the ground of human health most countries have imposed limits for nitrate in drinking water of 25–50 mg/l (0.4–0.8 mM) [6].

Spectro photometric methods for the determination of nitrate have been developed over the past several decades [7–10]. Ion exchange chromatography combined with spectrometric, conductimetric or electrochemical detection has recently become popular for the detection of nitrate [4,11–13]. However, even though they have high sensitivity and good reproducibility, these methods require large and expensive instrument and extensive pre-treatment of the sample. Nitrate ion-selective electrodes

* Corresponding author. Tel.: +33 472186243; fax: +33 478433717.
E-mail address: Nicole.Jaffrezic@ec-lyon.fr (N.J. Renault).

0039-9140/$ – see front matter © 2005 Elsevier B.V. All rights reserved.
doi:10.1016/j.talanta.2005.10.014

(ISEs) and ion-sensitive field-effect transistors (ISFETs), which provide fast response time, simplicity, low cost, and can be easily adapted to flowing streams and in situ measurements but suffer from poor stability and severe interference effect caused by other anions present in the sample [14].

Biosensors incorporating enzymes have also been used for nitrate analysis in real samples with reasonable sensitivity and selectivity. The majority of biosensors reported for the measurement of nitrate ions are molecular-based systems using a nitrate reductase enzyme purified from plant, fungal or bacterial sources. Both electrochemical and optical biosensors have been reported [15–19].

Nitrate reductases (NR) are produced by a variety of animals, plants, and microorganisms including fungi. The enzyme is a homodimer composed of two identical subunits of approximately 100 kDa, each of which contains three cofactors, flavin adenine dinucleotide (FAD) which is the site for NAD(P)H oxidation, heme-iron (heme-Fe) and Mo-molybdopetrin (Mo-MPT) which is the site for nitrate reduction in a 1:1:1 ratio [20]. NR can be reduced by NADH, NADPH or both nucleotides, in the case of abispecific enzyme, which catalyzes the reduction of NO_3^- to NO_2^-:

$$\text{NAD(P)H} + NO_3^- + H^+ \rightarrow \text{NAD(P)}^+ + NO_2^- + H_2O \quad (1)$$

While the NAD(P)H dependent activity of assimilatory enzymes is more rapidly lost than its nitrate reduction activity, it was shown that assimilatory nitrate reductases can be supplied with redox equivalents by redox mediators like methyl viologen, that work as artificial electron donor [21]. Methyl viologen can be reductively regenerated with sodium dithionite or electrochemically [22].

Several reports addressed the development of amperometric biosensor devices for nitrate have also used suitable redox mediators including oxygen or nitrogen heterocycles, triphenyl methane dyes and sulphonphtaleine dyes mainly as the electron transfer between the electrode and the enzyme [6,23–25]. To our best knowledge, no previous work describing the coimmobilization of nitrate reductase enzyme and redox mediator at an electrode surface resulting in conductometric biosensors has been reported to date. Conductometric sensors for biosensing devices have been introduced by Watson et al. [26]. The device consisted of a planar glass support with interdigitated gold electrode pairs on one surface in a planar configuration. The principle of the detection is based on the fact that many biochemical reactions in solution produce changes in the electrical resistance.

Conductance measurements involve the resistance determination of a sample solution between two parallel electrodes. For the direct assaying of many enzymes and their substrates, the conductometric biosensors present a number of advantages: (a) the planar conductometric electrodes are simple and relatively cheap which suits for miniaturization and large scale production, and therefore promising for practical use, (b) they do not require a reference electrode, (c) the applied voltage can be sufficiently small to minimize substantially the sensor's power consumption, (d) large spectrum of analytes of different nature can be determined on the basis of various reactions and mechanisms.

In the present study, we present a conductometric nitrate biosensor by the coimmobilization of nitrate reductase from *Aspergillus niger* (EC 1.6.6.2) and methyl viologen in the Nafion® films at an interdigitated thin-film electrodes surface. Since viologens are highly water soluble and toxic, any practical device containing these electron mediators should be based on immobilized viologens [27,28]. Methyl viologen's electrochemical behavior involves reduction of MV^{2+}. The structure of MV^{2+} consists of a hydrophobic part that is capable of hydrophobic–hydrophobic interaction with Nafion® and two cationic pyridinium groups that undergo ion exchange with the sulphonate sites of Nafion® polymer chains, according to:

$$MV_{aq}^{2+} + 2(SO_3^-Na^+)_{film} \rightarrow [(SO_3^-)_2MV^{2+}]_{film} + 2Na_{aq}^+ \quad (2)$$

This interaction results in accumulation of MV^{2+} in Nafion® films. In the presence of dithionite as electron donor, the biocatalyzed reduction of NO_3^- to NO_2^- is stimulated. The reaction was as follows.

$$NO_3^- + 2MV^{\bullet+} + 2H^+ \rightarrow NO_2^- + 2MV^{2+} + H_2O \quad (3)$$

The subsequent local changes of conductance inside the membrane is dependent on the reaction (3), and thus the conductometric nitrate sensor devices are assembled. The performance of the conductometric nitrate biosensor was evaluated by the detection of nitrate in water solution.

2. Experimental

2.1. Reagents

Nitrate reductase (EC 1.1.6.6.2) from *A. niger*, bovine serum albumin (BSA) and aqueous solutions (25%, w/v) of glutaraldehyde (GA), methyl viologen, Nafion® (perfluorosulfonated ion-exchange resin, 5% (w/v) solution in a solution of 80% aliphatic alcohol and 20% water mixture) and sodium hydrosulfite were purchased from Sigma–Aldrich Chemie GmbH. Sodium nitrate and sodium dithionite were from Merck. All other chemicals were of analytical grade.

2.2. Apparatus

The conductometric transducers were fabricated at the Institute of Chemo- and Bio-sensorics (Munster, Germany). Two pairs of Au (150 nm thick) interdigitated electrodes were made by the lift-off process on the pyrex glass substrate (10 mm × 30 mm). A 50 nm thick intermediate Ti layer was used to improve the adhesion of Au to the substrate. The central part of the sensor chip was closed by epoxy resin to define the electrode sensitive area. Both the digit width and interdigital distance were 10 μm, and their length was about 1.0 mm. As a result, the "sensitive" area of each electrode was about 1.0 mm^2.

The internal generator of a Stanford Research System SR 830 lock-in amplifier (Sunnyvale, CA, USA) was employed to

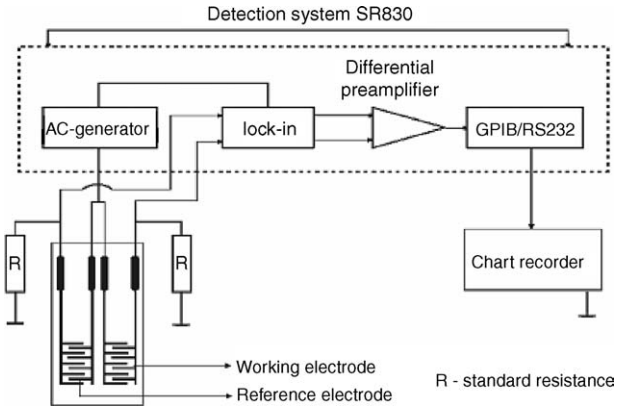

Fig. 1. Schematic diagram of the experimental set-up for conductometric measurements.

generate a sinusoidal wave with a frequency of 100 kHz and a peak-to peak amplitude of 10 mV around a fixed potential of 0 V to each pair of electrodes, forming a miniaturized conductance cell. The schematic diagram of the experimental system was shown in Fig. 1.

2.3. Enzyme immobilization

The enzymatic membrane was prepared on the transducer surface by the cross-linking of enzyme with bovine albumin in saturated glutaraldehyde vapor [29]. The enzyme was deposited together with methyl viologen and with Nafion® in order to obtain a stable and active enzyme layer. A mixture of 10% (w/w) NR enzyme and BSA, 3% (v/v) Nafion® (5% in alcohol), 10% (w/w) methyl viologen, 10% glycerol in 20 mM phosphate buffer (pH 7.5) was deposited on the sensitive area of the sensor using a drop method, while another mixture of 10% (w/w) BSA, 3% (v/v) Nafion® (5% in alcohol), 10% (w/w) methyl viologen and 10% (w/w) glycerol in 20 mM buffer (pH 7.5) was deposited on the other electrode. The later electrode was considered to be the reference sensor. The sensor chips were placed in a saturated glutaraldehyde vapor for 60 min followed by drying in air for 15 min at room temperature.

Milipore Mlli-Q nanopure water (resistivity 18.2 MΩ cm) was used throughout for the preparation of solutions.

2.4. Measurements

Measurements were conducted in daylight at room temperature (25 °C) in a glass cell. The sensor chip was immersed in a measuring cell filled with 5 ml of magnetic-stirred phosphate buffer solution (5.0 mM with 0.5 mM EDTA), pH 7.5, containing specific sodium nitrate concentration. Sample concentrations (nitrate standard solution) were adjusted by adding defined volumes of an appropriate concentrated stock solution. After stabilization of the output signal, 25 μl of freshly prepared sodium dithionite (200 mM) was added in the vessel, which initiated the reaction (3). The differential output signal between the measuring and reference was logged by the SR 830 lock-in amplifier and ΔS ($\Delta S = (S_n - S_0)$, where S_n is the conductive value in the presence of nitrate and S_0 is the conductive value obtained in the absence of nitrate) of the steady-state response of the biosensor was plotted as a function of nitrate concentrations.

The buffer in the measuring cell and nitrate solutions were bubbled separately with nitrogen for at least 15 min to remove the dissolved oxygen before use in all experiment.

3. Results and discussion

3.1. Optimization of enzymatic membrane formation

The performance of the biosensor, in terms of sensitivity and long-term stability is strictly depended on the enzyme loading and the amount of BSA. The effect of the enzyme loading in immobilization mixture on nitrate reductase biosensor response to nitrate was investigated. Different ratios NR/BSA were tested to optimize the amount of loaded nitrate reductase with sensor response (Fig. 2). It can be seen that the maximum of the enzyme activity in membrane can be achieved for enzyme/BSA ratio equal to 2:1, generally containing 10% protein. The further increase in the amount of enzyme loading might be leading to the increase of the diffusional resistance for the substrate to arrive to the electrode surface and then to the decrease in the biosensor response. On the other hand, if enzyme concentrations are too low, there is no enough enzyme involved in the reaction which leads only to a slight conductance variation. In addition, a high concentration of BSA may effectively decrease the NR activity [30].

An increase of the mediator concentration in the enzyme mixture solution did not lead to an increase of the sensor response. We concluded that the applied methyl viologen concentration of 10% (w/w) is sufficient to saturate the electrode surface with mediator molecules.

Without enzyme or mediator no signal was observed, implying that no direct reduction of NO_3^- occurs. This indicates that the conductometric biosensor detection process is nitrate-dependent and is enzyme-catalyzed.

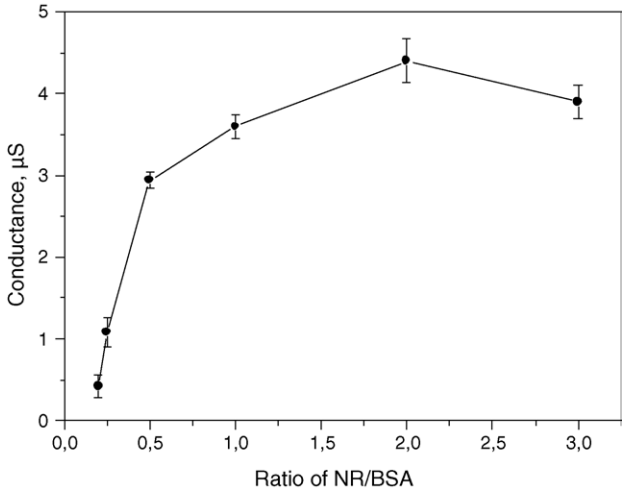

Fig. 2. Dependence of biosensor response on enzyme loading (NR/BSA ratio) for 0.1 mM sodium nitrate. Measurements were conducted in 5.0 mM phosphate buffer at pH 7.5, exposure time to glutaraldehyde vapor, 60 min. Membrane solution composition: 3% Nafion®, 10% methyl viologen.

Table 1
Effect of Naflon® ratio on the biosensor response to nitrate

Ratio of Nafion® (%, v/v)	Response time (s)	Detection range (mM)	Detection limit (mM)
0	–	–	
1	8	0.01–0.15	–
2	10	0.02–0.18	0.005
3	15	0.02–0.25	0.005
4	35	0.15–.030	0.10

Nafion® has hydrophobic fluorocarbon backbone and hydrophilic cation-exchange site, thus rendering moderate hydrophobicity. The effect of different Nafion® content in the membrane on NR biosensor response to nitrate was investigated (Table 1). The observed maximum conductance response of the biosensor occurs at the ratio of 3% (v/v). This results indicates that an initial increase of the Nafion® content in the composite up to 3% results in the increased stability of the enzyme and hence the increased sensitivity. The biosensor response times, i.e. times necessary to reach 95% of the steady-state amplitude were about 15 s. However, further increase in the Nafion® content in the composite increases the hydrophobicity of the membrane, thus the bioelectronic systems suffer from basic limitations associated with diffusion barriers of the substrates through the polymer membranes, resulting in, respectively, slow response times and moderate sensitivities. In addition, since the stabilization of Nafion® occurs in ethanol, the increase in Nafion® ration in the composite could lead to a greater deactivation of NR by ethanol.

In the case of the effect of exposure time on the residual NR enzyme activity, the calibration curves for nitrate determinations with different times of exposure to GA vapor is shown in Fig. 3. It can be seen that best results were obtained for 60 min of exposure. For longer immobilization time, a dramatic decrease of response values was obtained. This phenomenon can be connected to the formation of a large number of covalent bounds between glutaraldehyde and the enzyme molecules, which lead to blocking of enzyme active centers. Moreover, such

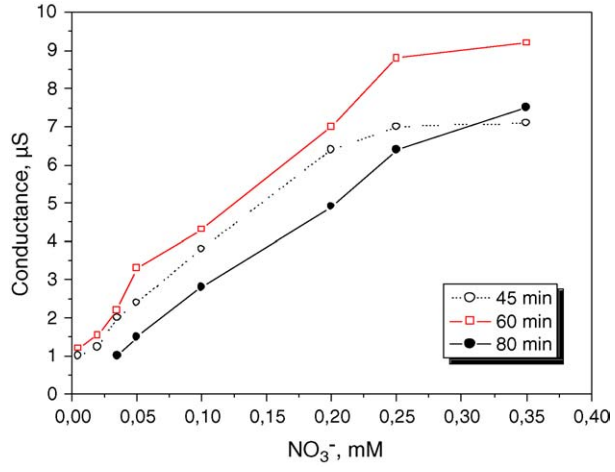

Fig. 3. Dependence of the nitrate conductometric biosensor response vs. the time of exposure to GA vapor. Measurements were conducted in 5.0 mM phosphate buffer, pH 7.5.

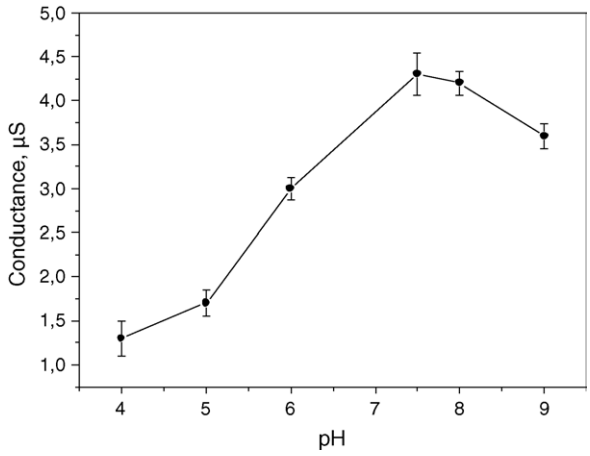

Fig. 4. Dependence of conductometric biosensor response on pH for 0.1 mM sodium nitrate concentration. Measurements were conducted in 5.0 mM phosphate buffer with pH 7.5.

dense membrane can reduce a diffusion of substrates and products of the biochemical reaction that can result in broadening of dynamic range of nitrate determination. On the other hand, for short exposure times (<30 min) the enzyme leakage through the membrane can take place due to an insufficient covalent bonding thus, the stability of the biosensor become poor, and the response of the sensors have decreased markedly with the number of measurements (not shown).

3.2. *Effect of experimental variables*

Since the enzyme activity is strongly affected by the solution pH, the effect of pH on the biosensor response was examined with 0.10 mM nitration in 5.0 mM phosphate buffer (with 0.5 mM EDTA). The result is displayed in Fig. 4. The maximum responses of the biosensors based on methyl viologen/Nafion® occur at pH 7.5. This result is strongly correlated with those obtained using NR-based methods for determination of nitrate [31,32]. This fact indicates that the immobilization of NR in the methyl viologen/Nafion® composite film has not much changed the microenvironment of the enzyme. We used the buffer solution of pH 7.5 throughout the experiments to obtain the maximum sensitivity.

Fig. 5 shows the effect of temperature on the sensitivity of the same biosensor. Like the majority of enzymes, the activity of NR is related to temperature, it can be seen that with increasing temperature the biosensor sensitivity increased. The temperature optimum was reached about at 60 °C, beyond which NR denaturation takes place.

It has been reported that the oxygen interference is not negligible during the detection, because the reduced viologens ($MV^{\bullet+}$) can be autooxidated by air. We found that although the relative standard deviation obtained is higher in air-saturated buffer (8%) than in nitrogen-saturated buffer (6%), the difference is not very significantly, which is due to the excessive sodium dithionite that depletes the oxygen in the buffer solution.

In order to study the effect of sodium dithionite concentration on the substrate response, its concentration was varied between 0

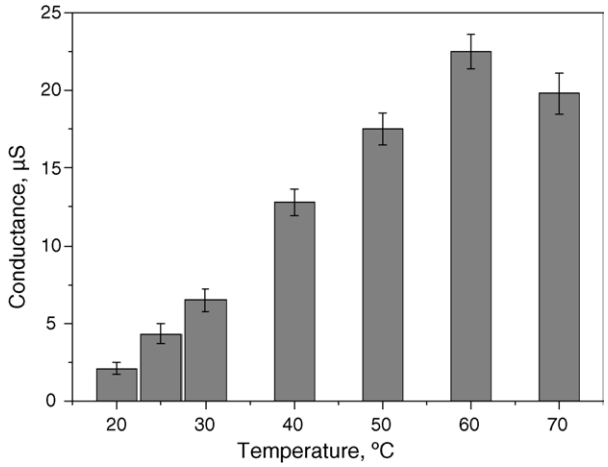

Fig. 5. Dependence of conductometric biosensor response on temperature for NO_3^- concentration of 0.1 mM. Measurements were conducted in 5.0 mM phosphate buffer with pH 7.5.

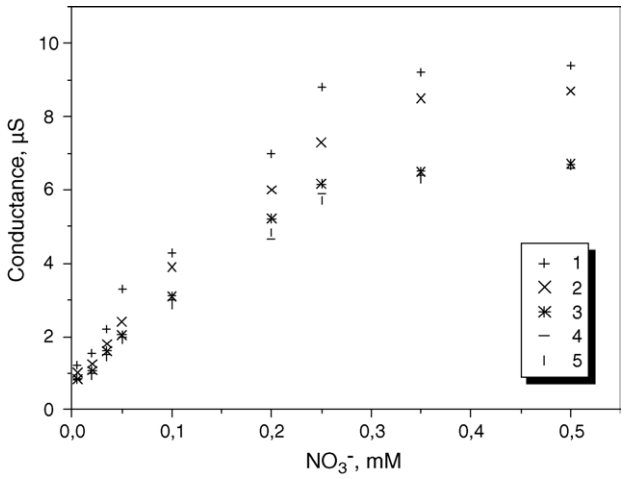

Fig. 7. Five sequential calibration curve measurements with newly prepared electrodes. Measurements were conducted in 5.0 mM phosphate buffer with pH 7.5. The number indicates the order of the measurements.

and 5.0 mM. In the absence of sodium dithionite no response was observed while for concentrations higher than 1.0 mM sodium dithionite the response became constant, and this concentration (25 μl of 200 mM sodium dithionite) was used for all the experiments.

Using the optimum conditions obtained in the above studies, calibration curve of the conductometric nitrate biosensor was obtained over a nitrate concentration range of 0.02–0.5 mM using the biosensor in 5.0 mM phosphate buffer at pH 7.5. As shown in Fig. 6, the methyl viologen/Nafion® modified electrode showed a linear response in a concentration range of 0.02–0.25 mM, the linear regression equation was $\Delta S\,Y = 1.1058 + 30.469\ [NO_3^-]$ (mM), $R = 0.9975$. The detection limit of 0.005 mM of nitrate was obtained with a signal-to-noise ratio of 3, these ranges being quite sufficient for the real samples (drinking water, wastewater) analyzed.

3.3. Stability of the biosensor

Long-term storage stability is one of the key factors of a biosensor performance. The low storage and operational stability of NR electrodes is a well-known fact. An exponential time decay of the enzyme activity, possibly caused by thermal or chemical inactivation of the enzyme, has been reported [33]. The methyl viologen/Nafion® modified electrodes were rinsed with 5 mM phosphate buffer solution (with 0.5 mM EDTA) at pH 7.5 and stored at 4 °C. In order to evaluate the long-term stability of this sensor, one calibration curve was recorded every day and the conductance at a fixed nitrate concentration of 0.1 mM was measured. There is a rather large initial decrease of response magnitude (Figs. 7 and 8). After 3 days there was a decrease of 30% from the initial response. The sensor gave a stable response conductance after this period,

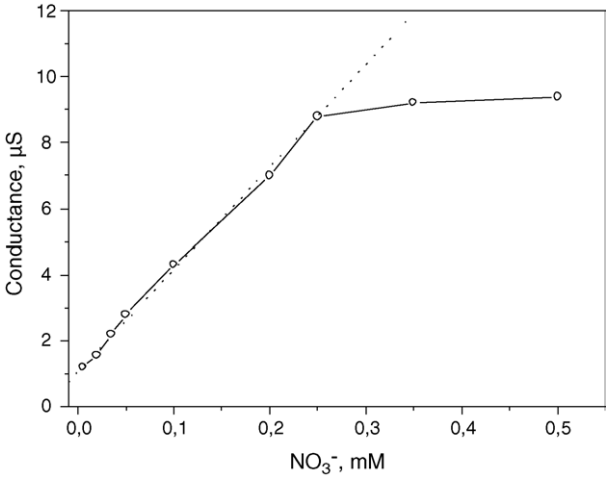

Fig. 6. Typical calibration curve for MV/Nafion/NR electrode for increasing nitrate concentrations. Measurements were conducted in 5.0 mM phosphate buffer with pH 7.5 at 25 °C.

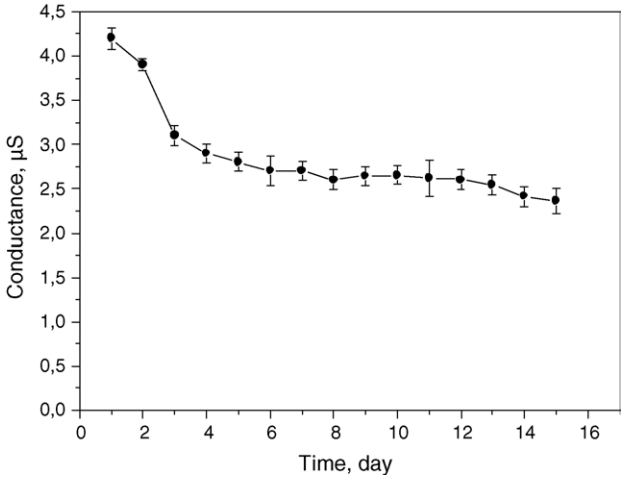

Fig. 8. Storage stability of the NR biosensor. Measurements were conducted in 5.0 mM phosphate buffer with pH 7.5, NO_3^- concentration, 0.1 mM.

which remained stable for more than 2 weeks. This fact indicates that the biocompatibility of Nafion® with NR enzyme provides substantial improvement in long-term stability of the nitrate biosensor.

The reproducibility of the methyl viologen/Nafion®/NR electrodes was evaluated by measuring the responses of five replicate biosensors to 0.1 mM nitrate. A relative standard deviation (R.S.D.) of around 6% was obtained. Thus, the sensor showed a good, reproducible behavior and can be used for reproducible measurements.

It is interesting to compare our rather simple and economic conductometric nitrate biosensor with recent reports on amperometric sensors with other nitrate reductases. Glazier et al. [16] reported a Nafion®-coated glassy carbon electrode with the *Zea mays* nitrate reductase trapped behind a dialysis membrane. Nitrate was measured under anaerobic conditions. The nitrate detection limit was 3 μM and a linear range was <200 μM, the half lifetime of the electrode was of the order of 1 day. While the lower detection limits of our enzyme electrodes are comparable we found a wider linear range and a better stability of our enzyme electrode for nitrate determination. These advantages of the conductometric nitrate biosensor, together with its simplicity, low cost, etc. are still considerably superior to that of the *E. coli* [15,34], *P. stutzeri* [6] nitrate reductases based amperometric biosensors.

4. Conclusion

In this paper, a novel conductometric mediated biosensor for nitrate determination has been developed. Nitrate reductase was immobilized together with the redox mediator, methyl viologen, and Nafion® through cross-linking with glutaraldehyde in presence of BSA. The biosensor proposed is relatively inexpensive, easy to operate. The main analytical characteristics of the biosensor created depend on conditions of membrane deposition. A low detection limit of 0.005 mM and a wide linear range up to 0.25 mM was obtained. Moreover, the biosensor presented here has a long star age stability and operational stability as well as a good thermal stability. The practical use of this biosensor in determination of nitrate in real samples (wastewater, river water, etc.) is being investigated.

Acknowledgements

This work was supported by Région Rhone-Alpes (in the frame work of Priority Thematic Actions) as well as France-China P2R research programs.

References

[1] P.M. Vitousek, J.D. Aber, R.W. Howarth, G.E. Likens, P.A. Matson, D.W. Schindler, W.H. Schlesinger, D.G. Tilman, Ecol. Appl. 7 (1997) 737.
[2] S.R. Carpenter, N.F. Caraco, D.L. Correll, R.W. Howarth, A.N. Sharpley, V.H. Smith, Ecol. Appl. 8 (1998) 559.
[3] J.H. Galloway, E.B. Cowling, Ambio 31 (2002) 64.
[4] D. Connolly, B. Paull, Anal. Chim. Acta 441 (2001) 53.
[5] A.M. Fan, V.E. Steinberg, Reg. Toxicol. Pharmacol. 23 (1996) 35.
[6] D. Kirstein, L. Kirstein, F. Scheller, H. Borcherding, J. Ronnenberg, S. Diekmann, P. Steinrücke, J. Electroanal. Chem. 474 (1999) 43.
[7] M. Belz, P. Dress, K.F. Klein, W.J.O. Boyle, H. Franke, K.T.V. Grattan, Water Sci. Technol. 37 (12) (1998) 279.
[8] M.S. Finch, D.J. Hydes, C.H. Clayson, B. Weigl, J. Dakin, P. Gwilliam, Anal. Chim. Acta 377 (1998) 167.
[9] M.N. Abbas, G.A. Mostafa, Anal. Chim. Acta 410 (2000) 185.
[10] M.A. Ferree, R.D. Shannon, Water Res. 35 (1) (2001) 327.
[11] M.R. Stratford, Methods Enzymol. 301 (1999) 259.
[12] V.D. Matteo, E. Esposito, J. Chromatogr. A 789 (1997) 213.
[13] J.M. Monaghan, K. Cook, D. Gara, D. Crowther, J. Chromatogr. A 770 (1997) 143.
[14] M.S. Patel, R.S. Patel, K.C. Patel, Acta Cincia Indica 29 (2) (2003) 75.
[15] S. Cosnier, B. Galland, C. Innocent, J. Electroanal. Chem. 433 (1997) 113.
[16] S.A. Glazier, E.R. Campbell, W.H. Campbell, Anal. Chem. 70 (1998) 1511.
[17] L.M. Moretto, P. Ugo, M. Zanata, P. Guerriero, C.R. Martin, Anal. Chem. 70 (1998) 2163.
[18] J.W. Aylott, D.J. Richardson, D.A. Russell, Analyst 122 (1997) 77.
[19] C. Huber, I. Klimant, C. Krause, T. Werner, O.S. Wolfbeis, Anal. Chim. Acta 449 (2001) 81.
[20] G.G. Barbier, R.C. Joshi, E.R. Campbell, W.H. Campbell, Protein Expr. Purif. 37 (2004) 61.
[21] R.B. Mellor, J. Ronnenberg, W.H. Campbell, S. Diekmann, Nature 355 (1992) 717.
[22] D.R. Senn, P.W. Carr, L.N. Klatt, Anal. Biochem. 75 (1976) 464.
[23] S. Da Siva, D. Shan, S. Cosnier, Sens. Actuators B 103 (2004) 397.
[24] S. Cosnier, C.H. Gondran, Analysis 27 (7) (1999) 558.
[25] I. Willner, E. Katz, N. Lapidot, Bioelectrochem. Bioenerg. 29 (1992) 29.
[26] L.D. Watson, P. Maynard, D.C. Cullen, R.S. Sethi, J. Brettle, C.R. Lowe, Biosensors 3 (1987/88) 101.
[27] A.L. De Lacey, M.T. Bes, C.G. Moreno, V.M. Fernandez, J. Electroanal. Chem. 390 (1995) 69.
[28] L. Mao, J. Jin, L. Song, K. Yamamoto, L. Jin, Electroanalysis 11 (1999) 499.
[29] S.V. Dzyadevich, A.P. Soldatkin, A.A. Shul'ga, V.I. Strikha, A.V. El'skaya, J. Anal. Chem. 49 (1994) 789.
[30] G. Giovannoni, J.M. Land, C. Keir, E.J. Thompson, S.J.R. Heales, Ann. Clin. Biochem. 34 (1997) 193.
[31] H. Borcherding, S. Leikefeld, C. Frey, S. Diekmann, P. Steinrucke, Anal. Biochem. 282 (1–9) (2000) 1.
[32] N.F. Ferreyra, V.M. Solis, Bioelectrochemistry 64 (2004) 61.
[33] W.D. Mac Donald, A. Coddington, Eur. J. Biochem. 46 (1974) 169.
[34] I. Willner, A. Riklin, N. Lapidot, J. Am. Chem. Soc. 112 (1990) 6438.