Pollution Control of Nitrate-selective Membrane by the Inner Solution and On-site Monitoring of Nitrate Concentration in Soil

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A liquid-membrane type nitrate-selective electrode was improved to lower the influence of contaminants by modifying its inner electrode system from Ag|AgCl|Cl– to Ag|Ag+. The NO3–-selective electrode displayed a linear response to the concentration of NO3– with a Nernstian slope of –53 ± 1 mV decade–1, in the concentration region between 10–6 and 2 mol dm–3 (M). The NO3– detection limit was about 10–5 M. The electrochemical response of this electrode was stable for more than 30 days. The deterioration in responding characteristics due to the coexistence of Cl– was suppressed by use of the Ag|Ag+ redox couple in the absence of Cl– inside the NO3–-selective electrode.

Keywords Nitrate, tetraheptylammonium ion, ion-selective electrode, monitoring, soil

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**Experimental**

**Chemicals**

LiCl, LiNO₃, Li₂SO₄, NaCl, NaNO₃, Na₂SO₄, NaH₂PO₄, NaH₂PO₃, KCl, KNO₃, K₂SO₄, MgCl₂, MgSO₄, AgNO₃, tetrahydrofuran (THF), and PVC were purchased from Wako Pure Chemical Ind., Ltd. The Bromide salt of THA⁺ (THABr) and GR reagent (Kishida Kagaku) were used for spectrophotometric determination of NO₃⁻. The concentration of NO₃⁻ in soil was evaluated according to the conventional procedure using a UV-Vis spectrophotometer (Shimadzu, MultiSpec-1500). All chemicals were reagent grade and were used without further purification.

**Preparation of the nitrate-selective membrane**

The nitrate salt of THA⁺ (THANO₃) was produced by mixing an ethanol solution containing THABr with that containing excess AgNO₃. Then AgBr precipitated from the solution was removed by filtration. By adding pure water to the filtrate containing THANO₃ while heating to about 70°C, THANO₃ precipitated in the filtrate. The white solid THANO₃ was separated from the solution via filtration, and it was washed with pure water several times to remove residual AgNO₃. It was then dried under vacuum for several hours.

All the aqueous solutions were prepared using high-purity water (σ = 18.2 MΩ cm).

**Apparatus**

The electrochemical cell utilized in this study is presented in Fig. S1a (Supporting Information), and it was also used as a hydroponic vessel. The potential between the NO₃⁻-sensing electrode and the reference electrode was measured using a handmade electrometer using an instrumentation amplifier (Texas Instruments Inc., INA116) and an A/D converter (Graphitec Corp., Midi Logger GL 900).

All measurements were performed at 25 ± 1°C.

**Preparation of the nitrate-selective electrode**

As a structural material for the NO₃⁻-ISE, a urethane tube (inner diameter: 4 mm, outer diameter: 6 mm, length: about 10 cm) was used. A sensitive membrane was attached to one end of this tube. An aqueous solution containing 50 mM AgNO₃ and 50 mM Mg(NO₃)₂ was used as an internal solution. A Ag wire (diameter: 0.5 mm) was inserted into the internal solution and fixed with a paraffin film, as shown in Fig. 1a. The composition of the NO₃⁻-sensitive membrane was made of the mass ratios of PVC, NPOE, and THANO₃ set to be 0.2:0.4:0.001, respectively. These were dissolved in 2 mL of THF (1 mL per 0.1 g of PVC) and used to prepare the NO₃⁻-sensitive membrane. Approximately 50 μL of the THF solution was dropped on a flat glass plate with a syringe, and THF evaporated day and night. After peeling the membrane off, the membrane was fixed to the end of the urethane tube with a small amount of the THF solution.

**Preparation of the reference electrode**

An overview of a reference electrode (RE) is indicated in Fig. 1b. The RE was prepared by fixing a porous ceramic block (diameter: 2.7 mm, length: 5 mm, ZR-11 Muff rod, Nikkato Co. Ltd.) to one end of a perfluoroalkoxyalkane (PFA) tube with a heat shrink tube. An aqueous solution containing 50 mM MgCl₂ and 2 M MgSO₄ was used as an internal solution and a silver wire coated with AgCl (hereinafter referred to as the Ag|AgCl electrode) was inserted and fixed with a paraffin film.

**Results and Discussion**

**Improvement of the reference electrode**

Because it became clear that the potential response of the NO₃⁻-selective membrane was inhibited by the presence of Cl⁻ concentrations of >10⁻³ mol dm⁻³, an aqueous solution saturated with MgSO₄ was used as a junction between the inner solution of the Ag|AgCl electrode and a test solution. Here, MgSO₄ was utilized as an electrolyte because it is nontoxic and the liquid junction potential within the passage of the porous ceramic was almost constant. Although the potential of the double junction-type RE used in the previous study was very stable, the electrode structure was complicated and the Nafion membrane was often broken. The structure of the RE in the present study was very simple, and it is easy to make the electrodes. In addition, the potential shift of the RE was as small as the RE used in the previous study. The potential of the RE indicated about +10 mV to the calomel electrode, and its fluctuation was about several mV per day at maximum. The potential fluctuation of the RE was within ±10 mV during the first 30 days.

**Characteristics of the NO₃⁻-sensor**

Electrochemical measurements were carried out using the aqueous solution of 5.0 × 10⁻⁷ M to 1 M Mg(NO₃)₂. The calibration curve of the NO₃⁻-ISE was measured from the low-concentration side. After the sensor was washed with pure water and the surface was wiped off, each measurement was conducted. In the NO₃⁻ concentration region higher than 10⁻³ M, the response time was shorter than 10 s. Even when the concentration of NO₃⁻ was lower than 10⁻³ M, the response time was within 60 s. Measurement time was 120 s for each measurement and the average value of the potential (E) obtained from 60 to 120 s was finally recorded. Figure 2 shows the potential response of the NO₃⁻-ISE. The NO₃⁻-ISE indicated a linear response with a slope of −49 ± 1 mV dec⁻¹ to NO₃⁻ in the concentration range from 10⁻⁵ to 0.1 M. In addition, the NO₃⁻-ISE showed a nearly linear response in the high concentration range up to 2 M, as shown in Fig. S2. The detection limit was experimentally estimated at about 10⁻⁵ M. The E value is expressed by Eq. (1).

\[
E = (0.367 \pm 0.001) - (0.049 \pm 0.001) \log(c_{NO_3}/\text{mol L}^{-1}) \tag{1}
\]

Here, c_{NO_3} is the concentration of NO₃⁻.

On the other hand, each ISE was found to actually respond to activity rather than concentration. Therefore, the activity of
NO₃⁻ in each solution was calculated and the ISE potential was linearly changed with its logarithm. Here, using the activity constant of NO₃⁻, γ, the activity of NO₃⁻, \( a_{\text{NO}_3^-} \), in the aqueous solution can be defined by the following equation,

\[
a_{\text{NO}_3^-} = \gamma_{\text{NO}_3^-} c_{\text{NO}_3^-}
\]

(2)

The mean activity coefficient, \( \gamma_s \), is expressed by Eq. (3) based on the Debye–Hückel rule:²²

\[
\log \gamma_s = \frac{A z_i z_j \sqrt{I}}{1 + B d \sqrt{I}} + b I
\]

(3)

Here, \( A = 0.509 \text{ mol}^{1/2} \text{ (298 K)} \), \( B = 0.3291 \times 10^8 \text{ cm}^{-1} \text{ mol}^{-1/2} \text{ kg}^{1/2} \), \( d = 3.78 \times 10^4 \text{ cm}^{1/2} \text{ z}_i (\text{charge number of Mg}^{2+}) = +2, z_j (\text{charge number of NO}_3^-) = -1, I (\text{ion strength of Mg(NO}_3)_2 \text{ solution}) = 1.5 \text{ c}_{\text{NO}_3^-}, b = 0.0585 \text{ mol}^{-1} \text{ kg}. \) By use of \( a_{\text{NO}_3^-} \) derived from Eqs. (2) and (3), the slope of Eq. (1) is \(-53 \pm 1 \text{ mV dec}^{-1}\), which is more close to the theoretical value (\(-59 \text{ mV dec}^{-1}\)). The potential response of the NO₃⁻-ISE was not affected by the existence of SO₄²⁻. Similarly, the potential responses of the NO₃⁻-ISE were not affected by the other three anions (CO₃²⁻, H₂PO₄⁻, and HPO₄²⁻), as shown in Fig. 4. On the other hand, the potential of the NO₃⁻-ISE responded to K⁺ in the presence of more than 10⁻³ M K⁺, as presented in Fig. 5. The influence of the coexisting ion on the resting potential of the NO₃⁻-ISE (\( E_{\text{ISE}} \)) is evaluated by the Nicsky–Eisenman equation, as expressed by Eq. (4):²⁴

\[
E_{\text{ISE}} = E^* + \frac{RT}{z_iF} \ln \left( a_i + \sum k_{i,j}^{\text{pot}} a_j^{z_j} \right)
\]

Here, \( E^* \) is a constant value that depends on the properties of an objective ion \( i \) and on the cell composition, \( a_i \) and \( a_j \) are the activities of the ions \( i \) and \( j \); \( z_i \) and \( z_j \) are the charge numbers of \( i \) and \( j \); \( F \) is the Faraday constant, \( k_{i,j}^{\text{pot}} \) is the selectivity coefficient of \( j \); \( R \) is the gas constant, and \( T \) is the temperature. The \( k_{\text{NO}_3^-}^{\text{Cl}^-} \) values for Cl⁻, SO₄²⁻, HCO₃⁻ (at pH 7), H₂PO₄⁻ (at pH 6), and HPO₄²⁻ (at pH 8.5) previously reported by the author’s research group are 0.01, 0.0009, 0.0007, 0.0005, and 0.0006, respectively.²⁹ Figure 3 shows the potential responses of the NO₃⁻-ISE to Li⁺ salt of NO₃⁻, Cl⁻, and SO₄²⁻, respectively. Although SO₄²⁻ did not affect the NO₃⁻-ISE at all, the influence of Cl⁻ appeared around 10⁻³ M and above. Based on the Nikolsky–Eisenman equation, \( k_{\text{NO}_3^-}^{\text{Cl}^-} \) is evaluated with 0.01
solution was metabolized and converted to the plant body. The decreased by the absorption and the NO₃⁻ concentration locally thought that 2.5
solution (200 mL of tap water containing 1 mM KNO₃), and the chrysanthemum coronarium was immersed in the culture
coronarium (Glebionis coronaria solution owing to the intake by a green plant, chrysanthemum
The initial concentration was about 1 mM, and it decreased
during 48 h. In this case, the potential was recorded, as it was.

\[ \Delta c / \Delta t = -0.03 \text{ mM h}^{-1} \]

(i = NO₃⁻, j = Cl⁻) and it is in good agreement with the previous value. In the present case, the \( k_{NO_3} \) value is less than 10⁻⁶. Figure 4 indicates the potential response of the NO₃⁻-ISE to the other anions such as CO₃²⁻, HPO₄²⁻, and H₂PO₄⁻ that often exist in the natural environment. Thus, these hydrophilic anions did not interfere with the NO₃⁻-ISE. Figure 5 expresses the potential response of the NO₃⁻-ISE to salts of SO₄⁻. It turned out that K⁺ affected the NO₃⁻-ISE around 10⁻³ M, while Mg²⁺ did not have an influence, similarly to Na⁺. That is, the \( k_{NO_3} \) value was estimated at about 10⁻³. As for anion-selective electrodes, the selectivity is usually evaluated for coexisting anions, but it is suggested that some kinds of coexisting cations can also influence the liquid-membrane type ISE.

Real-time monitoring

The variation of the concentration of NO₃⁻ in the culture solution owing to the intake by a green plant, chrysanthemum coronarium (Glebionis coronaria), was monitored. The root of chrysanthemum coronarium was immersed in the culture solution (200 mL of tap water containing 1 mM KNO₃), and the NO₃⁻-ISE and RE were inserted in the culture solution, as shown in Fig. S1b. The temperature was kept at 25°C using a plant incubator (Biotron LH200 NK system, Nippon Medical & Chemical Instruments Co., Ltd.), and the cultivation was carried out under illumination. A control experiment was carried out using the same container containing only tap water. The water volume of the control experiment was varied from 200 to 188 mL. After the cultivation, the volume of the culture solution changed from 200 to 123 mL. The difference seems to be caused by the stomatal transpiration. The weight of the green plant before and after the experiment was measured after wiping off drops of water on the surface. The plant weight increased from 5.14 to 7.68 g due to the cultivation. Figure 6 expresses the time-course of the concentration of NO₃⁻ in the culture solution during 48 h. In this case, the potential was recorded, as it was. The initial concentration was about 1 mM, and it decreased linearly with time. Since the concentration of NO₃⁻ rapidly decreased by the absorption and the NO₃⁻ concentration locally became heterogeneous, the culture solution was stirred during the real-time monitoring. It seems that the fluctuation of the NO₃⁻ concentration during the measurement was caused by the stirring and the absorption of NO₃⁻. After 35 h, NO₃⁻ was almost exhausted. Thus, we succeeded in monitoring the concentration of NO₃⁻ in the culture solution in real time. It is thought that 2.5 × 10⁻³ g of nitrogen atoms in the culture solution was metabolized and converted to the plant body. The

\[ \text{weight corresponds to 1.0\% of the total weight change, and it matches with the percentage of nitrogen atoms in general plants.}^{25} \]

Measurement of Nitrate Concentration in Soil

The dry soil sample was prepared as described in the SI. Soil samples were prepared by mixing the dry soil samples with tap water and adding a small amount of NaNO₃. The water contents were prepared at about 20 wt%. The concentration of NO₃⁻ within the soil samples was directly measured using the NO₃⁻-ISE and the reference electrode. About 30 g of a soil sample was measured and then dried at 200°C for 24 h. Then, 10 mL of distilled water was mixed with about 30 g of each dry soil sample, and the concentration of NO₃⁻ of the filtrate was spectrometrically determined by use of a GR reagent. Table 1 shows the concentration of NO₃⁻ of five soil samples. There was not a significant difference between the data obtained by the two measuring methods. Thus, the ISE method is very useful and powerful for practical use. The concentration of NO₃⁻ in the aqueous solution is often measured by a spectrophotometric method.\(^{35,26}\) Since this method requires a considerable amount of time and effort, an alternative method that can measure the concentration of NO₃⁻ easily and on-site is required. The ISE method seems to be optimum, because it does not need any pretreatment and the concentration can be directly evaluated.

Soil was filled into a cylindrical vessel (inner diameter: 10 cm, depth: 15 cm) that had multiple holes in the bottom, and 1 mM NaNO₃ was filled onto a saucer, as shown in Fig. S3. Since the soil usually contains NO₃⁻ and water easily evaporates at the surface, it is thought that the concentration of NO₃⁻ near the soil surface was 20 - 50 mM. Next, the depth dependence of the concentration of NO₃⁻ was estimated by use of the NO₃⁻-ISE. Figure 7 indicates the depth dependence of the concentration of NO₃⁻ of 6 h later. Although the concentration of NO₃⁻ in soil samples with a depth of 9 and 10 cm is about 3 mM, the

| Method   | Sample 1 | Sample 2 | Sample 3 | Sample 4 | Sample 5 |
|----------|----------|----------|----------|----------|----------|
| ISE      | 17       | 43       | 21       | 43       | 17       |
| Spectroscopy | 20       | 48       | 22       | 37       | 11       |

Fig. 7 The concentration of NO₃⁻ in soil samples of which depth ranged from 0 to 10 cm. The concentration of NO₃⁻ was directly measured by the NO₃⁻-ISE.
the concentration of NO$_3^-$ around the surface increased. Since the dry soil sample contained NO$_3^-$ derived from tap water, the NO$_3^-$ in soil samples was concentrated around the bottom. It is considered that this behavior is caused by drawing water by capillary action and evaporation of water at the surface.

**Conclusions**

NO$_3^-$-ISE for detecting the concentration of NO$_3^-$ in the region between $10^{-3}$ and 0.1 M was constructed. Although it is slightly affected by Cl$^-$ and K$^+$, no other ions coexisting in the usual environmental water affected the ISE, showing that the NO$_3^-$-ISE is applicable to real time monitoring. The direct measurement of soil was carried out with this ISE. As for the on-site analytical method, the measurement using optical sensors is well known. Since the concentration of nutrient ions on the soil surface is directly measured, it is very convenient to evaluate the concentration rapidly without needing pretreatment, as with the ISE method. It is thought that the fluctuation of the optical data is larger than those observed by use of the ISE. This might be because the surface condition significantly fluctuates with time.

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**Supporting Information**

This material is available free of charge on the Web at http://www.jsac.or.jp/analsci/.

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