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## **Evaluation of Ion-Selective Membranes for Real-Time Soil Nutrient Sensing**

**Hak-Jin Kim, Graduate Research Assistant**

Biological Engineering Department, University of Missouri, Columbia, Missouri, USA, 65211,  
[kimhj@missouri.edu](mailto:kimhj@missouri.edu)

**John W. Hummel, Agricultural Engineer**

Cropping Systems & Water Quality Research Unit, USDA-ARS, Columbia, Missouri, USA,  
65211, [hummelj@missouri.edu](mailto:hummelj@missouri.edu)

**Stuart J. Birrell, Assistant Professor**

Department of Agricultural and Biosystems Engineering, Iowa State University, Ames, Iowa,  
USA, 50011-3080, [sbirrell@iastate.edu](mailto:sbirrell@iastate.edu)

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**Abstract.** *A key to developing a real time, automated soil nutrient sensor depends on the ability to effectively extract soil nutrients from a soil sample and precisely detect them in a very short time period. An ion-selective field effect transistor (ISFET) chip has proven to be a good candidate for use in real-time soil nutrient sensing because of its rapid response and low sample volume. This paper describes the evaluation of nitrate ion-selective membranes and the investigation of the interaction between the ion-selective membranes and soil extracting solutions. The response characteristics of the membranes and their suitability for use for real-time soil nutrient sensing were investigated through evaluation of their sensitivity and selectivity using 16 ion selective electrodes (ISEs) in a computer-controlled system. All membranes showed an approximately linear Nernstian response when nitrate concentrations were above  $10^{-3}$  M, irrespective of extracting solution type. At low nitrate concentrations, below  $10^{-4}$  M, both the extracting solution and ligand types significantly affected the sensitivity and selectivity of each membrane. A TDDA-NPOE membrane showed greater sensitivity to nitrate*

*than did the MTDA-NPOE and MTDA-TOTM membranes. In addition, the selectivity of the TDDA membrane was superior to that of the MTDA membranes when bicarbonate, chloride, and bromide were considered as the interfering ions.*

**Keywords.** *Ion Selective Electrode (ISE), Ion Selective Field Effect Transistor (ISFET), Ion-Selective Membrane, Soil Nutrient Sensing, Nernst Response, Sensitivity, Selectivity*

## Introduction

Chemical fertilizers have been applied to soils as essential sources of nutrients for intensive agricultural production. But the excessive use of these substances can lead to runoff of nutrients into surface and ground water, causing an undesirable environmental impact (Artigas, et al., 2001). Recently, one of the environmental issues related to nitrogen has been water contamination with nitrates, which is a possible cause of oxygen depletion (hypoxia) in a large zone of the Gulf of Mexico along the Louisiana-Texas coast (Kaiser, 2001). Nitrate in drinking water has been reported to affect human health, and high concentrations have been blamed for causing the “Blue-baby syndrome” in infants less than six months of age (Illinois Department of Health, 1994). In this condition, the infant develops a syndrome where its blood system cannot effectively carry oxygen to the brain and other parts of the body.

Accumulation of nutrients in excess of crop needs may also increase the potential for eutrophication of surface waters. Eutrophication means that nutrient levels in water, especially phosphorus (P), are high and excessive algae growth occurs, which could create imbalances in the water ecosystem. This problem is compounded because soils of many grain crop production areas already have soil-test P levels that are at or above optimal grain yield levels (Mallarino, 1998).

Of the soil nutrients required in plants, macronutrients including nitrogen (N), phosphorus (P), and potassium (K) are some of the most important components for crop production. Nutrient uptake in plants is accomplished by combinations of root interception, mass flow and diffusion processes involving ionic forms such as  $\text{NO}_3^-$ ,  $\text{H}_2\text{PO}_4^-$ , and  $\text{K}^+$  in the soil.

Monitoring nutrient levels in soils, such as traditional soil testing, can provide useful information for the reduction of environmental impact, as well as for the efficient use of fertilizers resulting in the optimization of crop production. In addition, if the information were obtained in real time, it would allow the collection of data on a much finer spatial resolution to characterize more accurately within-field variability (Birrell and Hummel, 2000; Artigas, et al., 2001).

The development of a real-time soil nutrient sensor that could accurately estimate soil nutrient levels could result in the minimization of the chemical fertilizers impact on the environment, as well as the cost reduction of use of chemical fertilizers (Price, 2003).

Currently, systems that measure ions directly in the soil are not commercially available. Present soil nutrient measurements are carried out through soil sampling in the field and chemical analysis in the laboratory. All the standard methods are based on extraction processes, pre-treatment of the sample, and analysis using atomic absorption spectrometry, chromatographic techniques, etc. (Bigham, 1996). These are complex, time-consuming methods, which require expensive facilities and instruments (Artigas et al., 2001).

Ion-selective microelectrode technology, such as Ion Selective Electrodes (ISEs) and Ion Selective Field Effect Transistors (ISFETs), has been widely used in environmental monitoring and the biomedical field (Ammann, 1986). Much research on soil nitrate sensing has been focused on the use of ISEs to detect nutrient ions such as  $\text{NO}_3^-$ ,  $\text{Ca}^{+2}$ , and  $\text{K}^+$  in the soil (Dahnke, 1971; Pungor and Buzas, 1981; Adamchuck, 2000). Additionally, the application of an ISFET chip combined with Flow Injection Analysis (FIA) has represented a great advance and has proven to be an interesting alternative to ISEs since ISFETs have several advantages such as small dimensions, low output impedance, high signal-to-noise ratio, fast response and the ability to integrate several sensors onto a single electronic chip (Artigas et al., 2001; Birrell and Hummel, 2000). The rapid response and low sample volumes required by an ISFET/FIA system

would make it a promising candidate for use in real-time soil nutrient sensing (Birrell and Hummel, 2001).

A key to developing a real time, automated soil nutrient sensor depends on the ability to effectively extract soil nutrients from a soil sample and precisely detect them at one time in a very short time period. Considerable research has demonstrated the good capability of ISEs to determine nitrate concentrations in soil (Nielson et al., 1976; Hansen et al., 1977; Birrell and Hummel, 2001). However, most of the research related to real time sensing for soil analysis has not shown satisfactory results on an automated soil sampling and extraction system because most systems have problems with maintaining constant flow parameters and precise amounts of soil sample (Adsett et al., 1999; Birrell and Hummel, 2001).

## Review of Literature

As potentiometric sensors, ISEs that respond selectively to one analyte in the presence of other ions in a solution have been widely used in clinical analysis and analytical chemistry. Several researchers have expended considerable effort in attempting to apply ion-selective microelectrodes to the field of soil analysis that requires the determination of soil nitrates (Dahnke, 1971; Hansen et al, 1976; Li and Smith, 1984).

Dahnke (1971) used a nitrate ISE for rapid determination of nitrate in soil extracts while changing several factors, including interfering anions, extracting agents, soil to solution ratios, and reference electrodes. The results showed that the lower limit of accurate detection of the  $\text{NO}_3$  electrode was about 1-2 ppm  $\text{NO}_3\text{-N}$ . This level of extreme sensitivity proved to be useful for measuring the nitrate ions in the soil. Consequently, it was reported that the nitrate ion electrode provided a rapid and convenient method of determining the amount of  $\text{NO}_3\text{-N}$  in the solution and was well suited for use in routine soil testing.

Li and Smith (1984) investigated the rapid determination of nitrate at low concentrations in soil, by extracting solutions and using ISEs. Saturated  $\text{CaSO}_4$  was used as a soil extracting solution. By using a known addition of nitrate to the soil extract and the calibration extract, the response of the electrodes at low concentrations could be improved. Correlation between the electrode method and the continuous flow colorimetric method was high ( $R=0.9430$ ) and the ratio between the two methods was 0.94. However, the results were not produced in real time but in the laboratory so that the use of an on-going sensor was impossible. Since the 1990s, several researchers (Adsett and Zoerb, 1991; Adsett et al, 1999; Adamchuck, 2000) have reported on studies on real-time soil nutrient sensing using ion-selective electrodes and on development of soil samplers.

Adsett and Zoerb (1991) developed a tractor-mounted field monitoring system to directly measure soil nitrate levels. This system, consisting of a soil sampler, nitrate extraction unit, flow cell, and controller, was tested in the laboratory and field. The ISE technology was adaptable to automated field monitoring of soil nitrate levels. However, field tests indicated that the system did not produce repeatable results, and only 40% of the nitrate readings were accurate because its performance was affected by inadequate mixing of the soil and extraction solution. To solve this nitrate extraction problem, Adsett et al. (1999) redesigned the soil sampler using a wood saw blade and belt-conveying unit to gather and transport samples of known volume and density to the extraction and analysis unit. The results from lab testing indicated that the actual nitrate level could be predicted with 95% accuracy after 6 s of measurement. However, there were still several mechanical and electrical problems during field testing, such as clogging of the extractor outlet with plant residue and unacceptable levels of noise in the electrode signal.

Adamchuck et al. (1999) developed an automated sampling system for measuring soil pH by using a flat-surface combination pH electrode. The pH electrode measures the hydrogen ion concentration in soil solution. The system consisted of a lever situated below a soil tine, which collected a sample of soil and then rotated to press the soil slurry against the surface of a pH electrode. They reported that the automated measurement of soil pH using the flat surface combination electrode was possible. The test showed a high correlation ( $r^2=0.92$ ) between the electrode voltage output and soil pH in the laboratory, whereas a standard error ( $r^2 = 0.83$ ) of prediction of soil pH of 0.45 existed in the field.

Based on the above results, an automated soil pH mapping system was implemented by Collings et al. (2003). A soil sampling system consisting of a cutting shoe and a sampling trough was built to collect soil samples. The pH measurements were carried out with a glass pH electrode and a reference electrode, which were contacted with soil samples brought by the soil sampler. A microcontroller was used for controlling rinsing of the pH electrodes and communicating with a logging instrument. The accuracy of the system was evaluated by comparing collected pH data to laboratory analysis. The results showed that sensor readings were correlated well with laboratory measurements, with a correlation coefficient of 0.7972.

In the 1970's, a dramatic advance in the miniaturization of ion-selective membrane technology occurred when Bergveld (1970, 1972) reported on ISFETs. Since these initial reports on ISFETs, the majority of studies using ISFETs have been conducted in the medical field using ion-selective polymeric membranes, which allow the ISFET to respond selectively to the chemical species of primary interest. The chemistry of the ion-selective membrane on the ISFET is the same as that of conventional ISEs. Recent advances in microelectromechanical systems (MEMS) have also contributed to the application of ISFETs to soil analysis. The MEMS technology can result in batch fabrication of a sensor having all electronic and mechanical components on a single silicon chip (Birrell and Hummel, 2001).

Birrell and Hummel (2000) investigated several PVC matrix membranes to develop a nitrate ISFET chip that can be integrated with a FIA system for real-time soil analysis. As a result, the membranes tested proved to be viable candidates for ISFET use, with selectivity levels that were at least 40 times greater for nitrate than for chloride and bicarbonate.

Failures of individual ISFETs generally result from detachment of the membrane from the ISFET gate. Various membrane compositions for ion-selective electrodes were studied for their adhesion characteristics to the gates of field effect transistor devices used for the construction of ISFETs (Moody et al., 1988; Tsukada et al., 1989). They optimized the composition of the PVC membrane cocktail to improve the membrane adhesion and produced ISFETs with a useful life of greater than two months without compromising chemical response.

Artigas et al. (2001) reported on the fabrication of pH,  $\text{Ca}_2^+$ ,  $\text{NO}_3^-$ , and  $\text{K}^+$  ISFETs with photocurable polymeric membranes and their evaluation in aqueous solutions to investigate the application of ISFET technology to soil analysis. The photocurable polymeric membrane provided better adhesion to the surface of the ISFET and longer lifetime than PVC-based membranes. Sensor response characteristics were stable for two months. During that time no membrane damage occurred and no peel off was observed in the laboratory.

A more direct approach to measure nitrates in the soil by using an ISFET chip was achieved by Birrell and Hummel (2001). In order to overcome the limitations of laboratory use of ISFET chips, they attempted to develop a real-time multi-ISFET system combined with a FIA system and an automated soil extraction system. The results showed that the multi-ISFET sensor was successful in predicting soil nitrates in manually extracted soil solutions with correlation coefficients greater than 0.9. The rapid response of the system allowed samples to be analyzed within 1.25 s with sample flow rates less than 0.2 m/s. However, a prototype automated soil

extraction system did not consistently provide soil extracts that could be analyzed by the ISFET/FIA due to blockages in the filtration process. They suggested that considerable effort would be required for the development of an automated soil extraction system enabling the soil to be well mixed with extracting solution and the nutrients to be effectively extracted from the soil solution.

Price et al. (2003) reported on an intact core extraction system that might be used in the field for real-time prediction of soil nitrates using ISFETs developed by Birrell and Hummel (2001). Several design parameters affecting the nitrate extraction of the soil cores and output data of the ISFETs were studied. The results showed that nitrate extraction of the soil cores were successful. In addition, data descriptors based on the peak and slope of the ISFET nitrate response curve might be used in a real-time prediction system.

## Objectives

The overall objective of this research was to investigate the suitability of different ion-selective membranes for sensing important soil macronutrients such as  $\text{NO}_3^-$ ,  $\text{H}_2\text{PO}_4^-$  and  $\text{K}^+$  in order to develop a multi-ISFET chip integrated with an automatic soil extraction system for real-time soil analysis. As a first step to achieve this goal, this paper describes the evaluation of ion-selective membranes for soil nitrate sensing and the investigation of the interaction between ion selective membranes and extracting solutions. Specific objectives included:

- Evaluate the capabilities of ion selective membranes for soil nitrate sensing with respect to their sensitivity, lower detection limits, and selectivity against interferences from other ions.
- Investigate the effect of soil extracting solution on the response characteristics of ion selective membranes to predict nitrate concentrations in the solution.
- Determine an ion selective membrane and a soil extracting solution combination that is suitable for use with a real-time ISFET sensor.

## Materials and Methods

### *Reagents and Solutions*

For the determination of the nitrate, quaternary ammonium compounds were used as ligands in non-porous PVC –based nitrate ion-selective membranes. These membranes selectively bind the nitrate ion as a complex, and transport this nitrate through the membrane by a carrier mechanism. The ligands – tetradodecylammonium nitrate (TDDA) and methyltridodecylammonium chloride (MTDA); and the plasticizers – nitrophenyl octyl ether (NPOE) and tri-(2-ethylhexyl) trimellitate (TOTM) were selected for testing based on previous studies (Nielson et al. 1976; Tsukada et al., 1989; Birrell and Hummel, 2000).

Many of the soil testing procedures use different extracting solutions to determine soil nutrients. In a continuous flow-through system, a universal extracting solution would be advantageous as this would reduce the amount of soil preparation required for detecting different nutrients. Distilled water was recommended as an extractant for the soil nitrate test (Dahnke, 1971). A sulfate solution, 0.01M  $\text{CuSO}_4$ , was proven to be a suitable extractant because it causes little interference at low concentrations (Yu and Ji, 1993). Van Lierop (1986) showed that the Kelowna multiple ion extractant (0.25M  $\text{CH}_3\text{COOH}$  + 0.015M  $\text{NH}_4\text{F}$ ) could be used when determining soil nitrate concentrations, as well as when extracting phosphorus and potassium. Hence, these three different extracting solutions, distilled water, 0.01M  $\text{CuSO}_4$ , and Kelowna,

were utilized as base components of standard solutions for calibrating the response characteristics of ion selective membranes.

### ***Preparation of Ion Selective Membranes and Electrodes***

The ion selective membrane cocktail mixtures were prepared in the identical manner to those used in previous research by Birrell and Hummel (2000). A mixture of 30 mg of ligand (TDDA or MTDA), 80 mg of plasticizer (NPOE or TOTM) and 90 mg of high-molecular-weight polyvinyl chloride (PVC) was dissolved in 2 ml tetrahydrofuran (THF). After being stirred in a vial, the mixture was poured into a 23-mm glass ring resting on a polished glass plate, and allowed to evaporate for 24 h at room temperature. After removing the membrane, which formed as a film on the polished glass plate, three disks with a diameter of 2.5 mm were cut from each membrane. The membrane disks were attached to the end of PVC Hitachi ISE electrode bodies with the THF. Prior to testing, the ISE electrodes were conditioned in a 0.01M NaNO<sub>3</sub> solution for at least 6 h so that steady potentials could be obtained.

Each ISE electrode was filled with an internal solution consisting of 0.01M NaNO<sub>3</sub> and 0.01M NaCl. An Ag/AgCl electrode was immersed as the inner reference electrode. For the evaluation of ISEs in aqueous solutions, a double junction Ag/AgCl electrode (Omega Model PHE 3211) was used as an outer reference electrode, which had a 1M KCl reference electrolyte and 1M LiOAc.

### ***Apparatus***

An automated test apparatus was designed for simultaneously measuring the electromotive forces (EMFs) of 16 ISE electrodes (Fig 1). For controlling the system and recording values obtained from the ISE electrodes, a program was developed by using Microsoft Access and Visual Basic. A Daqbook 200 portable PC-based data acquisition system and a 400 MHz Pentium computer were used to collect and store ISE electrode voltage outputs. Eight different solutions, contained in Teflon-coated buckets, were controlled by a multi-channel peristaltic pump and eight solenoid valves. The program automatically activated valves to control injection of solutions into the test stand, and controlled pan motor speed to either move solution past the test electrodes (slow speed) during data collection, or to expel solutions from the pan (high speed) between tests.

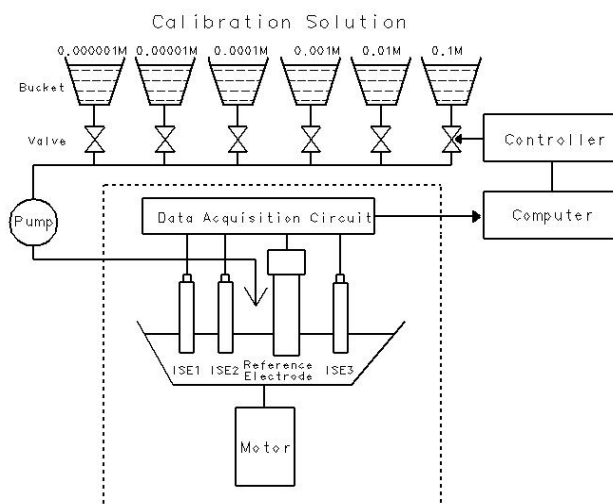


Figure 1. Schematic representation of ion selective electrode test apparatus.

To minimize current leakage, capacitive loading, and noise pickup of EMFs generated from the electrodes (Fig. 2), a buffering circuit module consisting of 16 operational amplifiers was used.

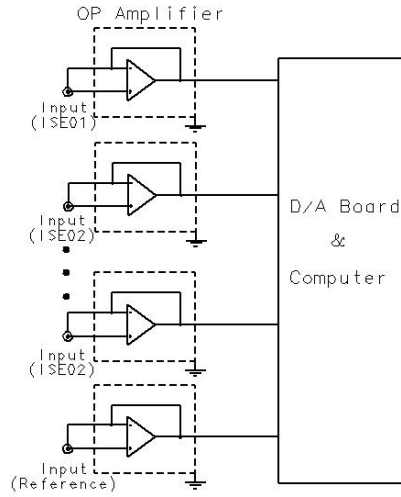


Figure 2. Block diagram of an operational amplifier module with high impedance input.

### Sensitivity Test

Two different sets of membrane combinations were prepared. The initial set included two TDDA-NPOE (A, B) membranes, two MTDA-NPOE (A, B) membranes, and one MTDA-TOTM (A) membrane. For the second test set, two TDDA-NPOE (A, B) membranes, one MTDA-NPOE (B) membrane, and two MTDA-TOTM (A, B) membranes were selected. Sensitivity tests were conducted with three electrodes of each membrane type.

For investigating the effect of soil extracting solution on sensitivity characteristics of each membrane, the following three solutions having possible multi-element extraction capacity were used: 1) deionized (DI) water, 2) 0.01M  $\text{CuSO}_4$ , and 3) Kelowna (0.25M  $\text{CH}_3\text{COOH}$  + 0.015M  $\text{NH}_4\text{F}$ ). Six standard nitrate solutions per extracting solution were produced with different concentrations of sodium nitrate ( $10^{-6}\text{M}$ ,  $10^{-5}\text{M}$ ,  $10^{-4}\text{M}$ ,  $10^{-3}\text{M}$ ,  $10^{-2}\text{M}$  and  $10^{-1}\text{M}$ ).

The effects of membrane composition and soil extraction solution on sensitivity were investigated by linear regression of the experimentally determined Nernstian slope response against the logarithm of the ionic activities of nitrate in the solution using SAS statistical software.

The Nernst equation was used to calculate the sensitivity:

$$EMF = E_o + E_j + S \log a_i \quad (1)$$

where

EMF = electromotive force  
 $E_o$  = standard potential  
 $E_j$  = liquid-junction potential  
 $S$  = Nernstian Slope (59.16 mv/ $Z_i$  for  $\text{H}_2\text{O}$  at 25 °C)  
 $a_i$  = activity of single-ion



The concentration can be converted to activities using single-ion activity coefficients in equation (2):

$$a_i = \gamma_i C_i \quad (2)$$

where  $a_i$  = single-ion activity (M)  
 $\gamma_i$  = single-ion activity coefficient  
 $C_i$  = ion molar concentration (M)

The single-ion coefficients are determined from the mean activity coefficients of the electrolyte, which are estimated using the Debye-Huckel formula (Morf, 1981). The Debye-Huckel equation is given as follows:

$$\log \gamma_{\pm} = \frac{-A|Z_+ \times Z_-|\sqrt{I}}{1 + Ba\sqrt{I}} \quad (3)$$

where A and B are constants with values of  $0.51(10^9)$  and  $3.3(10^9)$ , respectively, at  $25^\circ\text{C}$ ,  $a$  is the ion size parameter, and  $z$  is the charge on the ion. The ionic strength,  $I$ , is a measure of the total ions in solution, weighted according to their charges and concentrations, as in the following equation:

$$I = \frac{1}{2} \sum c_i Z_i^2 \quad (4)$$

Liquid-junction potentials are always generated when electrolytic solutions of different ionic compositions are in contact (Amman, 1986). A typical reference electrode has a liquid-junction potential at the junction of the reference electrode with the sample solution. For this experiment, the potential was assumed to be constant.

### **Selectivity Test**

Selectivity tests were conducted with two sets of membranes and three extracting solutions. Each set included membranes of each of the three ligand-plasticizer combinations (TDDA-NPOE, MTDA-NPOE, and MTDA-TOTM), and the three extracting solutions (DI water, 0.01M  $\text{CuSO}_4$ , and Kelowna) which are the same as those used in the sensitivity test.

The separate solution method (SSM) was used to determine the selectivity coefficients of the interfering ions for each membrane.

The Nernst equation used in the sensitivity test assumes that the membrane is ideally specific to a certain ion. However, in most cases the membrane responds to other interfering ions and the measured EMF is the sum of the membrane potentials. The extent of interference is expressed in the Nikolski-Eiseman equation (Eqn. 5) in terms of the electrode potential and a selectivity coefficient as follows:

$$EMF = E_o + E_j + S \log[a_i + \sum K_{ij} (a_j)^{z_i/z_j}] \quad (5)$$

where

$E_o$  = standard potential  
 $E_j$  = liquid-junction potential  
 $S$  = Nernstian Slope (theoretically, 59.16 mv/ $Z_i$  for  $H_2O$  at 25 °C)  
 $a_i$  = activity of primary ion  
 $a_j$  = activity of interference ion  
 $Z_i$  = charge of primary ion  
 $Z_j$  = charge of interference ion  
 $K_{ij}$  = selectivity coefficients

The selectivity coefficient values of  $K_{i,j}$  can be determined by using the following equation:

$$K_{i,j} = 10^{\frac{E_j - E_i}{S}} \cdot \frac{a_i}{a_j^{Z_i/Z_j}} \quad (6)$$

where

$a_i$  = activity of primary ion  
 $a_j$  = activity of interfering ion  
 $E_i$  = EMF measured with solution of primary ion  
 $E_j$  = EMF measured with solution of interfering ion  
 $S$  = Nernstian Slope

The selectivity of each membrane and base solution for nitrate over the sodium salts of interference ions was investigated in the following order; bicarbonate ( $NaHCO_3$ ), chloride ( $NaCl$ ), and bromide ( $NaBr$ ).

The test sequence (Fig. 3) consisted of eight solutions that included the primary ion, nitrate, and three interfering ions such as bicarbonate, chloride, and bromide. At the beginning of the test sequence, two sodium nitrate solutions of 0.1M and 0.01M concentrations, respectively, were used to determine the sensitivity response of each membrane. The responses of the 0.1 M nitrate solutions and 0.1 M interfering ion solutions were measured to calculate the selectivity coefficients of each interfering ion. The GLM procedure (SAS, 1999) was used to determine whether the selectivity factors of the membranes were significantly different, using Duncans Multiple-Range Test.

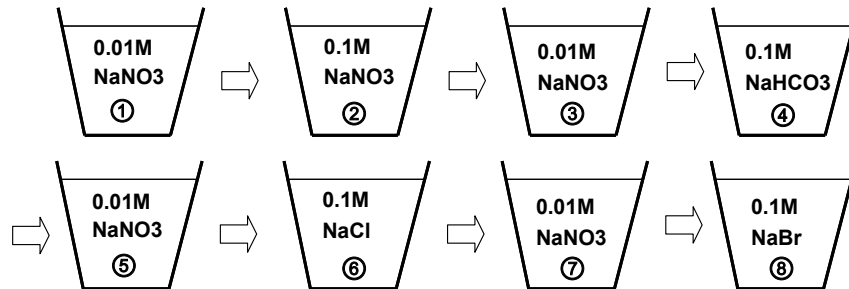


Figure 3. Test sequence of selectivity test.

## Response Behavior

The response time of a sensor is one of the most critical factors in developing a real-time sensing system and the measurement errors of the sensor must be below a certain level, so that repetitive measurements are reproducible within a certain range.

To investigate the response characteristics of ISE electrodes to changes in nitrate concentration, four solutions with different nitrate concentrations ( $10^{-5}\text{M}$ ,  $10^{-4}\text{M}$ ,  $10^{-3}\text{M}$ , and  $10^{-2}\text{M}$ ) were used. The voltage outputs of electrodes were recorded at 100 Hz with the Daqbook data collection system, while sequentially introducing the sodium nitrate solutions starting with the lowest concentration ( $10^{-3}$ ). The same solutions were then introduced in the reverse order, and the voltage outputs were recorded.

## Results and Discussion

### Sensitivity Tests

Figure 4 shows the response of the electrodes of three different membranes (TDDA-NPOE, MTDA-NPOE, and MTDA-NPOE) as the nitrates dissolved in DI water range in concentration from  $10^{-6}\text{M}$  to  $10^{-1}\text{M}$ . The EMFs of the electrodes were dependent on the logarithm of the nitrate concentration (ionic activity). The sensitivity response of typical electrodes of each membrane showed an approximately linear Nernstian response when the nitrate concentrations were above  $10^{-5}\text{M}$ . However, there appeared to be little change in voltage readings in the range of  $10^{-6}\text{M}$  to  $10^{-5}\text{M}$  nitrate concentrations. All of the electrodes exhibited a linear response over a range of  $10^{-5}\text{M}$  to  $10^{-1}\text{M}$  nitrate concentrations and their detection limits were about  $10^{-5}\text{M}$ . There was no difference in sensitivity between individual membranes when the DI water was used as the extracting solution.

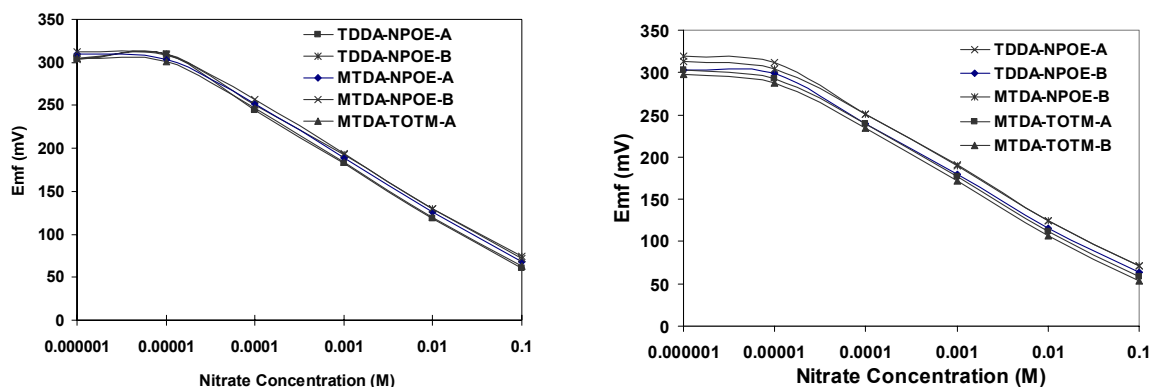


Figure 4. Electrode voltage potential vs. sample concentration for two sets of membrane compositions in DI water (left: set 1, right: set 2).

When the electrodes were used in  $0.01\text{M}$   $\text{CuSO}_4$  extracting solution (Figure 5), the response of the electrodes varied depending on membrane type. Although the decrease in sensitivity occurred at the nitrate concentration of  $10^{-4}\text{M}$  across all membranes, the TDDA membranes showed higher sensitivity at low concentrations than did the MTDA membranes. The linear response range of the TDDA-NPOE membrane was  $\sim 10^{-5}\text{M}$  -  $10^{-1}\text{M}$ , whereas the linear range of the MTDA membranes was  $\sim 10^{-4}\text{M}$  -  $10^{-1}\text{M}$ . Thus, it was found that a lower detection limit exists for the TDDA membranes than for the MTDA membranes.

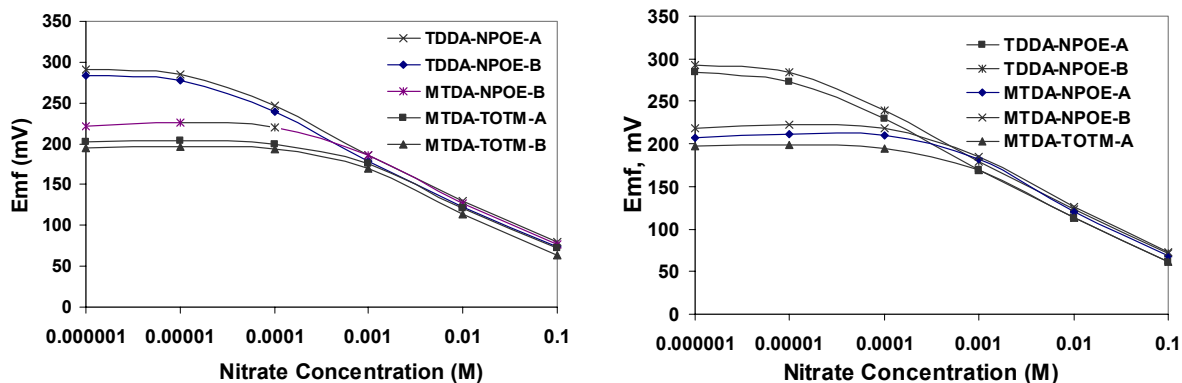


Figure 5. Electrode voltage potential vs. sample concentration for two sets of membrane compositions in 0.01M  $\text{CuSO}_4$  extracting solution (left: set 1, right: set 2).

The use of Kelowna solution, which contains 0.25M  $\text{CH}_3\text{COOH}$  and 0.015M  $\text{NH}_4\text{F}$ , affected the response curves of the membranes more significantly than did the DI water and the 0.01M  $\text{CuSO}_4$  extracting solutions (Fig. 6). The sensitivities of all membranes were decreased when using the Kelowna extracting solution (Table 1), as compared to the other two extracting solutions (DI and 0.01M  $\text{CuSO}_4$ ). The detection limits of the TDDA-NPOE membranes existed in the range of  $10^{-5}\text{M}$  to  $10^{-4}\text{M}$  nitrate concentration and those of the MTDA-NPOE and MTDA-TOTM membranes were almost  $10^{-4}\text{M}$ . The results showed that the presence of  $\text{CH}_3\text{COO}^-$  and  $\text{F}^-$  ions, which were dissolved in the solution, had a great effect on the response characteristic of nitrate selective membranes.

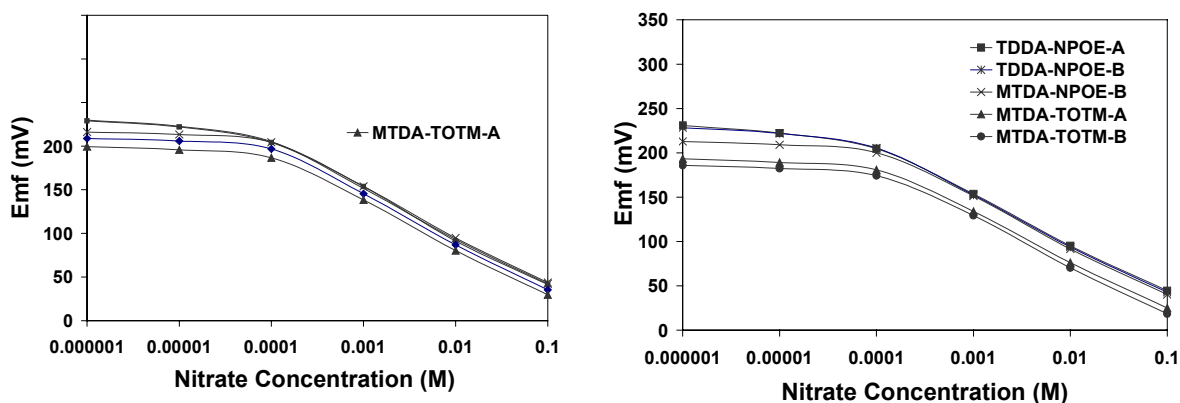


Figure 6. Electrode voltage potential vs. sample concentration for two sets of membrane compositions in the Kelowna extracting solution (left: set 1, right: set 2).

Table 1 shows the comparison of membranes sensitivity for different nitrate concentrations in the presence of each extracting solution. The results from the GLM analysis showed that there was no significant difference in sensitivity between the same kinds of membranes. In addition, at high concentrations ranging from  $10^{-3}\text{M}$  and  $10^{-1}\text{M}$ , all of the membranes showed identical sensitivity responses. When low concentrations such as  $10^{-4}$  and  $10^{-5}\text{M}$  were included, the sensitivities of the TDDA-NPOE membranes were higher than that of the MTDA-NPOE and MTDA-TOTM membranes.

The sensitivity of the membranes varied considerably between different base solutions containing different chemicals. The membrane sensitivities obtained in the DI water extracting

solution were higher than those with CuSO<sub>4</sub> and Kelowna solutions. At the nitrate concentration range of 10<sup>-1</sup> to 10<sup>-4</sup> M, in the 0.01M CuSO<sub>4</sub> solution, the sensitivities were significantly different depending on membrane type, whereas there were no significant differences in sensitivity between the membranes in the DI water and Kelowna solution. However, when the lowest concentration of 10<sup>-5</sup> M was considered, their sensitivities were significantly affected by membrane type.

Table 1. Comparison of sensitivity responses of membranes by extracting solution type.

Concentration Range	10 <sup>-1</sup> M to 10 <sup>-5</sup> M	10 <sup>-1</sup> M to 10 <sup>-4</sup> M	10 <sup>-1</sup> M to 10 <sup>-3</sup> M
-----sensitivity (mV/decade)-----			
<u>DI Water Solution</u>			
TDDA-NPOE (A)	-63.45 <sup>a*</sup>	-63.31 <sup>a</sup>	-63.48 <sup>ab</sup>
TDDA-NPOE (B)	-61.01 <sup>b</sup>	-61.56 <sup>b</sup>	-61.89 <sup>c</sup>
MTDA-NPOE (A)	-61.41 <sup>b</sup>	-63.97 <sup>c</sup>	-63.85 <sup>b</sup>
MTDA-NPOE (B)	-61.40 <sup>b</sup>	-63.58 <sup>abc</sup>	-63.86 <sup>ab</sup>
MTDA-TOTM (A)	-61.57 <sup>b</sup>	-63.42 <sup>abc</sup>	-63.14 <sup>ab</sup>
MTDA-TOTM (B)	-61.40 <sup>b</sup>	-63.08 <sup>a</sup>	-62.62 <sup>abc</sup>
<u>0.01M CuSO<sub>4</sub> Solution</u>			
TDDA-NPOE (A)	-53.85 <sup>a</sup>	-56.71 <sup>a</sup>	-54.85 <sup>a</sup>
TDDA-NPOE (B)	-54.09 <sup>a</sup>	-56.88 <sup>a</sup>	-55.18 <sup>a</sup>
MTDA-NPOE (A)	-38.05 <sup>b</sup>	-49.51 <sup>b</sup>	-58.41 <sup>b</sup>
MTDA-NPOE (B)	-39.44 <sup>b</sup>	-49.92 <sup>b</sup>	-56.83 <sup>c</sup>
MTDA-TOTM (A)	-35.27 <sup>c</sup>	-45.52 <sup>c</sup>	-54.59 <sup>a</sup>
MTDA-TOTM (B)	-34.86 <sup>c</sup>	-45.28 <sup>c</sup>	-54.53 <sup>a</sup>
<u>Kelowna Solution</u>			
TDDA-NPOE (A)	-47.13 <sup>a</sup>	-54.82 <sup>a</sup>	-55.96 <sup>a</sup>
TDDA-NPOE (B)	-47.33 <sup>a</sup>	-55.03 <sup>a</sup>	-55.36 <sup>a</sup>
MTDA-NPOE (A)	-45.31 <sup>b</sup>	-54.67 <sup>a</sup>	-55.68 <sup>a</sup>
MTDA-NPOE (B)	-44.94 <sup>b</sup>	-54.38 <sup>a</sup>	-55.90 <sup>a</sup>
MTDA-TOTM (A)	-43.73 <sup>c</sup>	-53.03 <sup>b</sup>	-55.00 <sup>a</sup>
MTDA-TOTM (B)	-43.32 <sup>c</sup>	-52.94 <sup>b</sup>	-55.96 <sup>a</sup>

Membrane sensitivities within a nitrate concentration range and within an extracting solution comparison with the same letter are not significantly different at the 5% level, based on Duncans Multiple-Range Test.

## Selectivity Tests

The mean selectivity factors (log K<sub>ij</sub>) with respect to the interference ions, bicarbonate (HCO<sub>3</sub>), chloride (Cl), and bromide (Br), in different extracting solutions are shown in Table 2. In the tests using the CuSO<sub>4</sub> solution, results for the bicarbonate ion were not obtained because the bicarbonate chemical was not completely dissolved and formed a precipitate in 0.01M CuSO<sub>4</sub> solution.

The results obtained from the GLM analysis showed that the selectivity responses of the membranes were affected considerably by both membrane ligand and extracting solution type (Table 2). The TDDA membranes displayed greater selectivity for nitrate against the interfering species than did the MTDA membranes. Also, the highest selectivity of the tested interference ions was obtained when using the 0.01M CuSO<sub>4</sub> extracting solution. The selectivity of the membranes for nitrate over bromide was the lowest (largest selectivity factor (log K<sub>ij</sub>)), followed by chloride and bicarbonate. There were no significant differences in selectivity among membranes of the same composition, and uniform results were obtained.

Table 2. Comparison of selectivity responses of membranes by extracting solution type.

Ionic Species	HCO <sub>3</sub>	Cl	Br
	-----selectivity factor (log K) -----		
<u>DI Water Solution</u>			
TDDA-NPOE (A)	-2.78 <sup>a*</sup>	-1.51 <sup>a</sup>	-0.00 <sup>a</sup>
TDDA-NPOE (B)	-2.73 <sup>a</sup>	-1.49 <sup>a</sup>	0.02 <sup>a</sup>
MTDA-NPOE (A)	-1.78 <sup>b</sup>	-0.89 <sup>b</sup>	0.31 <sup>b</sup>
MTDA-NPOE (B)	-1.86 <sup>b</sup>	-0.92 <sup>b</sup>	0.27 <sup>c</sup>
MTDA-TOTM (A)	-1.63 <sup>c</sup>	-0.82 <sup>c</sup>	0.33 <sup>bd</sup>
MTDA-TOTM (B)	-1.58 <sup>c</sup>	-0.78 <sup>c</sup>	0.34 <sup>d</sup>
<u>0.01M CuSO<sub>4</sub> Solution</u>			
TDDA-NPOE (A)	-	-1.85 <sup>a</sup>	-0.13 <sup>a</sup>
TDDA-NPOE (B)	-	-1.79 <sup>a</sup>	-0.09 <sup>b</sup>
MTDA-NPOE (A)	-	-1.16 <sup>b</sup>	0.21 <sup>c</sup>
MTDA-NPOE (B)	-	-1.13 <sup>b</sup>	0.19 <sup>c</sup>
MTDA-TOTM (A)	-	-1.04 <sup>c</sup>	0.26 <sup>d</sup>
MTDA-TOTM (B)	-	-0.95 <sup>d</sup>	0.29 <sup>e</sup>
<u>Kelowna Solution</u>			
TDDA-NPOE (A)	-2.34 <sup>a</sup>	-1.11 <sup>a</sup>	-0.06 <sup>a</sup>
TDDA-NPOE (B)	-2.26 <sup>a</sup>	-1.11 <sup>a</sup>	-0.04 <sup>a</sup>
MTDA-NPOE (A)	-1.95 <sup>b</sup>	-0.84 <sup>b</sup>	0.24 <sup>b</sup>
MTDA-NPOE (B)	-1.95 <sup>b</sup>	-0.85 <sup>b</sup>	0.20 <sup>c</sup>
MTDA-TOTM (A)	-1.80 <sup>c</sup>	-0.77 <sup>c</sup>	0.25 <sup>b</sup>
MTDA-TOTM (B)	-1.76 <sup>c</sup>	-0.70 <sup>c</sup>	0.27 <sup>b</sup>

Membrane sensitivities within an ionic species range and within an extracting solution comparison with the same letter are not significantly different at the 5% level, based on Duncans Multiple-Range Test.

## Response Behavior

The potential of an ISE electrode decreased in regular intervals of 50~60 mV as nitrate concentration increased from the lowest ( $10^{-5}$  M) to the highest ( $10^{-2}$  M) in 10-fold increments (Fig. 7(left)). When the order of nitrate concentration was reversed, the apparent responses of the electrodes were almost identical. However, as shown in Fig. 7 (right), the potential change towards lower concentration was smaller than expected. This is because residues of nitrate ions, which were previously used, still remain in the membrane being tested. The results indicate the importance of rinsing of the electrode prior to data collection to remove residues from a previous sample.

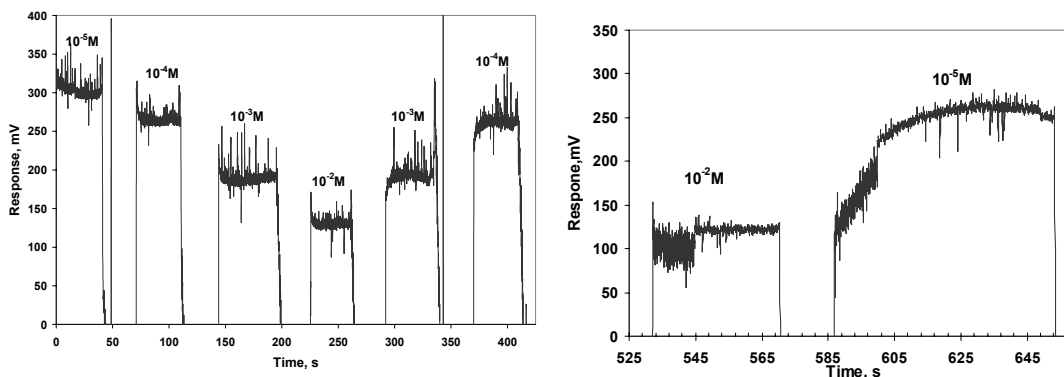


Fig. 7 Response behavior of an ISE electrode when exposed to different concentrations of nitrate in DI water solution (left: sequential response, right: intermediate response).

## Conclusions

To determine a nitrate selective membrane and a soil extracting solution combination for real-time soil nitrate sensing, three kinds of nitrate ion selective membranes were tested in the presence of different soil extracting solutions, and the effect of the soil extracting solutions on the response characteristics of each membrane was investigated.

All membranes showed an approximately linear Nernstian response when nitrate concentrations were above  $10^{-3}$  M, irrespective of which extracting solution was used. However, at low nitrate concentrations, i.e., below  $10^{-4}$  M, the sensitivity response curves of each membrane were different when 0.01M  $\text{CuSO}_4$  and Kelowna extracting solutions were used.

The TDDA-NPOE membrane showed greater sensitivity to nitrate than did the MTDA-NPOE and MTDA-TOTM membranes. The linear detection limits for the TDDA-NPOE membrane were lower than those of the MTDA membranes. Also, the membranes having TDDA as the ligand in the membrane displayed superior selectivity as compared to the membranes having MTDA, when bicarbonate, chloride, and bromide were considered as possible interfering ions of nitrate. Thus, the TDDA-NPOE membrane proved to be a good candidate for a nitrate ion-selective membrane for soil nitrate sensing.

The sensitivity and selectivity responses of each membrane were significantly affected by base solution type. The sensitivity slopes of all membranes obtained in DI water were higher than those with 0.01M  $\text{CuSO}_4$  and Kelowna extracting solutions. However, the  $\text{CuSO}_4$  extracting solution showed the highest selectivity for all tested interference ions among the three different extracting solutions.

Assuming that nitrate concentrations in soil typically exist between  $10^{-5}$  M and  $10^{-4}$  M, a soil extracting solution would be one of the most critical factors in determining an ion-selective membrane for soil nutrient sensing.

When an ISE electrode was exposed to different nitrate concentrations, the dynamic response obtained in the electrode was fast and reproducible, demonstrating proper sensor action.

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