Construction of Nitrate-selective Electrodes and Monitoring of Nitrates in the Hydroponic Solutions

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Abstract

A liquid-membrane type nitrate-selective electrode was constructed, in which the responding membrane contained polyvinylchloride, o-nitrophenyloctylether and tetraheptylammonium nitrate. The NO$_3^-$-selective electrode displayed a linear response to the concentration of NO$_3^-$ with a Nernstian slope of $-53 \pm 1$ mV decade$^{-1}$, in the $10^{-5} - 10^{-1}$ mol dm$^{-3}$ (M) NO$_3^-$ concentration range. The NO$_3^-$ detection limit was about $10^{-6}$ M. The electrochemical response of this electrode was stable for more than 30 days. Measurements performed using the NO$_3^-$-sensor indicated that in the presence of green plants, the concentration of NO$_3^-$ in a hydroponic solution decreased from 0.20 to 0.05 mM over a three-day period.

**Keywords:** Nitrate, Tetraheptylammonium ion, Ion-selective electrode, Monitoring, Hydroponic solution, Green plants.
Introduction

Generally, soil in farmland contains a variety of ions such as Ca$^{2+}$, Mg$^{2+}$, K$^+$, NO$_3^-$, H$_2$PO$_4^-$, HPO$_4^{2-}$, and Cl$^-$. The concentration of essential nutrients in the soil fluctuates in response to these nutrients’ absorption and release by plants and animals, to fertilization, to the discharge of artificial products, etc. Given that NH$_4^+$, amino acids, urea, and NO$_3^-$ are the main natural sources of nitrogen for plant growth, NH$_4^+$ or NO$_3^-$ are sometimes used as plant nutrients. Recently, hydroponic culture is drawing attention from all over the world because it can provide a stable supply of fresh vegetables. It has been recognized that hydroponic systems using an aqueous solution containing nutrients such as K$^+$, H$_2$PO$_4^-$, and NO$_3^-$ are easy to handle and maintain. When the solution’s nutrients are consumed, the nutrients are replenished by adding a condensed hydroponic solution. Although the concentration of NO$_3^-$ in the hydroponic solution is important, especially for the growth of green vegetables, the direct monitoring of the concentration of NO$_3^-$ using an ion-selective electrode (ISE) is not commonly implemented. Instead, the parameters that have often been monitored in this context are pH, electrical conductivity, and temperature. Since NO$_3^-$ concentration is usually roughly estimated based on only the solution’s electrical conductivity, the construction of a stable NO$_3^-$-ISE is a desirable research objective.

Several research groups have already developed NO$_3^-$-ISEs and a few of these electrodes have become commercially available. These responding membranes of the NO$_3^-$-ISEs contained NO$_3^-$ salts of various hydrophobic cations to immobilize NO$_3^-$ within the membranes. However, several practical problems have been reported for these electrodes, e.g., with respect to their stability, their reproducibility, and their narrow linear range. Milham, et al. mentioned the influence of the coexistence of Cl$^-$ escaped from the reference electrode. Therefore, NO$_3^-$-ISEs have not been used often as practical monitoring tools. Conversely, Zuther and
Cammann reported the alleviation of such problems by preparing a sensing membrane comprising polyvinylchloride (PVC), o-nitrophe nyloctylether (NPOE), and a quaternary ammonium salt.\textsuperscript{23,24} Regrettably, the exact composition of this membrane and its construction protocol were not made clear, so reproducing the construction of Zuther and Cammann’s NO\textsubscript{3}\textsuperscript{−}-ISE is a difficult task. Furthermore, considering that this NO\textsubscript{3}\textsuperscript{−}-ISE requires a pretreatment like soaking in a solution of 0.1-mol dm\textsuperscript{−3} KNO\textsubscript{3} for 8-12 h, simplification of the method is quite possible.

In the present article, we report the development of a NO\textsubscript{3}\textsuperscript{−}-ISE using a PVC membrane immersed in NPOE containing tetraheptylammonium (THA\textsuperscript{+}) nitrate. In order to obtain a stable and highly sensitive sensor, we optimized the composition of the ion-responsive membrane and the electrolyte composition of the internal solutions of the sensing and reference electrodes. To reduce the interference in measurements due to ions like K\textsuperscript{+} and Cl\textsuperscript{−}, some modifications were applied to the NO\textsubscript{3}\textsuperscript{−}-ISE with respect to the electrode developed by Zuther and Cammann: the electrolyte in the inner solution, KCl, was replaced with LiCl and that in the salt bridge between the reference electrode and the sample solution, KCl, was replaced with MgSO\textsubscript{4}, respectively. Subsequently, the linear range, the detection limit, the slope, the selectivity, and the long-term stability of the NO\textsubscript{3}\textsuperscript{−}-sensing system were examined, and the concentration of NO\textsubscript{3}\textsuperscript{−} in a hydroponic solution was monitored using the newly developed NO\textsubscript{3}\textsuperscript{−}-ISE. In these experiments, the intake of NO\textsubscript{3}\textsuperscript{−} by green vegetables was confirmed.

**Experimental**

**Reagents and chemicals**

Lithium chloride, lithium nitrate, lithium sulfate, sodium chloride, sodium nitrate, sodium sulfate, sodium carbonate, disodium hydrogenphosphate, sodium dihydrogenphosphate,
potassium chloride, potassium nitrate, potassium sulfate, magnesium chloride, magnesium sulfate, silver nitrate, THF, and PVC were purchased from Wako Pure Chemical Ind., Ltd. The chloride salt of THA⁺ (THACl) was obtained from Sigma-Aldrich Co. LLC. NPOE was purchased from Dojindo Chemicals. All chemicals were reagent grade and were used without further purification.

The nitrate salt of THA⁺ (THANO₃) was prepared by mixing an ethanol solution of THACl with an ethanol solution containing excess AgNO₃. Upon mixing, the highly insoluble AgCl precipitated from the solution, the mixture thus obtained was filtered and the precipitate was discarded. By adding pure water to the filtrate containing THANO₃ and excess AgNO₃, THANO₃ precipitated in its crystalline form. AgNO₃ remained dissolved in solution. The white solid THANO₃ was separated from the solution via filtration, and it was washed with pure water several times to remove any residual AgNO₃. It was then dried under vacuum for several hours.

High-purity water (ρ = 18.2 MΩ cm) was used to prepare all the aqueous solutions.

**Apparatus**

The electrochemical cell, which also acted as a hydroponic vessel, utilized in this study is depicted in Fig. 1. The potential of the NO₃⁻-sensing electrode against the reference electrode was measured using a handmade electrometer and an A/D converter (Graphtec Corp., Midi Logger GL 900).

All measurements were performed at 25 ± 1 °C.

**Preparation of the nitrate-selective electrode**

PVC membranes were prepared as NO₃⁻-sensing membranes by dissolving PVC, THANO₃, and NPOE in several mL of THF (0.1 g of PVC, 3.75-150 mg of THANO₃, 0.05-0.2 g of NPOE in 1.5 mL of THF). A 30-μL aliquot of the THF solution just obtained was spread
over a glass plate, where it was allowed to dry, so as to make it a flat membrane. The thickness of the NO$_3^-$-selective membranes thus prepared was measured with a digital micrometer (MDQ-30, Mitutoyo Co.). Three types of NO$_3^-$-sensing membranes (0.03 ± 0.01 mm, 0.08 ± 0.02 mm, and 0.17 ± 0.03 mm in thickness) were attached to one end of PVC tubes (thickness: 5 mm; inner diameter: 3.18 mm, Saint-Gobain Com.), which looked like PVC cups. In order to avoid the presence of Cl$^-$ and K$^+$ ions in the inner solution in contact with the NO$_3^-$-selective membrane, one side of a polypropylene tube comprising the inner Ag|AgCl electrode was covered with a porous polytetrafluoroethylene resin film coated with Nafion (referred to as Nafion membrane hereafter), as depicted in Fig. 1(a). The Nafion membrane was formed by the repetition of both impregnating 5% Nafion dispersion solution (Wako Pure Chemical Ind., Ltd.) and drying it. The polypropylene tube was then filled with the inner aqueous phase I containing 0.1 mol dm$^{-3}$ of LiCl. Here, Li$^+$ was selected as a hydrophilic cation. The PVC cup was attached to the end of another polypropylene tube. The inner Ag|AgCl electrode was inserted into this tube after the tube was filled with a 0.1-mol dm$^{-3}$ LiNO$_3$ aqueous solution (inner solution II). The potential difference across the Nafion membrane was measured to have an almost constant value of about 0 V. Since only Li$^+$ ions can move across this membrane, these data confirmed that the presence of Li$^+$ had no effect at all on the potential response. Notably, a similar lack of potential response was observed by the presence of Mg$^{2+}$ and Na$^+$. 

**Preparation of the reference electrode**

Because it turned out that the potential response of the NO$_3^-$-selective membrane was influenced by the presence of Cl$^-$ concentrations of $>10^{-3}$ mol dm$^{-3}$, a saturated MgSO$_4$ aqueous solution was used instead of one containing KCl as the electrolyte within the junction phase IV between the inner solution III of an Ag|AgCl electrode (containing MgCl$_2$ at a concentration of 0.1 mol dm$^{-3}$) and a test solution. Here, MgSO$_4$ was used as the electrolyte because this salt is
nontoxic and the liquid junction potential within the porous glass was almost constant when MgSO₄ was employed. The structure of the reference electrode is depicted in Fig. 1(b). In this electrode, one end of the Ag|AgCl electrode was encased within the Nafion membrane. Furthermore, the same end of the electrode was inserted in another polypropylene tube filled with a saturated aqueous solution of MgSO₄. Finally, the joint was coated with a paraffin film. The other end of the tube was filled with a porous vycor glass cylinder (3.4 mmφ in diameter and 4 mm in length, EC Frontier Co., BG-1A). The phase IV inside the polypropylene tube and the porous glass cylinder served as a salt bridge. The potential of this reference electrode was checked using a saturated calomel reference electrode. The time course of the resting potential of the reference electrode is shown in Fig. SI-1. After about two days, the potential settled at an approximately constant value.

**Results and Discussion**

3.1. *Properties of the NO₃⁻-sensor by using the liquid-membrane-type electrode*

Figure 2(a) shows the typical response of the potential difference (open circles), $E$, between the NO₃⁻-ISE and the reference electrode as a function of the concentration of NO₃⁻ ($c_\text{NO}_3^-$) in the sample solution. The $E$ value is described by eqn. (1):

$$E = (0.005 \pm 0.001) - (0.0533 \pm 0.0010) \log (c_\text{NO}_3^-/\text{mol L}^{-1}).$$  \hspace{1cm} (1)

The potential of the NO₃⁻-ISE gradually approached a constant value 30 s after the exchange of the sample solution. The average $E$ value and its standard deviation were evaluated using 2-s data (200-202 s after the start of each measurement). Figure SI-2 indicates the changes of the calibration curves with respect to time constant throughout the measuring period (30 days). Table SI-1 indicates the values of the fluctuation of the NO₃⁻-ISEs as a function of the thickness of the NO₃⁻-selective membrane, of the concentration of THANO₃ and of the weight ratio.
between PVC and NPOE. Data indicate that as the concentration of THANO$_3$ in the membrane increased, so did the stability of the potential of the NO$_3^-$-ISE. However, the detection limit of NO$_3^-$ became $>10^{-5}$ mol dm$^{-3}$ when the concentration of THANO$_3$ in NPOE was 100 mg cm$^{-3}$. In the case of the same concentration of THANO$_3$ in NPOE, the potential of the NO$_3^-$-ISE characterized by the smallest weight ratio between PVC and NPOE (0.5) was the most stable. The potential response of the sensing membrane characterized by a thickness of 0.08 $\pm$ 0.02 mm was better than that with a thickness of 0.17 $\pm$ 0.03 mm. Conversely, the thinnest membrane tested (0.03 $\pm$ 0.01 mm) was physically weak. Based on these results, the authors used a sensing membrane with a thickness of 0.08 $\pm$ 0.02 mm and decided the weight proportion ratio of PVC powder: NPOE: THANO$_3$ was 0.5: 1.0: 0.003. The results reported in Fig. 2 were obtained using the NO$_3^-$-ISE prepared according to the specifications just described. The response time of each NO$_3^-$-ISE ranged 5-30 s and the detection limit was $10^{-6}$ mol dm$^{-3}$. Figure SI-3 presents a schematic of the ion transfer taking place at the interface between the NO$_3^-$-sensing membrane and the sample solution, based on voltammetry for the ion transfer at the interface between the two immiscible electrolyte solutions such as aqueous|nitrobenzene, aqueous|1,2-dichloroethane interfaces.\textsuperscript{29,30} Except for the effect of the coexisting ions and of interfering ions in particular, the membrane potential depends on the ratio of the activity of NO$_3^-$ in the inner solution to that in the outer sample solution [see eq. (1)].

Conversely, as the concentration of THANO$_3$ in the membrane decreased, the resting potential of the solution became more unstable. Notably, a decrease in the concentration of THANO$_3$ in the membrane causes an increase in the resistance to the solution within the membrane. Similarly, the potential of the NO$_3^-$-ISE fluctuated when the membrane became thicker or the weight ratio between PVC and NPOE became larger, despite the membrane being characterized by the same concentration of THANO$_3$ in NPOE. Considering the trends just described, the composition of the PVC membrane of the NO$_3^-$-ISE was as follows: weight
proportion ratio of PVC powder: NPOE: THANO 
3 was 0.5: 1.0: 0.003 and the membrane
thickness selected was 0.08 ± 0.02 mm.

3.2. Selectivity

The potential responses of the NO₃⁻-ISE to five anions (Cl⁻, SO₄²⁻, CO₃²⁻, H₂PO₄⁻, and
HPO₄²⁻) were investigated [see data reported in Fig. 2(b)]. The influence of coexisting ions on
the potential of the NO₃⁻-ISE, E_{ISE}, is usually evaluated using the Nicolsky–Eisenman equation,
as expressed by eq. (2). 31

\[
E_{ISE} = E_i^- + \frac{RT}{z_i F} \ln \left( a_i + \sum_{j \neq i} k_{ij}^{pot} a_j^{z_j} \right)
\]  

Here, \( E_i^- \) is a constant value that depends on the identity of ionic species \( i \) and on the cell
construction, \( z_i \) and \( z_j \) are the charge numbers of the ions \( i \) and \( j \), \( F \) is the Faraday constant, \( a_i \)
and \( a_j \) are the activities of ions \( i \) and \( j \), \( k_{ij}^{pot} \) is the selectivity coefficient of ion \( j \), \( R \) is the gas
constant, and \( T \) is the temperature. The \( k_{NO_3^-}^{pot} \) values for Cl⁻, SO₄²⁻, CO₃²⁻ (at pH 7), H₂PO₄⁻
(at pH 6), and HPO₄²⁻ (at pH 8.5) are evaluated as 0.01, 0.0009, 0.0007, 0.0005, and 0.0006,
respectively, in conditions whereby the concentration of these ionic species is 0.1 mol dm⁻³.
Therefore, the value of \( E_{ISE} \) is affected by the presence of Cl⁻ in the sample solution when the
concentration of Cl⁻ is > 10⁻³ mol dm⁻³. Because the inner solution of the conventional NO₃⁻-ISE
usually contains not only NO₃⁻ ions but also Cl⁻ ions when measuring the potential difference
with an Ag|AgCl electrode, the response of the membrane can easily be affected by the presence
of Cl⁻. In addition, another Ag|AgCl|sat.-KCl electrode system is always used as a reference
electrode in the cell system. Therefore, we consider that the instability and/or deterioration of
the conventional NO₃⁻-ISE is due to the presence of Cl⁻ ions in the cell system. In recent years,
the interference of coexisting ions in the potential of ISEs has been explained by both the
relation between the transfer energy of the target ion and that of the coexisting ion and the
activity of the coexisting ion in the sample solution. The Gibbs energy for the transfer of NO$_3^-$ from water to NPOE (50.3 kJ mol$^{-1}$) is 14.4 kJ mol$^{-1}$ larger than that for Cl$^-$ (35.9 kJ mol$^{-1}$), thus, this fact may explain the observation that the zero-current potential of the NO$_3^-$-ISE seemed to change when the Cl$^-$ concentration was >10$^{-3}$ mmol dm$^{-3}$. As for the other anions, because the potentials for the transfer of SO$_4^{2-}$, HCO$_3^-$, H$_2$PO$_4^-$ and HPO$_4^{2-}$ from the aqueous phase to NPOE have not yet been reported, we compare the hydration energies of Cl$^-$, SO$_4^{2-}$, HCO$_3^-$, H$_2$PO$_4^-$ and HPO$_4^{2-}$ with that of NO$_3^-$. The hydration energies of NO$_3^-$, Cl$^-$, SO$_4^{2-}$, HCO$_3^-$, and H$_2$PO$_4^-$ have been reported to be –300, –340, –1080, –335, and –465 kJ mol$^{-1}$, respectively; therefore, data indicate that NO$_3^-$ is the most hydrophobic anion in this group. Although the hydration energy of HPO$_4^{2-}$ has not been reported, it is predicted to have an intermediate value between that of H$_2$PO$_4^-$ (–465 kJ mol$^{-1}$) and of PO$_4^{3-}$ (–2765 kJ mol$^{-1}$). These data may explain the observation that SO$_4^{2-}$, HCO$_3^-$, H$_2$PO$_4^-$ and HPO$_4^{2-}$ appeared to have hardly any influence on the potential of the NO$_3^-$-ISE. Similar to the case of Cl$^-$, these data justify the prediction that an interference of coexisting hydrophobic cations on the potential response will be observed. The hydration energies of Mg$^{2+}$, K$^+$, Na$^+$, and Li$^+$ were measured to be –1830, –295, –430, and –475 kJ mol$^{-1}$, respectively; thus, data indicate that K$^+$ is the most hydrophobic cation among these. Because interference from K$^+$ was observed when this ion was present in the cell system at concentrations >10$^{-2}$ mol dm$^{-3}$, Li$^+$ was chosen as a hydrophilic cation of the NO$_3^-$-ISE in the present study. For similar reasons, Mg$^{2+}$ was used as a hydrophilic cation within the salt bridge. Although the interference of coexisting cations on the measurement of the $E_{ISE}$ of the objective anion (NO$_3^-$) has been paid no attention until now, it turned out that the influence must be evaluated in the presence of hydrophobic cations more than K$^+$. As for K$^+$, since the concentration of K$^+$ is usually less than 1 mM in the hydroponic cultivation, it is thought that the influence of the coexistence of K$^+$ on the NO$_3^-$-ISE is negligibly small.
Since the ionic composition of the various solutions that are part of the NO$_3^-$-sensing system except for the sample solution is usually constant, the potential of the NO$_3^-$-ISE reflects a mixed potential determined by the distribution of both THA$^+$ and NO$_3^-$ between W1 and the liquid membrane (LM). In the absence of THA$^+$ in the sample solution, the potential of the NO$_3^-$-ISE is determined mainly by the ratio of the activity of NO$_3^-$ in W1 to that in the LM, as indicated by eq. (2). However, the influence of THA$^+$ in the LM can sometimes become apparent when the concentration of NO$_3^-$ in the LM is too high, as can be inferred from the graph in Fig. SI-3. The fact that the region characterized by linear potential is narrow in the presence of 0.1-g THA$^+$ NO$_3^-$ in the LM is a result of the mentioned influence.

3.3. Improvement of the reference electrodes

An Ag|AgCl|sat.-KCl electrode is usually utilized as a reference electrode to measure the potential of ISEs However, because the potential of the NO$_3^-$-ISE developed in the present study may be affected by the presence of KCl at concentrations $>$10$^{-3}$ mol dm$^{-3}$, it is predictable that in long-term measurements, the leakage of KCl from the reference electrode will end up affecting the response of the NO$_3^-$-ISE. Therefore, a saturated MgSO$_4$ solution was used in a salt bridge, and the tip of the tube was blocked by a porous vycor glass rod (3 mm in diameter and 3 mm in length). In order to prevent the outflow of Cl$^-$ from the inner site, a Nafion membrane was used. In practice, the potential difference reached a roughly constant value 1 h after the measurement started, as can be seen in the graph in Fig. SI-1.

3.4 Monitoring the concentration of NO$_3^-$ in the hydroponic solution

Using an artificial climate chamber, the temperature of the electrochemical cell-hydroponic vessel was kept constant at 20 °C and green vegetables were cultured under the irradiation of light. Fig. 3 indicates the time courses of the concentration of NO$_3^-$ in the
hydroponic solution that initially contained KNO$_3$ at a concentration of 0.25 mmol dm$^{-3}$. Notably, the value of the potential changed over time even in the absence of green vegetables. The initial changes in the ISE potential in the absence of any green vegetables were about 20 mV. Given that the response properties of the NO$_3^-$-ISE were almost constant for 21 days, this initial fluctuation seemed to result from changes in the potential of the reference electrode. Because it is predictable that a change in potential occurs in every case, the potential at the end of cultivation is treated as the standard. The value of the potential changed in the negative direction in the presence of green vegetables (Brassica rapa var. lacimifolia and Chrysanthemum coronarium), as they absorbed NO$_3^-$ from the hydroponic water through their roots. Except for the second measurement conducted in the presence of Brassica rapa var. lacimifolia, the concentration of NO$_3^-$ in the hydroponic solution decreased to 0 mmol dm$^{-3}$ within the 21 days of the experiment. In the case of the second measurement in the presence of Brassica rapa var. lacimifolia, the plant began to bloom about 50 h after the monitoring started. At precisely this point, the absorption of NO$_3^-$ was ceased, and the plant growth was the smallest when flowers opened.

**Conclusions**

An NO$_3^-$-ISE was constructed using a PVC membrane containing NPOE and THA$^+$ NO$_3^-$. This electrode displayed a linear response to NO$_3^-$ with a Nernstian slope of $-53.3 \pm 1.0$ mV decade$^{-1}$. This NO$_3^-$-ISE responded linearly to a NO$_3^-$ concentration in the $10^{-5}$–$10^{-1}$ mol dm$^{-3}$ range and the detection limit of the target ion was measured to be $10^{-6}$ mol dm$^{-3}$. It was proven that this NO$_3^-$-ISE can be used for more than 4 weeks consecutively and that during this time it can be used to monitor the concentration of NO$_3^-$ in a hydroponic solution.
Acknowledgements

The authors thank Prof. T. Matoh of Kyoto University for his useful suggestions. This work was supported by the donation from Mr. Nobuo Takeshige.

Supporting Information

Supporting information includes a comparison of the standard deviation on the potential of the respective NO$_3^-$-ISEs (thickness, concentration of THANO$_3$ and weight ratio of PVC to NPOE), the time course of the resting potential of the reference electrode, the changes with time of the calibration curves obtained using the same NO$_3^-$-ISE and a conceptual illustration for the ion transfer at the interface between the NO$_3^-$-sensing membrane and the sample solution based on the voltammetry for the ion transfer at the interface between two immiscible electrolyte solutions. This material is available free of charge on the Web at http://www.jsac.or.jp/analsci/.

References

2004, 167, 635.


Figure Captions

Fig. 1. Schematics of a) NO$_3^-$-ISE, b) a reference electrode, and c) a hydroponic system (c)

Fig. 2. a) Calibration curve of the NO$_3^-$-ISE and b) dependence of the potential of the NO$_3^-$-ISE on the concentration of coexisting anions. Each electrolyte was used as a sodium salt (and/or a lithium salt).

Fig. 3. Time courses of the concentration of NO$_3^-$ in the culture solution during hydroponic cultivation. a) First test and b) second test.
Fig. 2

(a) and (b) show the relationship between the half-wave potential $E_{1/2}$ and the logarithm of the concentration $\log(c/M)$ for different ions, with a slope of 53.3 mV dec$^{-1}$. The graphs illustrate the effect of various ions on the potential.
Fig. 3

a) [Graph showing concentration of NO$_3^-$ over time for Control, Brassica rapa var. lacinifolia, and Chrysanthemum coronarium.]

b) [Graph showing concentration of NO$_3^-$ over time for Control, Brassica rapa var. lacinifolia, and Chrysanthemum coronarium.]
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