

MEMBRANE SELECTION AND ISFET CONFIGURATION EVALUATION FOR SOIL NITRATE SENSING

S. J. Birrell, J. W. Hummel

ABSTRACT. *Successful implementation of site-specific crop management relies on accurate quantification of spatial variation of important factors. Data collection on a finer spatial resolution than is feasible with manual and/or laboratory methods is often required but cost prohibitive. Therefore, there is a need for the development of sensors to more accurately characterize within-field variability. The objective of this research was to investigate matrix membranes produced from different combinations of ligand and plasticizer materials using ion-selective electrode (ISE) technology, and to use selected membranes to develop a nitrate ion-selective field effect transistor (ISFET) which might be integrated with a flow injection analysis (FIA) system for real-time soil analysis. Several ion-selective membranes were tested, and all of the evaluated membranes proved to be viable candidates for the development of a nitrate ISFET. Membranes using methyltridodecylammonium chloride (MTDA) as the ligand showed a better response to nitrates at low concentrations while those using tetradodecylammonium nitrate (TDDA) ligand showed superior selectivity for the nitrate ion. A multi-ISFET nitrate sensor was successfully developed. The electrical responses of the ISFETs were consistent and predictable. While significant difficulty was found in preparing a multi-ISFET chip with all four sensors operational, once prepared, the multi-ISFET chips were reliable and performed through extensive tests without failure. The sensitivities of the nitrate ISFETs (38-46 mV/decade) were lower than the theoretical Nernst sensitivity. The nitrate ISFETs proved to be viable sensors for the development of a real-time soil nitrate analysis system, under the conditions of our tests.*

Keywords. Soil, Site-specific management, Nitrate.

Site-specific management (SSM) is a strategy that addresses within-field variability by optimizing inputs such as pesticides and fertilizers on a point-by-point basis rather than applying them according to the field average. Successful implementation of the concept relies on accurate quantification of the spatial variation of important soil factors. There is a tremendous need for the development of sensing technologies that will allow automated collection of soil, crop, and pest data. The full benefit of SSM will only be realized if the spatial variation across the field is accurately determined. Data collection on a finer spatial resolution than is feasible with manual and/or laboratory methods is required but cost prohibitive. In-field, real-time sensors will allow the collection of data on a much finer spatial resolution to characterize more accurately within-field variability. Real-time sensors can provide a sampling intensity several orders of magnitude greater than traditional methods, allowing significant reductions in sampling errors.

Ion-selective field effect transistors (ISFETs) have advantageous inherent features such as small dimensions, low output impedance, high signal-to-noise ratio, low sample volumes, and the potential for mass production,

which are all required for a real-time soil sensor. However, ISFETs have long-term drift, a disadvantage that is diminished by the use of a Flow Injection Analysis (FIA) system (Bergveld, 1991). In fact, FIA and ISFETs are complementary since the small sample volumes and rapid response of ISFETs allow the miniaturization of the FIA system, dramatically decreasing sample dispersion and thereby increasing both sample resolution and sample frequency.

The development of a nitrate ion-selective field effect transistor depends on the ability to deposit a nitrate ion-selective membrane on the ISFET. The majority of previous research on ISFETs, predominantly in the medical field, has been conducted using PVC matrix membranes. The objective of this research was to investigate matrix membranes produced from different combinations of ligand and plasticizer materials using ion-selective electrode (ISE) technology, and to use selected membranes to develop a nitrate ISFET which might be integrated with an FIA system for real-time soil analysis.

LITERATURE REVIEW

Ion-selective electrodes have been widely used in clinical analysis, water analysis, oceanographic research, process control, and soil fertility testing (Rechnitz, 1967; Durst, 1969; Dahnke, 1971; Freiser, 1978; Pungor and Buzas, 1981; Yu, 1985). Glass electrodes, solid-state or precipitate electrodes, and liquid-liquid membrane electrodes are the three major classes in which ISEs fall (Rechnitz, 1967). Liquid-liquid membrane electrodes are composed of liquid ion exchangers with mobile trapped sites (Buck, 1981), and numerous neutral carriers have

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been developed to respond to many cations and anions (Stefanic and Simon, 1967; Wegmann et al., 1984; Buhrer et al., 1988). The electrodes consist of either the liquid ion exchanger that is separated from the sample using an inert porous membrane, or a liquid exchanger that is cast into membrane films using a polymer support matrix (Moody et al., 1988). The majority of studies using ISFETs have been conducted using polymeric membranes (Sibbald, 1984; Tsukada et al., 1987; Tsukada et al., 1989).

Three different types of neutral carriers, quaternary ammonium compounds, phenanthroline chelates of iron, and phenanthroline chelates of nickel, have been used to develop nitrate ISEs. Electrodes based on iron chelates showed slightly inferior sensitivity and detection limits (Reinsfelder and Schultz, 1973). The nickel chelates displayed the theoretical Nernstian responses with the lower limit of the linear range of 10^{-4} to 10^{-5} M and selectivity factors ($\log K$) in the range of -1.6 to -2.6 (Hulanicki et al., 1978; Hwang and Cheng, 1978). Davies et al. (1972) used commercial nitrate ion exchangers incorporated in a poly(vinyl chloride) (PVC) matrix to obtain electrodes which performed as well as commercial liquid-liquid electrodes but with a much longer life. However, Nielsen and Hansen (1976) developed PVC membrane nitrate electrodes using different quaternary ammonium compounds with superior performance to those described previously. Their electrodes displayed a detectable response at lower than 10^{-6} M and a linear detection limit of 10^{-5} M with a selectivity factor ($\log K$) of approximately -2.3 . Quaternary ammonium salts have been used to develop nitrate ISFETs with comparable electrochemical responses (Covington and Whalley, 1986).

While the development of new ion-selective membranes continued, a dramatic change in the miniaturization of ion-selective membrane technology occurred when Bergveld reported on ion sensitive field effect transistors (Bergveld, 1970; Bergveld, 1972). The theoretical chemical response of an ISFET is the same as that of an ion-selective electrode except that an ISFET does not have an internal solution, and the membrane is directly on the solid silicon nitride surface of the field effect transistor. However, the long-term drift and hysteresis characteristics of ISFETs are significantly inferior to those of ion-selective electrodes (Janata and Huber, 1985; Catrall and Hamilton, 1984; Miyahara and Simon, 1991). Several studies have attempted to elucidate the electrochemistry of the membrane/silicon nitride interface of ISFETs and the associated drift problems (Zemel, 1975; Janata and Moss, 1976; Janata and Huber, 1979). While the theoretical operation of ISFETs was not completely resolved, the ISFET's potential as an extremely small, rapid-response sensor that could be fabricated using IC technology to develop a multi-ion sensor chip was recognized. Several researchers reported on the development of ISFETs to measure different ionic species including calcium, potassium, sodium, and ammonium ions (Matsuo and Wise, 1974; Esashi and Matsuo, 1975, 1978; Moss et al., 1975; Janata and Moss, 1976; McBride et al., 1978; Moss et al., 1978; Ramsing et al., 1980; Oesch et al., 1981). Soon other researchers were developing multiple sensor modules with integrated signal conditioning to buffer and amplify the signal, including in some cases, automatic temperature compensation (Sibbald et al., 1984;

Brown et al., 1985; Fung and Fu, 1985; Lauks et al., 1985). The use of dynamic measurement systems to alleviate the long-term drift of ISFETs was also reported (Ramsing et al., 1980; van der Schoot and Bergveld, 1985; Sibbald et al., 1984; Covington and Whalley, 1986; Alegret et al., 1989; Chandler et al., 1990).

OBJECTIVES

The development of a nitrate ion-selective field effect transistor depends on the ability to deposit a nitrate ion-selective membrane on the ISFET. The majority of the previous research on ISFETs has been conducted using PVC matrix membranes in medical applications, involving sodium, potassium and chloride ISFETs. The overall objective of this research was to investigate matrix membranes, produced from different combinations of ligand and plasticizer materials, which may be potential candidates for use as ISFET membranes. The membranes were then used to develop a nitrate ISFET to be integrated with an FIA system for real-time soil analysis. Specific objectives included:

1. Formulate and prepare different combinations of plasticizers and ligands to make PVC matrix ion-selective membranes that are compatible with ISFETs.
2. Evaluate the response characteristics of the membranes, including a comparison of their relative sensitivity to nitrate, and determination of the membrane's lower detection limits for nitrate. Membranes exhibiting a high sensitivity to nitrate, particularly at low concentrations were sought.
3. Evaluate the selectivity of the membranes. The response of the membranes to different interference ions could be critical since soil extracts contain many different ions.
4. Develop multi-ISFET nitrate sensors using the nitrate membranes identified as potential candidates. Multi-ISFET sensors provide redundant signals which allow a more robust analysis system to be developed, with automatic failure detection.
5. Evaluate the electrical and chemical response of the ISFET to nitrates.

THEORY OF ION-SELECTIVE MEMBRANES

Ion-selective electrodes allow the determination of the activity of certain ions in the presence of other ions in a solution based on galvanic half cells. The total electrical potential measured between an ion-selective electrode and a reference electrode is the sum of the voltage drops at the solid-solid, solid-liquid, and liquid-liquid interfaces. The potential across the ion-selective membrane determines the performance of an ion-selective membrane electrode. If a membrane is exclusively selective for ions of type i , the membrane potential is a direct measure of the respective activities in the solutions on either side of the membrane and is written in the form of the Nernst equation. However, in most cases the membrane will respond to other interfering ions, and the measured electromotive force (EMF) is the sum of the membrane response to the activity of each ion. The Nikolskii-Eisenman form of the Nernst

equation (eq. 1) includes the effects of interference ions (Nikolskii, 1937; Eisenman, 1967):

$$\text{EMF} = E_o + E_j + S \log \left[a_i + \sum K_{ij} (a_j)^{Z_i/Z_j} \right] \quad (1)$$

where

- E_o = standard potential
- E_j = liquid-junction potential
- S = Nernstian Slope (59.16 mV/ Z_i for H_2O as 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

For a particular set of electrodes, the reference potential, E_o , is constant at constant temperature. The liquid-junction potential, E_j , generated between the sample solution and reference electrode may change, contributing to the measured EMF. Therefore, the junction potential effects must be minimized by either maintaining the junction potential at a constant level or subtracting any changes in junction potential from the measured EMF. The former is achieved by using a reference electrolyte which closely resembles the sample solution or a highly concentrated equitransferent reference electrolyte. Also contributing to the measured EMF, the liquid junction potential, E_j , between the reference electrode and sample solution may vary considerably depending on the sample solution and is superimposed on the membrane potential. When the junction potential does not remain constant, the changes in the junction potential must be subtracted from any changes in measured EMF. The Henderson formula was used to estimate the junction potential (Henderson, 1907; Morf, 1981):

$$E_j = \frac{\sum_i Z_i U_i (a_i - a'_i)}{\sum_i Z_i^2 U_i (a_i - a'_i)} \frac{RT}{F} \frac{\sum_i Z_i^2 U_i a'_i}{\sum_i Z_i^2 U_i a'_i} \quad (2)$$

where

- Z_i = charge number of the ion I
- U_i = absolute mobility of the ion I ($\text{cm}^2\text{s}^{-1} \text{J}^{-1} \text{mol}$)
- a_i = single-ion activity of ion I in the sample solution (M)
- a'_i = single-ion activity of ion I in the reference electrolyte
- R = universal gas constant
- T = absolute temperature (Kelvin)
- F = Faraday constant

The Nikolskii-Eisenman form of the Nernst equation centers on the determination of the selectivity coefficients, which are generally derived empirically since theoretical derivation is possible only in some special cases. The selectivity factors depend on both the method used and the conditions of the measurement and do not characterize membrane selectivity under all measurement conditions (Ammann, 1986). Selectivity factors can be determined experimentally using the separate solution method, which

has the advantage of being very simple; however, the selectivity coefficients may not be representative for mixed sample solutions. Using the separate solution method, EMF values are determined when the electrodes are placed in pure single electrolyte solutions of the primary ion and interference ion. The Nikolskii-Eisenman equation is then used to compute the selectivity coefficients:

$$K_{ij} = \left[10^{(E_j - E_i)/S} \right] \times \left[\frac{a_i}{a_j^{(Z_i - Z_j)}} \right] \quad (3)$$

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

The EMF of an ISE cell is directly dependent on the single-ion activity and only indirectly dependent on the ion concentration. The concentration (molar) can be converted to activities using single-ion activity coefficients:

$$a_i = \gamma_i c_i \quad (4)$$

where

- a_i = single-ion activity (M)
- γ_i = single-ion activity coefficient
- c_i = ion concentration (M)

The single-ion coefficients are determined from the mean activity coefficients of the electrolyte, which are estimated using the Debye-Hückel formula (Morf, 1981).

$$\log \gamma_{\pm} = \frac{-A |Z_+ \times Z_-| \sqrt{I}}{1 + B a \sqrt{I}} \text{ with } I = \frac{1}{2} \sum_i c_i Z_i^2 \quad (5)$$

METHODS AND PROCEDURE

EVALUATION OF NITRATE ION-SELECTIVE MEMBRANES

Preparation of Nitrate Ion-selective Membranes.

Quaternary ammonium compounds have been used successfully as ligands in non-porous PVC-based nitrate ion-selective electrode (ISE) membranes. The ligands [tetradodecylammonium nitrate (TDDA) and methyltridodecylammonium chloride (MTDA)] and the plasticizers [tri-(2-ethylhexyl)trimellitate (TOTM), nitrophenyl octyl ether (NPOE), dibutylphthalate (DBP), and tri-(2-ethylhexyl) phosphite (TEHP)] were selected for testing based on previous studies (Nielsen and Hansen, 1976; Tsukada et al., 1989). Therefore, various membranes were prepared which contained either TDDA or MTDA as the ligand combined with one of the plasticizers DBP, TOTM, NPOE, or TEHP. Three disks (3.5 mm diameter) of each membrane composition were attached to the end of Hitachi ISE bodies. The ISEs were filled with a solution of 0.01 M sodium chloride (NaCl) and 0.01 M sodium nitrate (NaNO_3) with a silver/silver chloride internal reference wire. Ion-selective electrodes were conditioned by soaking them in a 0.01 M potassium nitrate (KNO_3) solution for at least three hours prior to testing. The reference electrode,

an ETH R44/2-SD/1 double junction electrode, was used with a 1 M KCl reference electrolyte and a 1 M lithium acetate (LiAc) outer solution. All electrodes were stored in 0.01 M KNO_3 during a testing sequence.

Membrane Test Apparatus and Procedure. The ion-selective membranes were tested in a computer-controlled test stand. The computer acquired and recorded digitized electrode potentials from digital voltmeters. The system could simultaneously evaluate the sensitivity and selectivity of multiple electrodes. The computer controlled the rotation and drainage of the sample holder and the operation of five peristaltic solution pumps according to the particular test being conducted and the parameter settings selected for the test. After signal conditioning and analog-to-digital conversion by the voltmeters, individual readings were automatically recorded by the computer for processing.

Control parameters were set to rinse the sample solution holder three times with fresh solution between each solution exchange. The sample holder was rotated throughout the measurement time, stirring the sample. The first sensor voltage measurement occurred 60 s after the solution exchange, and three measurements taken every 5 s were averaged and recorded for each standard solution in the testing sequence. Each test included five recorded iterations of the complete testing sequence.

Sensitivity Tests. Two different sets of membrane materials were prepared. The initial set included TDDA-NPOE, MTDA-NPOE, TDDA-TEHP, and MTDA-TEHP membranes. A second set included membranes of all eight combinations of the two ligands and four plasticizers. Sensitivity tests were conducted with three or more electrodes of each membrane type from each membrane set. Sensitivity tests of the membranes were conducted on the day that the electrodes were conditioned, and were repeated on the third and ninth day afterward. The sensitivity of each membrane to nitrate was tested with five standard solutions (0.00001 M, 0.0001 M, 0.001 M, 0.01 M, and 0.1 M KNO_3) in sequence. The raw voltage readings were corrected for the change in junction potential using the Henderson formula (Morf, 1981). The Debye-Hückel formula was used to calculate the ionic activities for each solution (Meier, 1982). The sensitivity of each membrane was determined by linear regression of the mean corrected electrode voltage for each standard solution against the logarithm of the ionic activities of the solutions. The effects of membrane composition, ligand, plasticizer, and the interaction between ligands and plasticizers on sensitivity were tested using the SAS statistical software (SAS, 1985).

Selectivity Tests. Two different sets of membrane materials were prepared. The initial set included four membrane compositions consisting of all combinations of the TDDA and MTDA ligands, and the NPOE and TEHP plasticizers. A second set included membranes of all eight combinations of the two ligands and four plasticizers (NPOE, TEHP, TOTM, and DBP). Selectivity tests were conducted with three or more electrodes of each membrane type from each membrane set within five days of being conditioned. The separate solution method was used to determine the selectivity coefficients of the membranes (Ammann, 1986). The selectivities of the membranes for nitrate over the sodium

salts of interference ions were determined in the following order: sulphate (Na_2SO_4), phosphate (Na_2HPO_4), acetate (CH_3COONa), bicarbonate (NaHCO_3), chloride (NaCl), bromide (NaBr), iodide (NaI), chlorate (NaClO_4), and thiocyanate (NaSCN). This order was selected so that the ion with the lowest interference would be tested first, followed by ions of increasing interference to reduce the effects of membrane poisoning by the earlier-tested ions. The extended Nernst equation, and the electrode response for each interference ion were used to determine the selectivity factors. The SAS General Linear Model Procedure (GLM) was used to determine whether the selectivity factors of the different membranes were different using Duncans Multiple-range Test.

DEVELOPMENT AND EVALUATION OF NITRATE ISFETs

Design of the FET Chip. The FETs used in this study were developed by Dr. Y. Miyahara and Dr. K. Tsukada of the Hitachi Central Research Laboratory, Hitachi Ltd., Japan. A photograph of the multi-FET chip (fig. 1) shows the interface circuits and bonding pads arranged along the edge, and the four FETs in the center of the 5 mm \times 5 mm chip. The method of production is described by Tsukada et al. (1989). A photosensitive polyamide layer was spin coated onto the chip wafer and then patterned by photolithography to form a well over the gate area of each FET. The dimensions of the wells were 250 μm \times 700 μm . The thickness of the polyamide layer was approximately 60 μm . The chip was mounted on a ceramic substrate with pins for an IC socket, and the bonding wires and FET surfaces (except for the gate regions) were protected using an epoxy resin. The lower portion of the flow cell attached to the top of the multi-FET chip, exposing the FET gate regions to the solution.

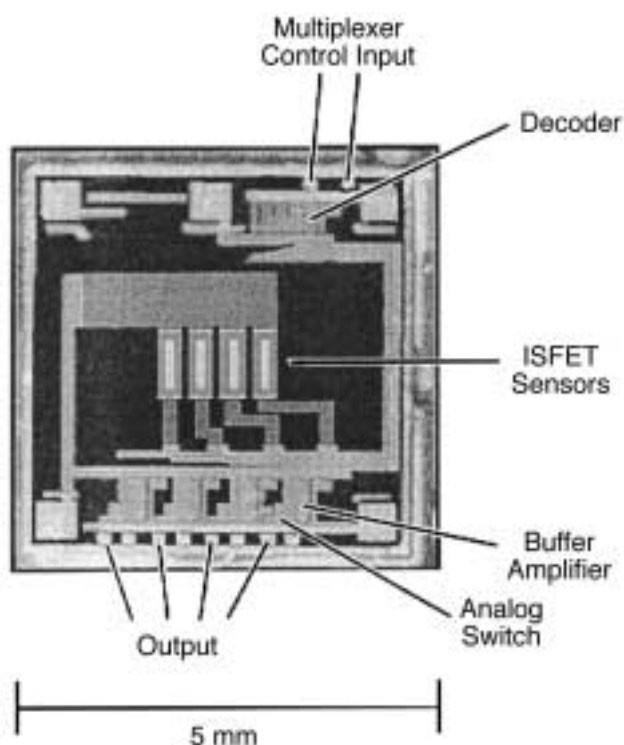


Figure 1—Image of integrated multi-ISFET sensor (size 5 mm \times 5 mm).

The integrated multi-FET chip consisted of the four FETs, operating with constant drain current, source follower circuits, buffer amplifier circuits, analog switches, and a decoder to multiplex the outputs. The outputs of the four FETs were multiplexed after impedance conversion to avoid switching delay during multiplexing from one FET signal to another FET signal (Tsukada et al., 1989). The individual non-multiplexed amplified FET outputs were available and used in this study instead of the multiplexed output.

Preparation of Nitrate ISFET Membranes. The ion-selective membrane mixtures were prepared in an identical manner to those used for the ion-selective electrode membrane tests; however, only two ligands (TDDA and MTDAA), and two plasticizers (TOTM and NPOE) were used to obtain four different membrane mixtures which were placed on the different sensor surfaces. A micro-syringe was used to carefully inject a single membrane mixture into each of the FET sensor wells. Whenever possible, a membrane mixture of different composition was placed on each of the four sensors on the multi-FET chip. The membranes were allowed to dry overnight under ambient conditions. After curing, the lower portion of the flow cell was carefully epoxied onto the chip holder ensuring the sample liquid was directed over the membranes.

ISFET Membrane Test Apparatus and Procedure. The ISFET membrane tests were conducted using a flow injection system. A variable-speed, multichannel, peristaltic pump was used to propel the carrier and sample solution to the injection valve. The injection valve was a two-position Valco Model EF60 valve, with "zero" dead volume, controlled by a Valco Cheminert C1-417-2460 electric actuator. During the load period, the carrier stream was sent through the flow cell while the sample solution was diverted to waste, and during the injection period the exact opposite occurred. The flow cell consisted of two parts; an acrylic spacer attached to the top of the multi-ISFET chip, and a 40 mm × 25 mm × 12.5 mm stainless steel block machined to match the acrylic spacer with minimal dead volume. The volume of the flow cell was approximately 0.8 µl. The stainless steel flow cell, which operated as the pseudo-reference, was maintained at some potential with respect to ground for two reasons. First, the total potential on the ISFET gate had to be maintained at a certain threshold level so that the ISFET buffer amplifiers were not in a saturated state. Second, any static charges caused by stray voltages and the operation of the peristaltic pump were reduced.

Since the ISFETs were operating in a constant drain current mode, the ISFET output potential with respect to ground followed the gate potential of the FET, which is the sum of the potential of the steel flow cell (pseudo reference potential, E_{pseudo}), the pseudo-reference junction potential (E^*_{j}) between the steel cell and sample solution, and the ISFET membrane potential (E_{m}). The ISFET output can be written in a form similar to the extended Nernst equation:

$$E_{\text{ISFET}} = E_{\text{pseudo}} + E^*_{\text{j}} + S \log \left[\sum K_{ij} (a_j)^{Z_i/Z_j} \right] \quad (6)$$

The pseudo-reference junction potential (E^*_{j}) is unknown and cannot be assumed to be constant. However, the reference electrode potential (E_{refer}) with respect to ground

is a function of the pseudo reference potential (E_{pseudo}), pseudo-reference junction potential (E^*_{j}), the standard potential (E_o) and "constant" reference electrode junction potential (E_{j}) between the reference electrode and sample solution. The reference electrode potential (E_{refer}) with respect to ground is given by:

$$E_{\text{refer}} = (E_{\text{pseudo}} + E^*_{\text{j}}) - (E_o + E_{\text{j}}) \quad (7)$$

Therefore, the difference in potential between the ISFET output and reference electrode, with respect to ground, is identical to the extended form of the theoretical Nernst equation:

$$E_{\text{ISFET}} - E_{\text{refer}} = E_o + E_{\text{j}} + S \log \left[a_i + \sum K_{ij} (a_j)^{Z_i/Z_j} \right] \quad (8)$$

A Metrabyte DAS-HRES 16-bit A/D board connected to a 386 computer was used to collect the data using SNAPSHOT STORAGE SCOPE (HEM Data Corp.) data collection software. The power for the multi-ISFET chip was supplied by the A/D board, and six analog channels were used in differential mode to collect the output voltages from the four ISFETs with respect to ground, the pseudo-reference voltage, and the valve position. The voltages were displayed on the screen of the computer and streamed to disk by the software for later analysis.

ISFET Amplifier Gain. The ISFETs were designed with an amplifier gain of one; however, the actual gains were checked for each ISFET. The gain was found by adjusting the pseudo-reference voltage while recording the pseudo-reference voltage and sensor output voltage using the A/D board. A pseudo-reference voltage above the threshold voltages and below the positive saturation voltages for all four ISFETs must be selected for all ISFETs to be simultaneously operational. The gain for each ISFET was found by linear regression of the output voltage versus the pseudo-reference voltage.

ISFET Sensitivity Tests. The effect of different flowrates on ISFET response was tested in the FIA system. The injection valve was manually set to direct the sample solution through the flow cell, and the pump was set at a specific flowrate (0.057, 0.120, 0.179, and 0.240 mL/s). A six-port rotary valve, upstream of the injection valve, was used to select one of the five standard solutions. The standard solutions were 0.1 M, 0.01 M, 0.001 M, 0.0001 M, and 0.00001 M NaNO_3 in a 0.01 M CuSO_4 solution. The response of the ISFET to each standard solution was recorded at 250 Hz for 20 s before the rotary valve was used to introduce another standard solution. The solutions were tested starting with the highest concentration, then sequentially decreasing to the lowest concentration, and then increasing back to the highest, while recording the ISFET outputs and the potential of the double junction reference potential.

Multiple ISFET and reference electrode voltages, recorded for each test solution concentration, were reduced to a single value for each solution introduced into the system. Voltage readings from 2.0 s after the new solution was introduced until 0.2 s before the next solution was

introduced were averaged, producing a voltage level that represented the ISFET output for each test solution.

The sensitivities of the ISFETs were calculated for each test using the SAS GLM procedure. The sensitivity was obtained using two methods. The first method assumed that the pseudo-reference junction potential was constant, i.e., the reference electrode potential was redundant and could be ignored, and the sensitivity was calculated using only the ISFET output directly (eq. 6). The second method adjusted for the unknown pseudo-reference junction potential by subtracting the reference electrode potential from the ISFET output before calculating the ISFET sensitivity (eq. 8).

RESULTS

EVALUATION OF NITRATE ION-SELECTIVE MEMBRANES

Although at least three membranes of each composition were tested, a complete set of results was not obtained for two reasons. First, a few membranes failed suddenly, mainly due to the incorrect placement of the membrane disk on the end of the electrode body. Second, incorrect placement of the electrode in the test apparatus sometimes caused contact between the membrane and sample solution to be broken. Unfortunately, this problem was not always recognized, and some operational electrodes that should have been retested were discarded.

Sensitivity Tests. The response of typical electrodes of each membrane type (fig. 2) shows an approximately linear Nernstian response when the nitrate concentrations were above 0.0001 M. However, at low concentrations (0.0001 M), the sensitivities of the TDDA membranes decreased more than did the sensitivities of the MTDA membranes. Although the decrease in sensitivity at low concentrations varied between individual membranes, in general, the TDDA membranes showed less sensitivity at low concentrations than did the MTDA membranes. The results from the GLM difference of means procedure reflect the higher sensitivity of the MTDA membranes at low concentrations. When all five solutions were included, the TDDA-TEHP membranes again showed the lowest response followed by TDDA-TOTM, TDDA-NPOE, TDDA-DBP, MTDA-TEHP, MTDA-NPOE, and MTDA-

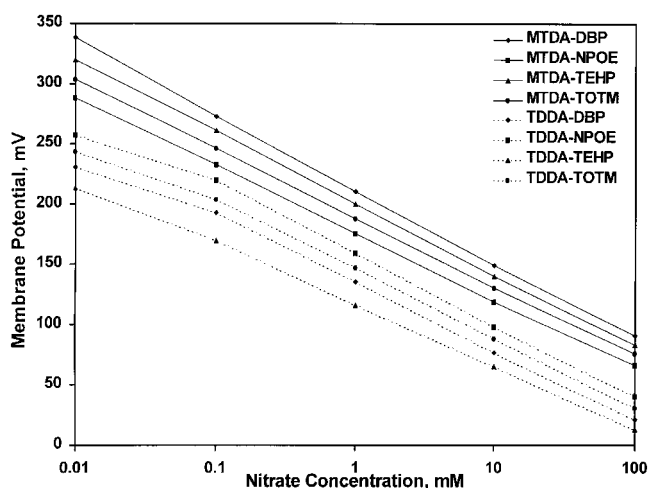


Figure 2—Electrode voltage potential vs sample concentration for electrodes representing each membrane composition.

TOTM with the MTDA-DBP membranes with the greatest sensitivity response. Differences between ligands were highly significant, and all membranes containing the TDDA ligand were less responsive than those with the MTDA ligand. The responses of the MTDA membranes were not significantly different than those of the TDDA membranes when low concentrations were excluded (table 1). When electrode sensitivity was compared by plasticizer type, those membranes containing TEHP plasticizer had the lowest sensitivity response, followed by membranes with NPOE and TOTM, and the DBP membranes had the highest sensitivity response.

Selectivity Tests. Three or more electrodes prepared with each membrane composition were tested within five days of being conditioned for the first time. The mean selectivity factors ($\log K_{ij}$) with respect to the interference ions phosphate (HPO_4), sulphate (SO_4), acetate (Ac), bicarbonate (HCO_3), chloride (Cl), bromide (Br), chlorate (ClO_4), iodide (I), and thiocyanate (SCN) are shown in table 2. The selectivities of the membranes were predominantly determined by the ligand type. The TDDA membranes displayed greater selectivity for nitrate over the interfering species than did the MTDA membranes (table 2).

The results of the GLM regression of electrode selectivity factors against membrane ligand, and plasticizer, showed that membrane ligand was the most significant factor. The mean selectivity factors ($\log K_{ij}$) for HPO_4 , Ac, SO_4 , HCO_3 , Cl, Br, I, SCN, and ClO_4 were -2.60 , -2.55 , -2.38 , -2.34 , -1.68 , -0.67 , 1.03 , 1.49 , and 2.70 , respectively, for the MTDA ligand and the mean selectivity factors ($\log K_{ij}$) for SO_4 , HPO_4 , Ac, HCO_3 , Cl, Br, I, SCN, and ClO_4 were -3.63 , -3.59 , -3.29 , -3.14 , -2.35 , -0.80 , 0.89 , 1.10 , and 1.80 , respectively, for the TDDA ligand. The differences between the means of the two ligands were significant at the 5% level for all of the interference ions except iodide. The TDDA membranes consistently showed

Table 1. Overall mean sensitivity response of membranes to nitrate solutions

Concentration Range:	Sensitivity (mV/decade)			N
	100 to 0.01 mM	100 to 0.10 mM	100 to 1.00 mM	
Membrane Composition				
TDDA-TEHP	-55.6 ab*	-58.3 a	-58.5 a	6
TDDA-TOTM	-58.4 abcd	-61.4 bc	-62.5 b	10
TDDA-NPOE	-58.4 bcd	-63.4 bcd	-63.4 b	13
TDDA-DBP	-59.6 bcde	-62.8 bcd	-63.3 b	9
MTDA-TEHP	-59.8 bcde	-62.3 bcd	-62.1 b	11
MTDA-NPOE	-61.1 def	-62.5 cd	-62.0 b	10
MTDA-TOTM	-62.2 def	-63.3 cd	-63.3 b	9
MTDA-DBP	-63.3 ef	-64.7 cd	-64.4 b	8
Ligand Comparison				
MTDA	-61.5 a	-62.9	-62.6	38
TDDA	-58.3 b	-61.9	-62.4	38
Plastizer Comparison				
TEHP	-58.3 a	-60.9 a	-60.8 a	17
TOTM	-60.2 ab	-62.3 ab	-62.8 b	19
NPOE	-59.6 ab	-63.0 b	-62.8 b	23
DBP	-61.4 b	-63.2 b	-63.3 b	17

* Membrane sensitivities within a solution concentration range and within a comparison group with the same letter are not significantly different at the 5% level, according to Duncans Multiple-range Test.

Table 2. Comparison of the means of selectivity factors by ligand and plasticizer for different interference ions

Ionic Species:	Mean Selectivity Factor (log K)									
	HPO ₄	SO ₄	Ac	HCO ₃	CL	BR	I	SCN	CLO ₄	N
Membrane Comparison										
MTDA-TOTM	-2.42 ab*	-2.15 a	-2.47 ab	-2.26 ab	-1.67 a	-0.65 ab	1.03 abc	1.43 abcde	2.62 abc	3
MTDA-TEHP	-2.58 ab	-2.31 a	-2.50 ab	-2.25 ab	-1.58 a	-0.63 ab	0.98 abc	1.61 abc	2.65 abc	5
MTDA-NPOE	-2.62 ab	-2.43 a	-2.57 ab	-2.37 ab	-1.70 a	-0.68 ab	1.07 ab	1.49 abcd	2.76 ab	11
MTDA-DBP	-2.70 abc	-2.50 a	-2.62 abc	-2.47 abc	-1.72 a	-0.70 abc	1.02 abc	1.38 abcdef	2.65 abc	4
TDDA-TOTM	-3.12 abcd	-3.32 b	-2.87 abc	-2.80 abcd	-2.27 b	-0.88 de	0.99 abc	0.63 g	1.91 cd	5
TDDA-TEHP	-3.37 bcde	-3.49 b	-3.26 bcd	-3.08 bcd	-2.27 b	-0.67 abc	0.73 bcd	1.20 cdef	1.89 cd	4
TDDA-DBP	-3.78 cde	-3.66 b	-3.21 bcd	-3.13 cd	-2.35 b	-0.77 bcd	0.40 d	1.33 bcdef	0.52 e	5
TDDA-NPOE	-3.76 cde	-3.76 b	-3.48 cd	-3.29 cd	-2.40 b	-0.81 cde	1.07 ab	1.16 def	2.20 bcd	14
Ligand Comparison										
MTDA	-2.60a	-2.38a	-2.55a	-2.34a	-1.68a	-0.67a	1.03	1.49a	2.70a	23
TDDA	-3.59 b	-3.63 b	-3.29 b	-3.14 b	-2.35 b	-0.80 b	0.89	1.10 b	1.80 b	28
Plasticizer Comparison										
TOTM	-2.86	-2.88	-2.72	-2.60	-2.05 abc	-0.79 b	1.01 a	0.93 a	2.18 a	8
TEHP	-2.93	-2.84	-2.84	-2.62	-1.89 ab	-0.65 a	0.87 ab	1.43 b	2.31 a	9
NPOE	-3.26	-3.18	-3.08	-2.88	-2.10 bc	-0.75 b	1.07 a	1.30 b	2.45 a	25
DBP	-3.30	-3.15	-2.95	-2.84	-2.07 bc	-0.74 b	0.68 b	1.35 b	1.47 b	9

* Membrane selectivity factors within an ionic species and within a comparison group with the same letter are not significantly different at the 5% level, according to Duncans Multiple-range Test.

a greater selectivity against any particular interference ion than did the MTDA membranes.

The differences among plasticizer types, based on mean selectivity factors, were more complex. There were no significant differences among the mean selectivity factors for different plasticizers when the interference ions were phosphate, sulphate, acetate, and bicarbonate. Mean selectivity factors for the TEHP plasticizer were significantly different from the NPOE and DBP plasticizers for chloride ions. However, the difference of means procedure did not consistently show an advantage of one plasticizer over another. In general, the NPOE and DBP plasticizers showed slightly better selectivity response than the TOTM and TEHP plasticizers, particularly for ionic species in which the log of the selectivity factor was less than zero.

EVALUATION OF NITRATE ISFETs

The development of an ISFET chip with four ISFETs that were simultaneously operational was a challenge. Typically, the threshold voltage for each ISFET was different and, although all four ISFETs were operational, those with a low threshold voltage would be in positive saturation before the ISFET with the highest threshold voltage became operational. The most likely cause of these problems was imprecise manual deposition of the membranes on the chip surface. The ISFETs were checked for response by varying the pseudo-reference voltage and recording the relative change in output.

ISFET Amplifier Gain. All ISFETs responded linearly to a change in pseudo-reference voltage, provided that the chip was not near saturation (fig. 3). When the output voltage level of one of the individual ISFETs approached the supply voltage level of the ISFET, the effective gain of the ISFETs began to decrease. Therefore, the pseudo-reference voltage required adjustment to maintain the output of all ISFETs at least 0.5 V lower than the supply voltage. All sensors on the multi-ISFET chips exhibited

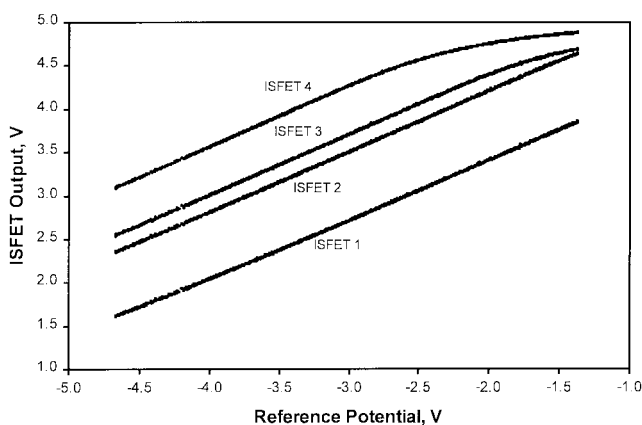


Figure 3—Typical ISFET sensor response to changes in quasi-reference potential.

nearly identical gains (0.69 ± 0.015) irrespective of the date tested.

ISFET Sensitivity Tests. ISFET 1 on the multi-ISFET chip was electrically operational; however, the ion-selective membrane was not very sensitive to nitrate and only showed a response at the highest concentration levels. The mean response of this ISFET was only -4.4 mV/decade when all five solution concentrations were considered and -7.1 mV/decade when the two lowest concentrations were eliminated. The low response could have resulted from an incomplete membrane or the membrane being isolated from the solution by epoxy. However, examination of the membrane under a low-power microscope did not reveal either of these problems. On a positive note, if the low sensitivity of ISFET 1 were reproducible, and if the ISFET was not sensitive to any other ions, it could be used as a reference electrode to account for the sample junction potentials.

The responses of the ISFETs to changes in nitrate concentration are shown in figure 4 for different flowrates. The individual curves have been shifted vertically for

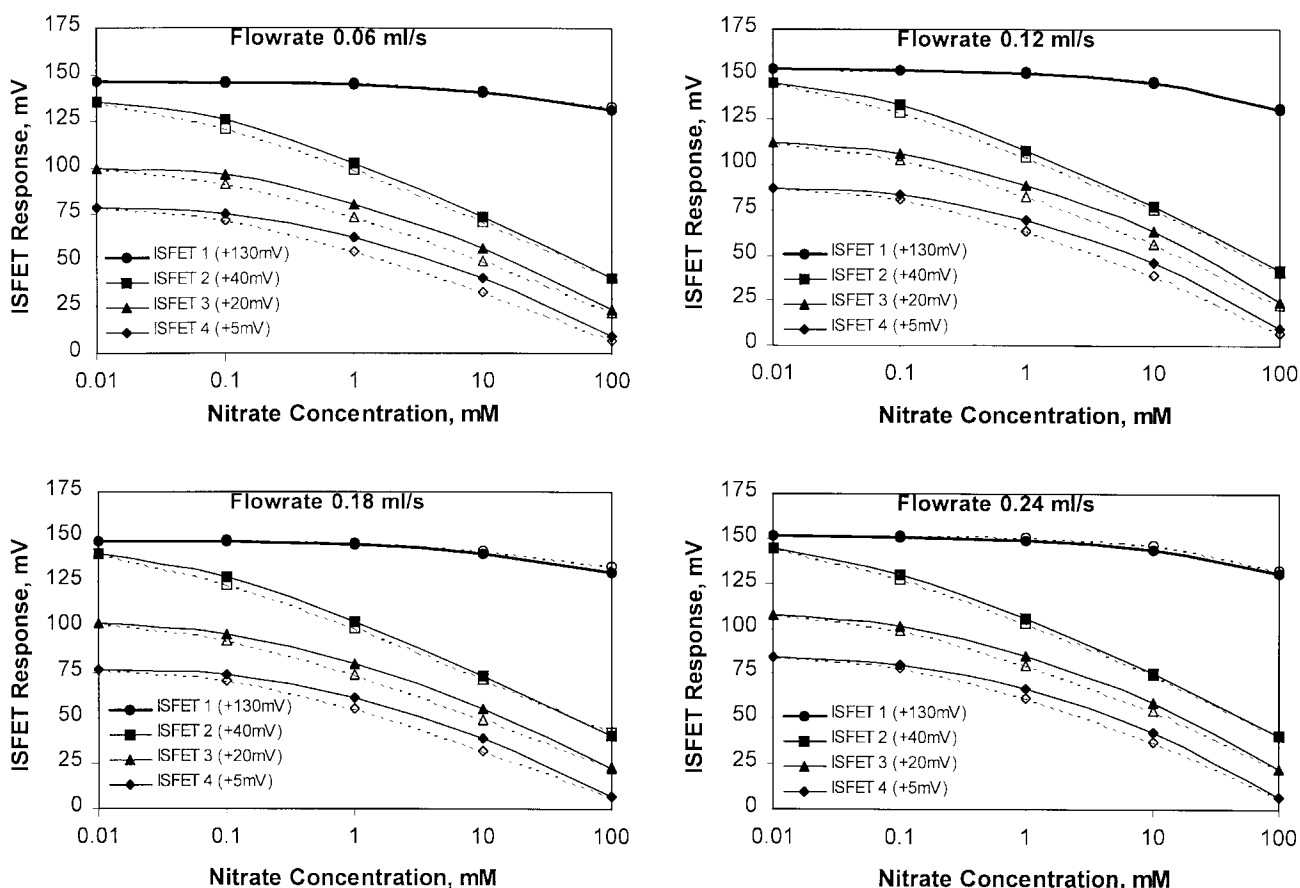


Figure 4—ISFET response to changes in nitrate standard solutions at four flow rates (0.06, 0.12, 0.18, and 0.24 mL/s), with increasing concentration gradients (solid line) and decreasing concentration gradients (broken line). All response curves have been vertically shifted for clarity.

clarity. The response of ISFET 2 was linear at concentrations above 0.001 M with decreasing sensitivity as the concentrations approached 0.00001 M. ISFETs 3 and 4 showed similar responses. The mean responses for ISFETs 2, 3, and 4 were -26.3 , -21.9 , and -19.9 mV/decade change in concentration, respectively, when all five standard solutions were included, and -30.1 , -27.6 , and -25.9 mV/decade, respectively, when the three highest concentration standards were used (table 3). When the amplifier gains (0.69) of the multi-ISFET chip was applied to the raw measured sensitivities, actual membrane sensitivities were approximately -44.5 , -40.0 , and -37.5 mV/decade, respectively. These sensitivities are lower than the theoretical Nernst sensitivity (-59 mV/decade) but are within acceptable limits, and all three ISFETs were considered operational.

The sensitivities of the ISFETs showed two trends. The sensitivities of the ISFETs were greater when the regression coefficients were calculated with an increasing concentration gradient than with a decreasing concentration gradient. The difference in sensitivity between the two gradients was approximately 2-3 mV/decade for ISFETs 2, 3, and 4. However, this trend was not as apparent if the slope was calculated for only the three highest concentration solutions. The difference in sensitivity with an increasing concentration gradient when compared to a decreasing concentration gradient, under dynamic conditions, is well documented. Generally it is a result of

the relative rates of ionic exchange between the bulk of the sample and the boundary layer to which the membrane is responding, and a theoretical time response model for ion-exchange membranes predicts a slower response with decreasing concentrations (Morf, 1981; Buck, 1981).

The other trend was that the sensitivities of the ISFETs were between 0.5 and 1.0 mV/decade higher when the reference electrode was ignored than when the reference electrode was included, and the standard deviation between replications was generally lower when the reference electrode was not included. Theoretically, the Nernst equation cannot be used without a thermodynamically defined system which would include a true reference electrode. However, these tests showed no advantage to using a reference electrode. Although the average potential of the reference electrode is relatively stable, the reference electrode displayed considerable high frequency noise with an amplitude of nearly 5 mV. The high frequency noise would not be a problem if static measurements were used since integrating the signal over time would remove the noise (as is done with commercial ISE systems). In a dynamic FIA system which relies on the determination of an instantaneous peak, the high frequency noise of the reference electrode is unacceptable. However, if the relative changes in the unknown pseudo-reference junction potential are slow with respect to the analysis period, the pseudo-reference electrode may be used instead of a reference electrode. The ideal case would be for the

Table 3. Mean estimates of ISFET Nernstian response parameters to flowrate and direction of concentration change of nitrate solutions

Concentration Range	Flowrate (mL/s)	Nernst Response Parameters			
		Nernst Slope (mV/decade)*		Nernst Slope (mV/decade)†	
		Mean (SD)‡	Mean (SD)§	Mean (SD)‡	Mean (SD)§
ISFET 1 (MTDA-NPOE)					
100-0.01mM	0.06	-3.63 (0.05)	-4.35 (0.10)	-2.66 (0.11)	-3.89 (0.06)
	0.12	-4.52 (0.62)	-5.86 (1.03)	-3.61 (0.76)	-5.20 (0.83)
	0.18	-3.59 (0.47)	-4.01 (0.58)	-2.51 (0.42)	-3.40 (0.86)
	0.24	-4.23 (0.61)	-4.88 (1.16)	-2.68 (0.65)	-4.12 (0.90)
100-1.00mM	0.06	-6.81 (0.03)	-6.05 (0.26)	-5.36 (0.10)	-5.35 (0.20)
	0.12	-8.53 (1.28)	-8.34 (1.61)	-7.18 (1.48)	-7.33 (1.41)
	0.18	-6.60 (0.83)	-5.64 (0.60)	-2.86 (3.32)	-4.70 (0.88)
	0.24	-7.70 (1.26)	-6.94 (1.83)	-1.97 (4.48)	-5.85 (1.51)
ISFET 2 (MTDA-TOTM)					
100-0.01mM	0.06	-24.06 (0.04)	-26.84 (0.19)	-23.09 (0.10)	-26.38 (0.15)
	0.12	-25.60 (0.72)	-28.39 (1.04)	-24.69 (0.85)	-27.73 (0.85)
	0.18	-25.05 (0.48)	-26.88 (0.55)	-23.97 (0.43)	-26.26 (0.83)
	0.24	-25.66 (0.55)	-27.54 (1.08)	-24.12 (0.56)	-26.78 (0.78)
100-1.00mM	0.06	-30.75 (0.08)	-28.64 (0.36)	-29.30 (0.15)	-27.95 (0.31)
	0.12	-31.94 (1.21)	-30.53 (1.54)	-30.58 (1.40)	-29.52 (1.33)
	0.18	-30.37 (0.79)	-28.24 (0.73)	-26.63 (3.30)	-27.31 (1.01)
	0.24	-31.26 (1.11)	-29.25 (1.55)	-25.53 (4.20)	-28.15 (1.19)
ISFET 3 (TDDA-NPOE)					
100-0.01mM	0.06	-19.21 (0.04)	-22.95 (0.14)	-18.24 (0.05)	-22.49 (0.10)
	0.12	-20.85 (0.90)	-25.12 (1.42)	-19.94 (1.03)	-24.46 (1.22)
	0.18	-19.67 (0.41)	-22.91 (0.58)	-18.60 (0.33)	-22.28 (0.85)
	0.24	-20.42 (0.74)	-23.92 (1.35)	-18.88 (0.75)	-23.16 (1.06)
100-1.00mM	0.06	-28.37 (0.04)	-25.16 (0.29)	-26.91 (0.12)	-24.47 (0.23)
	0.12	-30.57 (1.59)	-28.06 (2.06)	-29.21 (1.80)	-27.05 (1.85)
	0.18	-28.24 (0.76)	-24.80 (0.77)	-24.50 (3.37)	-23.87 (1.04)
	0.24	-29.42 (1.44)	-26.37 (2.02)	-23.70 (4.54)	-25.27 (1.66)
ISFET 4 (TDDA-TOTM)					
100-0.01mM	0.06	-17.30 (0.05)	-21.44 (0.17)	-16.32 (0.08)	-20.98 (0.12)
	0.12	-18.54 (0.73)	-23.20 (1.31)	-17.63 (0.88)	-22.54 (1.11)
	0.18	-17.34 (0.34)	-20.95 (0.71)	-16.26 (0.24)	-20.33 (0.98)
	0.24	-18.19 (0.87)	-21.90 (1.71)	-16.65 (0.82)	-21.14 (1.35)
100-1.00mM	0.06	-26.65 (0.01)	-23.77 (0.31)	-25.20 (0.09)	-23.07 (0.24)
	0.12	-28.57 (1.37)	-26.25 (2.03)	-27.22 (1.57)	-25.24 (1.81)
	.18	-26.57 (0.77)	-23.26 (0.75)	-22.83 (3.37)	-22.32 (1.03)
	0.24	-27.86 (1.49)	-24.61 (2.18)	-22.13 (4.56)	-23.52 (1.78)

* Nernst response parameters estimated without using double junction reference electrode (eq. 6).

† Nernst response parameters estimated using double junction reference electrode (eq. 8).

‡ Nernst response parameters estimated with decreasing concentration gradient and 3 replications of each test condition.

§ Nernst response parameters estimated with increasing concentration gradient and 3 replications of each test condition.

reference electrode to be an integrated part of the sensors on the ISFET chip.

The ISFET sensitivity was not greatly affected by the flowrate, provided the flowrate was maintained at a constant level during a test. A change in flowrate caused the ISFET output to shift to a new voltage level without any change in sample concentration. The most likely cause for this shift is a change in the pseudo-reference junction potential. This change in output level is not apparent in figure 4 since the individual curves have been shifted along the vertical axis. Therefore, the regression slopes of output versus concentration (log scale) were very similar for all flowrates, but the regression intercept varied between flowrates.

CONCLUSIONS

All of the membrane compositions included in these tests proved to be good candidates for the development of ion-selective nitrate sensors and, since they were PVC polymer membranes, they could be deposited on the gates

of ion-selective field effect transistors to make nitrate ISFETs. While any one of the ligand-plasticizer combinations could be used to sense nitrate, there were some differences in the selectivity of the membranes that would make some membrane compositions more suitable than others, depending on the application.

All membranes displayed an approximately Nernstian response to nitrates. However, the membranes based on the MTDA ligand showed slightly greater sensitivity to nitrate than did the TDDA membranes, particularly at low concentrations. The linear detection limits for the MTDA membranes were lower than those of the TDDA membranes, which is important if the membrane is to be used to test samples with low concentrations. The plasticizer did not have a great effect on the relative sensitivity of the membranes.

While the membranes formulated with the MTDA ligand showed greater sensitivity, those formulated with the TDDA ligand showed superior selectivity against interferences from other anions. The better selectivity of the TDDA membranes with respect to chlorides is important in the selection of a membrane for sensing soil nitrate since chloride ions may be present in soils in concentrations similar to those of nitrates. The plasticizer affected the selectivity to a much lesser extent than did the ligand. Although all membranes were more sensitive to I, SCN, and ClO₄ ions than to the nitrate ion, the membranes formulated with the TDDA ligand were always less sensitive to these interference ions than were the membranes formulated with the MTDA ligand. Therefore, from a selectivity point of view, the membranes formulated with the TDDA ligand are more suitable for applications where interference ions are present in the sample.

Both the MTDA and TDDA ligands warrant further testing for use in ISFET membranes. The MTDA ligand should be studied because it exhibited superior response in low nitrate concentrations, and the TDDA ligand should be studied because it showed superior selectivity. The NPOE and TOTM plasticizers deserve further investigation for nitrate sensing since the sensitivity and selectivity of the membranes were not significantly affected by the plasticizer, and these particular plasticizers had been used previously in ISFET membranes.

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