

RAPID NITRATE ANALYSIS OF SOIL CORES USING ISFETs

R. R. Price, J. W. Hummel, S. J. Birrell, I. S. Ahmad

ABSTRACT. *An intact core extraction procedure was tested that might be used in the field for real-time prediction of soil nitrates. An extraction solution was pushed through a soil core held between two filters, and an ion-selective field-effect transistor/flow injection analysis (ISFET/FIA) system was used to sense soil nitrates in real time. Laboratory tests were conducted using four soil types and two levels of nitrate concentration, soil moisture, core density, core length, core diameter, and extraction solution flow rate. The extraction solution flow was sampled at the exit face of the core and routed to the ISFET/FIA system. The ISFET output voltage was sampled at 100 Hz. Results of the test indicate that nitrate extraction of the soil cores was successful, and that data descriptors based on response curve peak and slope of the ISFET nitrate response curve might be used in tandem in a real-time prediction system.*

Keywords. *Environment, Extraction, Membrane, Precision farming, Real-time, Sensors, Site-specific farming.*

Nitrate fertilizer is a major factor in obtaining high yields of agricultural crops that feed the world's population. But the use of synthetic nitrogen fertilizer now disrupts the nitrogen cycle and is a major contributor to acid rain, nitrates and other compounds in waterways, and oxygen depletion in coastal waters (Kaiser, 2001). From a health standpoint, nitrate leaching has occurred in many areas of the country, causing contamination of the drinking water supply (Staver and Brinsfield, 1990; Fruhling, 1986; Hubbard et al., 1984; Hallberg, 1986). Currently, the U.S. Environmental Protection Agency (EPA) sets the maximum allowable nitrate-N concentration level in drinking water at 10 mg/L (Freese, 1988). Studies have shown that this level is exceeded in many rural water supplies, with levels as high as 133 mg/L (Hubbard et al., 1984). This concentration can cause methemoglobinemia ("blue baby" sickness), birth defects, cancer, and eutrophication in lakes and streams (Fruhling, 1986; Hubbard et al., 1991). Still, nitrate leaching can be controlled by irrigation scheduling, fertilization based on soil test, conservation tillage, cropping practice, and recommended manuring rates (Power and Schepers, 1989). Currently, the farmer has control of fertilizer application rates. Several

nitrate management plans have been developed to match commercial nitrogen fertilizer application levels to expected crop requirements. The University of Illinois Department of Agronomy (Anderson et al., 1992) recommended that Illinois corn producers establish the amount of nitrogen needed to attain 100% yield, adjust for any nitrogen supplied by the previous year's crop, and then make an N fertilizer recommendation. In this plan, other nitrogen sources such as soil mineralization are disregarded, which in certain weather conditions can result in over-application of fertilizer and increased probability of nitrate leaching.

Other plans for setting broadcast N fertilizer application rates have been developed. One plan incorporates a pre-side-dress nitrate test (PSNT), which measures the soil nitrate-N concentration late in the spring when the corn is 15 cm to 30 cm tall. Sampling is performed to a 30 cm depth (Magdoff et al., 1984). At this growth stage, conventional equipment can still operate to apply a side-dressing of fertilizer, but it is late enough in the growing season to get a good indication of the nitrate deficiencies in the soil for the remainder of the growing season (Blackmer et al., 1989). The research of Blackmer et al. (1989) indicated that at least 20 ppm of nitrate-N is needed in the top 30 cm of soil to achieve the 100% yield goal of corn. If the soil is below 10 ppm, then normal fertilizer application rates should be applied to attain the 100% yield goal. This test is currently finding widespread use in Vermont, Connecticut, and Pennsylvania and has reduced environmental problems associated with nitrogen fertilizers. Still, when using this plan, accurate estimates of soil nitrate level are needed to make the nitrogen fertilizer recommendation. Currently, few sensors exist to estimate soil nitrates in real time. The development of a real-time nitrate sensor that could accurately estimate soil nitrate levels could result in fertilizer application rates that more closely match crop needs and could minimize the impact of synthetic nitrogen fertilizer on the environment.

LITERATURE REVIEW

For over a decade, researchers at public institutions have pursued real-time soil nitrate sensing. Adsett and Zoerb

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(1991) reported on a mobile tractor-mounted system that involved three major components: (1) a device to collect soil samples, (2) a mechanism to mix and extract a liquid solution from soil, and (3) an ion selective electrode (ISE) to evaluate the nitrates in solution. A chainsaw blade cut a slot in the ground and conveyed soil up above the ground surface. Gravity separated the soil from the chain, and the soil dropped into the soil solution extraction and analysis system. The ion selective electrode analyzed the extracted solution. Field tests indicated that the system did not produce repeatable results, and only 40% of the nitrate readings were accurate. The poor results were attributed to inadequate mixing of the soil and extraction solution. In addition, the volume and consistency of the soil sample delivered to the mixing and extraction system from the slot cutter varied, depending upon the soil type and moisture content.

Thottan et al. (1994) reported on a laboratory investigation into the suitability of the ISE to detect nitrates in an automated soil nitrate monitoring system. The ISE was immersed in magnetically stirred deionized water until a constant reading was observed. A soil solution was extracted from a clay loam soil sample, which resembled a field-extracted soil core (no crushing or sieving). The ISE was placed in the solution, and data were collected to determine the time lag until the sensor output reached equilibrium. Results indicated that the ISE could indicate a large percentage of the nitrates in solution in less than 20 s (although detection of a final peak still took up to 2 min.). The researchers concluded that characterization of the NO₃ ISE response might allow sample nitrate level prediction as early as 6 s after the start of measurement.

Additional research (Adsett et al., 1999) indicated that the soil sampler had been redesigned, using a woodsaw blade and belt-conveying unit to gather and transport samples of known volume and density to the extraction and analysis unit. Although a major emphasis in the new design of the NO₃ extraction unit was electrical isolation of the extraction chamber from other components to eliminate interference with the NO₃ ISE signal, some problems were still present. Laboratory testing showed that, with the use of a field calibration routine, the NO₃-N reading at 6 s after start of measurement could be used to predict NO₃-N level in the soil solution.

McGrath et al. (1994) designed an automated soil sampler (AS) that operated in real time and a nitrate sensing system that processed one soil sample every minute after a 90-minute delay in initial preparation of the first sample. The sampler consisted of a two-wheel trailer, sampling mechanism, sample packer, sample collector, trace marker, control, and alert system. The AS stored soil samples in a continuous web of film. The trailer could be attached to a self-propelled sprayer or tractor, and up to 300 samples could be stored on the trailer. The sampling increment was >15 m.

Ehsani et al. (1999) investigated the use of near-infrared (NIR) reflectance spectroscopy to estimate soil mineral nitrogen. Using both spiked and field samples, they were able to correlate reflectance in the range of 1800 to 2300 nm with soil mineral nitrogen, provided that an appropriate calibration curve was available. However, the technique failed whenever a soil sample was encountered that contained an NIR responsive chemical constituent that was not represented in the original calibration dataset. Ehsani et al. (2000) determined that a peak in spectral reflectance response

occurred in the mid-NIR range (5000 to 12500 nm) that was associated with nitrate. They also were able to detect the response in a ground soil sample that was mixed with a non-absorbing KBr dilution matrix. Although the mid-NIR response could not be detected in a pure soil sample, additional processing using a wavelet technique made the response to nitrate detectable.

Birrell and Hummel (2000) investigated matrix membranes for sensing nitrates using ISE technology, and the use of those membranes with field-effect transistor technology to produce nitrate ion-selective field-effect transistor (ISFET) sensors. Both ISEs and ISFETs respond to the activity of the ions in the sample, and the response is linearly related to the logarithm of the ion concentration. ISFET technology offers inherent features such as small dimensions, low output impedance, high signal-to-noise ratio, low sample volumes, and the potential for mass production, all of which are required for a real-time sensor. 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 or bicarbonate.

The ISFET chip can be combined with a flow injection analysis (FIA) system to detect nitrate ion concentrations in real time. FIA (Ruzicka and Hansen, 1975) operates by pulsing a sample solution and carrier (base) solution to the sensor. This pulsing action allows a differential measurement between the two solutions, providing a baseline for each sample. Bergveld (1991) stated that the ability of the ISFET to measure ion fluxes makes it a very useful detector in an FIA system where dynamic signals occur. One problem that exists with ISFETs is long-term drift (Bergveld, 1991), but the electrical responses of ISFETs tested by Birrell and Hummel (2001) were consistent and predictable when used with an FIA system to minimize long-term output drift. Precision and accuracy of the system were dependent on maintaining precise, repeatable injection times and constant flow parameters during the calibration and testing cycle.

Considerable research (Clements et al., 1974; Li and Smith, 1984; Hansen et al., 1977) has demonstrated the capability of ion selective electrodes to determine nitrate concentration in soil. The ISFET has the same theoretical basis as ion selective electrodes, but it also has potential as an extremely small, rapid-response sensor that could be mass-produced using integrated circuit technology. This article presents a further step in the development of ISFETs for use as the sensing element in a real-time soil nitrate sensor system. Our objectives were to identify sample extraction system design parameters that significantly affect the extraction rate, and output data descriptors that might be used to accurately predict sample nitrate content while reducing the response time.

EQUIPMENT AND PROCEDURE

The factors thought to be critical in the development of a real-time nitrate extraction system were soil texture, moisture, core density, nitrate concentration, core diameter, core length, and extraction solution flow rate through the core. A 2ⁿ factorial statistical design was used to test the significance of all of the variables except soil texture. Since soil textures vary widely, four soils were chosen from the ten soil types selected and tested by Birrell and Hummel (2001). The soils

Table 1. Textural classification and organic carbon content of Illinois surface soils used in this study.

Soil Name	ID	Textural Class ^[a]	Textural Properties (%)			Organic Carbon (%) ^[b]
			Sand	Silt	Clay	
Ade	1	Loamy sand	86.5	7.3	6.2	0.34
Proctor	8	Clay loam	25.6	47.1	27.3	2.23
Drummer	24	Silty clay loam	9.0	63.4	27.6	1.42
Drummer	26	Silty clay loam	8.7	61.0	30.3	1.53

^[a] Textural classification and analysis data from Worner (1989).

^[b] Organic carbon data from Birrell (1995).

(table 1) were Ade loamy sand, Proctor clay loam, and two Drummer silty clay loams (which differed in organic carbon content). These soils represent a broad range of the agricultural soils found in Illinois. Soil moisture tension levels near field capacity (0.1 MPa) and at the wilting point (1.5 MPa) were selected to provide a broad range of moisture contents. Two levels (loose-fill and compacted) were selected to represent possible soil core densities. The nitrate levels (10 and 30 ppm) were chosen since they represent the ends of the range of soil nitrate concentrations where rates other than zero or the full rate might be used when fertilizing, according to the PSNT. Levels for core diameter and core length were 9.5 mm and 12.7 mm, and 6.25 mm and 12.7 mm, respectively, and extraction solution flow rates were 1.5 and 3.0 mL/s, so that with even the largest core volume, rapid extraction could occur. The entire design included 256 samples.

SOIL PREPARATION

Four 600-g lots of each soil were split from the same soils used by Birrell and Hummel (2001), which had been screened using a 2-mm sieve, air-dried, and stored in sealed containers at room temperature. The 16 lots of soil were leached with deionized water, dried, crushed, sieved through a 2-mm sieve, and stored in sealed containers at 5°C. Subsamples of each lot were analyzed for nitrate-N concentration using a diffusion analysis method (Mulvaney and Khan, 1999). The four lots for each soil were randomly assigned nitrate concentration and soil moisture levels. Based on the soil nitrate content of each lot after leaching, the desired amount of nitrate (NaNO₃) was added to the required amount of deionized water and added to the soil. The soil lots were kept in sealed containers at 5°C, except when tests were being conducted.

Four soil core holders, one for each combination of core diameter and core length, were machined from 5.08-cm dia. Delrin rod. A dual-layer support screen (63 × 63 strands per cm polypropylene screen installed over a 3.18-mm thick plastic sheet with 1.59-mm dia. holes) was placed in the bottom of the core holder to support the soil sample during packing, while still allowing solution to flow through the core during extraction.

Soil was measured and packed into the appropriate soil core holder, according to the treatment randomization. Soil was poured into the soil core holder and struck off flush with the upper surface of the soil core chamber to produce cores with a low soil density level. Applying a 27-kPa pressure to the soil before the strike-off, using a hand-held compaction tool, produced the high soil density level.

On each test day, a 10-g sample of each of the 16 lots of soil was dried at 105°C (48 h) for gravimetric moisture

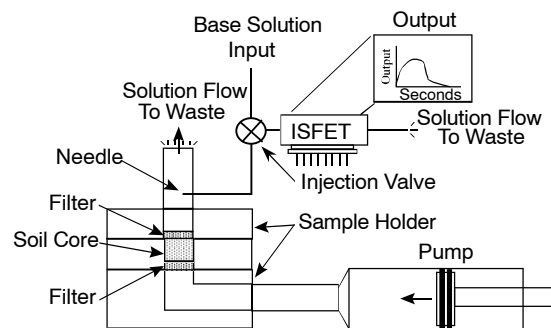


Figure 1. Schematic diagram of complete nitrate extraction system.

analysis. An additional 15-g sample was collected for nitrate-N analysis using diffusion (Mulvaney and Khan, 1999). Additional samples were drawn at a later date and analyzed using a KCl extraction and colorimetric analysis method (Parker, 1996).

EXTRACTION SYSTEM

A system was devised to clamp a soil core holder between two filters (fig. 1) during nitrate extraction. The clamping mechanism consisted of a vertical handle hold-down clamp (De-Sta-Co, Carrollton, Texas) mounted on a base and fitted with a base plate and filter holder for quick release of the core holders. Extraction solution flow was produced by an extraction pump, which consisted of a linear electric actuator and syringe. A frequency generator (model 33120A, Hewlett-Packard, Palo Alto, Cal.) was used to set duty cycle and cycle frequency of the stepper motor that controlled the extraction pump. This frequency was set to produce a single-period, 50%-duty-cycle square wave of either 21 or 42 ms duration, providing either a 12 or 24 s sampling duration, respectively, depending on the flow rate through the core. Price (2000) provided additional detail about the extraction pump and its operation. The extraction solution was introduced through the base plate below the soil core holder and had an upward flow through the extraction system. Flow rate was constant during extraction of each sample.

The upper filter holder, above the soil core, housed disposable filter media (Price, 2000) and supported a 25-μL glass disposable needle for sampling the extraction solution exiting the soil core. The needle extended to the vertical axis of the soil core and was positioned just above the upper surface of the filter.

INJECTION/DETECTION SYSTEM

An ISFET/FIA system (Birrell, 1995) was used to sense the nitrate concentration in the extraction solution exiting the core. The ISFET/FIA system consisted of an injection valve, an injection pump, and an ISFET/FIA flow cell. A base solution of known ionic strength (10⁻² M CuSO₄ in deionized water) was sent through the ISFET/FIA flow cell to provide a baseline output, and the sample solution was injected into the flow stream. Based on previous research by Birrell and Hummel (2000), the peristaltic injection pump (Masterflex model 7520-25, Cole-Parmer, Vernon Hills, Ill.) was set to a flow rate of 0.20 to 0.24 mL/s using a tubing size of 0.89 mm I.D. The ISFET output voltage, just prior to sample injection, provided the baseline level, and differences in voltage from the baseline, during sample injection, indicated the nitrate concentration in the sample stream. The ISFET chip (table 2)

Table 2. Electrical properties of the ISFET chip (Tsukada et al., 1989).

Supply voltage	+5V
Total power consumption	2 mW
Operational amplifier	
Slew rate	5 V/ μ s
Open loop gain	58 dB
Analog switch	
On resistance	\approx 1 k Ω
Switching rate	50 ns

was designed and developed by Hitachi (Tsukada et al., 1989). The injection valve controlled the source of fluid being drawn into the ISFET/FIA system. The valve was rated for switching between the base solution and the sample flow up to once each 0.56 s.

Several procedures were necessary to prepare the ISFET chip for use in the FIA system. First, the ISFET chip was coated with a polyamide layer (the gate area of the four transistors was left exposed to form a sensor well). The chip was then bonded to a circuit board, which had pins for an IC socket with a polyamide layer applied. An epoxy resin coating was applied over the thin bonding wires and the bonding pads for mechanical and chemical protection. After curing, a nitrate ion-selective membrane was applied by injecting a casting solution into the sensor well using a micro-syringe. The membrane was composed of the ligand (tetradodecylammonium nitrate, TDDA), the plasticizer (2-nitrophenyl octyl ether, NPOE), and a high molecular weight polyvinyl chloride (PVC). Tetrahydrofuran (THF, 99.98% purity) was used as the solvent to keep the membrane in suspension for application on the chip (Birrell, 1995). Only two of the four ISFETs were active on the chip that was used, and their outputs were accessed directly rather than through the multiplexer (Birrell, 1995).

A Daqbook 200 (Iotech, Cleveland, Ohio) portable PC-based data acquisition system and 400 MHz Pentium computer were used to collect and store ISFET output voltage data. The system also collected and stored data from a soil chamber pressure sensor and a syringe pump position sensor (an indicator of extraction solution flow rate).

DATA COLLECTION

Each soil core was prepared in the core holder immediately prior to testing. The soil core chamber, holding a soil core, was loaded onto the test stand, and data collection was initiated. When extraction solution flow was detected at the sampling needle, a capacitance sensor activated the injection valve to begin sample injection into the ISFET/FIA flow stream. During testing of each core sample, data were collected at a sampling rate of 100 Hz. A maximum of 5000 points was collected for each test.

Recalibration of the ISFET chip was performed after 16 soil samples, or hourly, whichever came first. Five calibration solutions and one baseline solution were used. The calibration solutions were $5.0(10^{-4})$ M, $2.5(10^{-4})$ M, $1.25(10^{-4})$ M, $6.25(10^{-5})$ M, and $3.125(10^{-5})$ M NaNO_3 (prepared in a 0.01 M CuSO_4 solution). The baseline solution was 0.01 M CuSO_4 . Calibration was performed by successive injections of the calibration solutions (duration of 5 s) and the baseline solution (duration of 10 s). The soil chamber pressure sensor and extraction pump position sensor were also calibrated to check linearity. A baseline reading, i.e., injection without a soil sample, was also made to ensure that electrical noise was not affecting ISFET response.

RESULTS AND DISCUSSION

ISFET RESPONSE DATA ANALYSIS

Typical test results (figs. 2 and 3) illustrate the response of the ISFET/FIA system. At 7 to 8 s after test initiation, changes in the pressure and position sensor outputs signal that the extraction solution pump has started to push extracting fluid through the soil core. Injection of the sample from the soil core usually began 10 to 15 s after data collection was initiated, which gave ample time to establish the ISFET baseline for the test. Data collection continued at 100 Hz for 50 s for each test, even though the sample injection time varied, depending on the flow rate selected. The ISFET response to nitrate in the injected sample is a reduction in the output voltage; thus, a minimum voltage corresponds to a maximum response. A noise-reduced ISFET response curve,

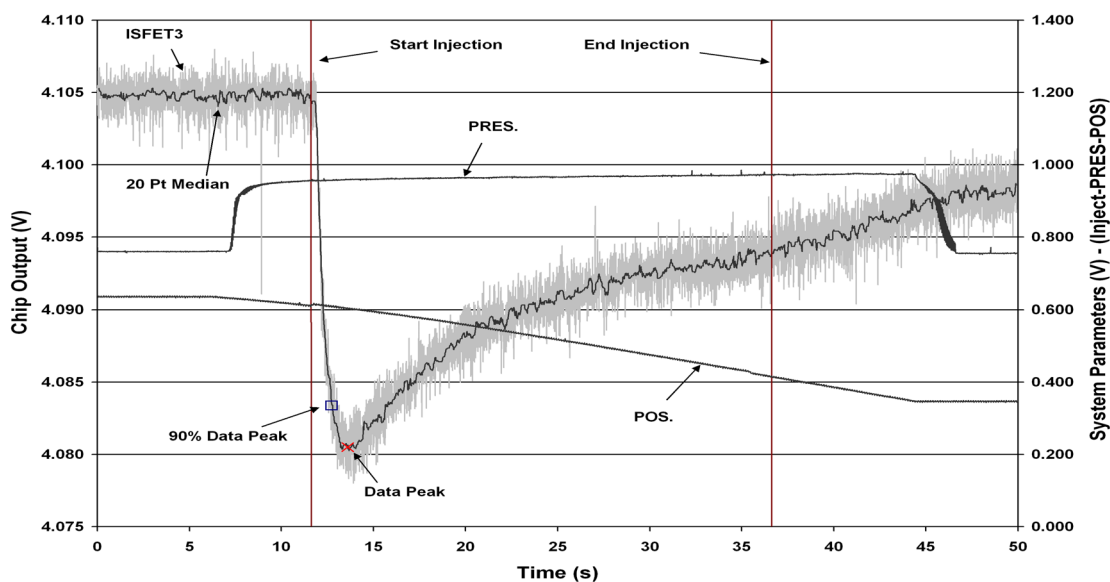


Figure 2. Nitrate extraction curve (ISFET3) for a low-nitrate Ade loamy sand soil (Test 336 - 31 ppm).

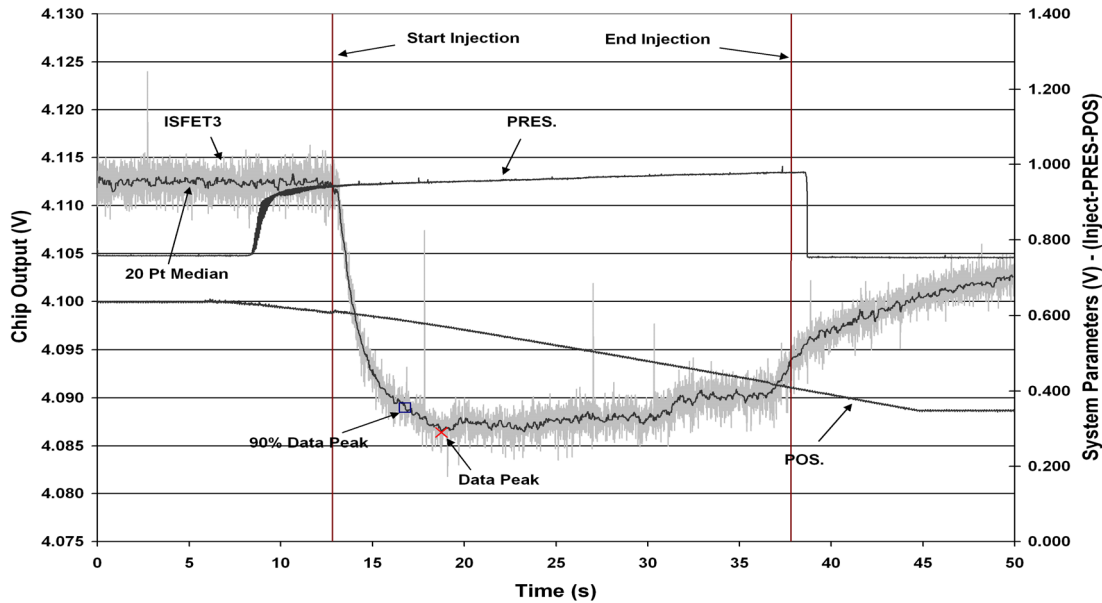


Figure 3. Nitrate extraction curve (ISFET3) for a high-nitrate Drummer #24 silty clay loam soil (Test 377 – 47 ppm).

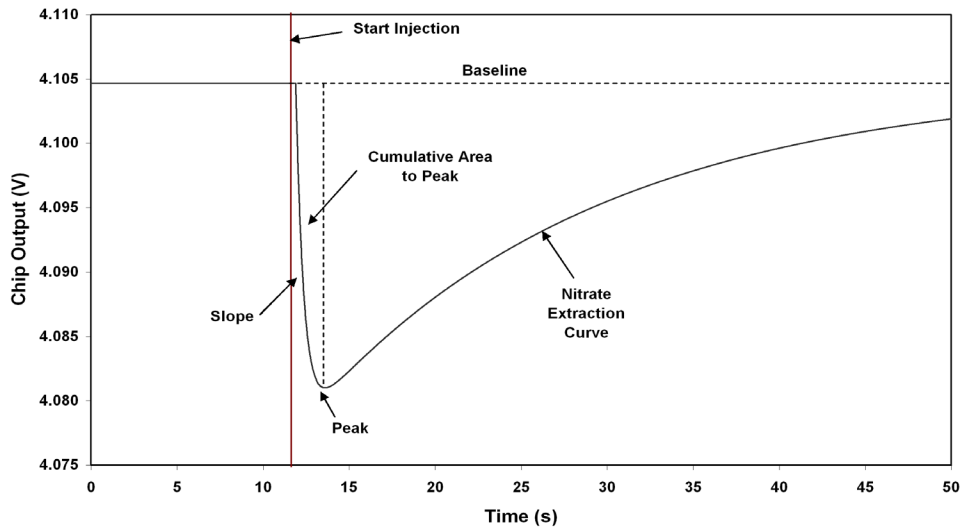


Figure 4. Illustration of nitrate extraction curve showing peak, slope, and cumulative area-to-peak data descriptors.

produced by conducting a 20-point running median filter of the data, is superimposed on the raw data.

A comparison of test results shows that soil type affected the shape of the ISFET response curve. A maximum response occurred for the coarser textured soil, Ade loamy sand (fig. 2), and then decreased quickly, even as extracting solution was continuing to flow through the soil core. In contrast, the ISFET response for the heavier textured Drummer silty clay loam (fig. 3) and Proctor clay loam (response not shown) soils did not exhibit a maximum response as quickly, nor did they decrease in response level as quickly as did those of the coarser textured soil.

A number of response data descriptors that might be useful for nitrate level prediction were included in the analysis. Obvious data descriptors to investigate included peak, slope, and cumulative area-to-peak (fig. 4). Although the use of the 20-point running median filter produced two response curves for each test, the data descriptors were only evaluated for the running-point median filter response curve. Peak response

was calculated as the difference between the baseline value prior to injection and the maximum ISFET response. In addition, since the ISFET response does not always exhibit a sharp peak value, a 90% peak response was also investigated as a data descriptor. The cumulative area-to-peak value could not be readily automated for the running-point median filter data, so it was not implemented.

Since a rapid, real-time indication of nitrate concentration was sought, attention was focused on the initial portion of the response curve. Visual observation suggested that the slope of the response curve might be related to nitrate concentration. The ISFET is responding to additional nitrate being extracted from the soil core throughout the flow-through extraction process, but in the interest of rapid prediction, some accuracy might be sacrificed. Three values of slope were calculated, based on the difference between the baseline value prior to injection and the response value at 0.5 s, 1.0 s, and 2.0 s, respectively, after injection was initiated.

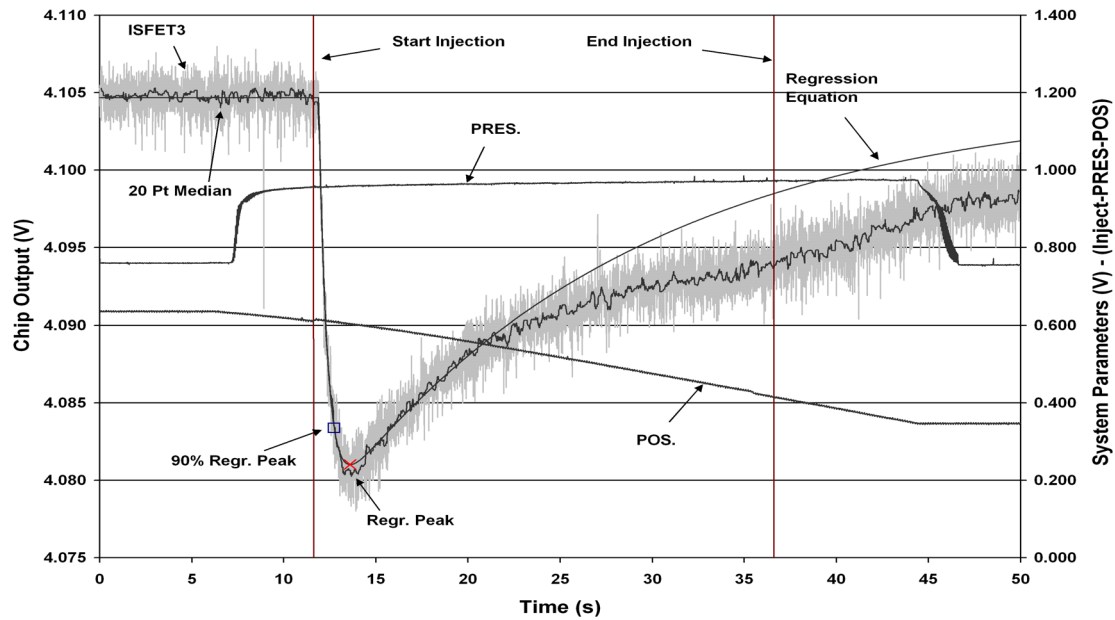


Figure 5. Nitrate extraction curve (ISFET 3) for Ade loamy sand soil illustrating regression line fit (Test 336 – 31 ppm).

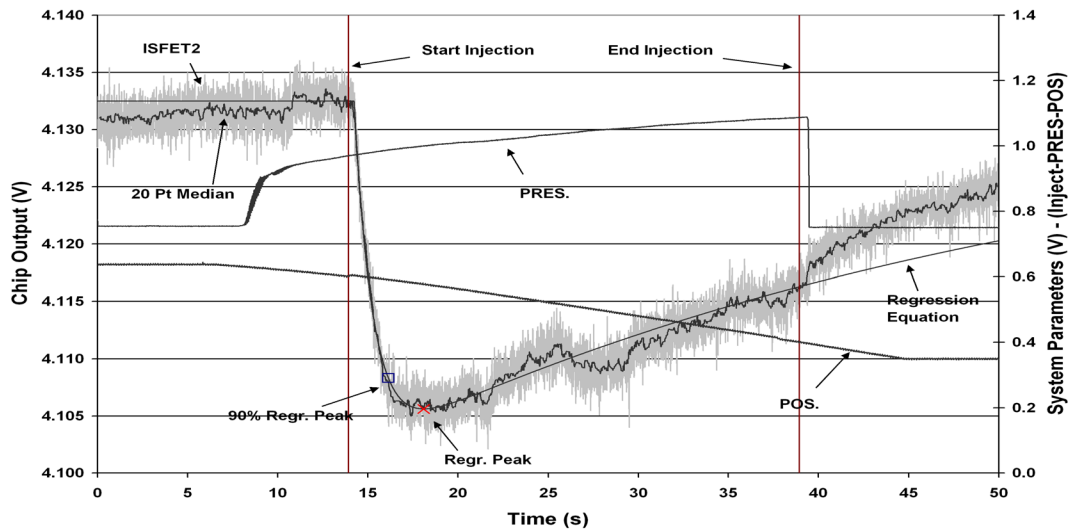


Figure 6. Nitrate extraction curve (ISFET2) for Drummer silty clay loam soil illustrating regression line fit (Test 476 – 40 ppm).

In a further attempt to reduce noise in the ISFET/FIA response, and considering the goal of an automated real-time analysis system, a regression curve was sought to describe the ISFET/FIA system response. Data analysis software (Peakfit, ver. 4.0, SPSS Inc., Chicago, Ill.) was used, and a subroutine called “Pulse Peak with Second Width Term (Amplitude)” was able to fit the response data quite well (figs. 5 and 6). The equation being fit to the data was able to produce the baseline, the injection initiation point, and the curvature-to-peak portion of the response curve accurately ($R^2 = 0.97$ to 0.99) when fitted to the portion of curve from 5 to 10 s before the start of injection to 3 to 5 s after peak amplitude. The regression curve did not accurately fit the ISFET response data beyond 3 to 5 s after peak amplitude was reached, but this portion of the curve was not being used in any of the response data descriptors. With the use of the regression curve, one additional slope data descriptor was added – slope at 0.25 s after injection was initiated. The use of the

regression curve also made it possible to automate the calculation of the cumulative area-to-peak data descriptor, using Simpson’s Rule applied to each 0.01 s interval from start of injection to maximum response.

Applying the five data descriptors (peak, 90% peak, and 0.5 s, 1.0 s, and 2.0 s slopes) to both the running-point median filtered data and the generated regression curve resulted in 10 data descriptors. The application of the 0.25 s slope and the cumulative area-to-peak data descriptors to the regression curve data added two additional data descriptors (table 3).

The ISFET response data for the first ten data descriptors were adjusted using the calibration data obtained from the standard calibration solutions and baseline solution that were injected into the flow stream to the ISFET/FIA system after each set of 16 soil samples.

Adjustments were made to compensate for any changes in ISFET response during the test sequence using only the

Table 3. Variables included in the stepwise multiple linear regression analysis.

Regression Variable	Symbol	Units
Soil type	S	
Nitrate-N	N	µg/g
Core diameter	D	cm
Core length	L	cm
Extraction solution flowrate	F	mL/s
Soil moisture content	M	% d.b.
Soil clay content	C	%
Soil density	ρ	g/cc
Soil organic carbon	O	%
Regression peak	var1	mV
90% regression peak	var2	mV
Raw data peak	var3	mV
90% raw data peak	var4	mV
Regression slope – 0.25 s	var5	V/s
Regression slope – 0.5 s	var6	V/s
Regression slope – 1.0 s	var7	V/s
Regression slope – 2.0 s	var8	V/s
Raw data slope – 0.5 s	var9	V/s
Raw data slope – 1.0 s	var10	V/s
Raw data slope – 2.0 s	var11	V/s
Regression cumulative area-to-peak	var12	mVs

running-point median filtered data, since the regression program could not fit a regression curve to the calibration data (data for all standard solutions were saved in a single file for each calibration set, resulting in a number of discontinuities). Standard solution calibration data collected before and after each set of 16 soil samples were grouped together. Using regression techniques, the calibration data were used to adjust the corresponding soil sample data, so data from all samples would represent response from a single ISFET/FIA system operating condition. The cumulative area-to-peak soil sample data could not be adjusted, since no corresponding data stream existed in the calibration data.

DESIGN FACTOR ANALYSIS

Statistical analysis was used to identify the design variables that significantly affected the values of the data descriptors. Data were analyzed using the PROC MIXED and PROC LSMEANS statistical analysis routines in SAS (SAS, 1999), since the data sets were classified as incomplete and unbalanced (data for 10 tests were missing due to high system pressure causing the extraction pump to stall). The data were also checked for normal distribution using the PROC UNIVARIANT routine. Prior to analysis, a logarithmic transformation was applied to the ISFET response data, since ISFET responses to ionic concentration are logarithmic. A code number (either 1 or -1) was assigned to each level of each variable (table 4) in the statistical design.

All of the data descriptors were analyzed with the goal of identifying all variables and all second- and third-order interactions between and among variables that significantly

Table 4. Variable levels used for statistical design.

Level	Compaction	Moisture (MPa)	Ni-trate-N (µg/g)	Core Diameter (cm)	Core Length (cm)	Flow Rate (mL/s)
-1	Compacted	1.5	10	0.95	0.64	1.5
1	Loose fill	0.1	30	1.27	1.27	3.0

affected each data descriptor. During the analysis of each descriptor, variables participating in insignificant higher order interactions were removed in a stepwise manner, starting with the least significant interactions until only those variables participating in significant effects were retained. All variables contributing to a significant interaction (but not as a main effect) were also retained in the model. This analysis continued until only those variables left in the model were significant ($\alpha = 0.05$) and/or highly significant ($\alpha = 0.01$). The residual sum of squares was used to test for significance of variables and interactions.

A typical statistical analysis for one of the data descriptors, the regression peak response (table 5), shows that nitrate-N level, soil type, core length, core diameter, and extraction pump flow rate were variables whose effects on the regression peak response were highly significant. In addition, second-order interactions between soil type and nitrate level, and between nitrate level and flow rate, were also highly significant. Additional results for other tests are in Price (2000). For both ISFET2 and ISFET3, and across all data descriptors, nitrate-N and soil type were the variables with the greatest effects on the ISFET data descriptors. This result suggests that *a priori* knowledge of soil type might be necessary for the ISFET technology to make accurate real-time measurements of soil nitrate-N when using core extraction. Soil core length and core diameter were highly significant variables in this experiment, and the values of these variables used in the design of a real-time sampler will be critical. The highly significant effect of the extraction pump flow rate indicates that high-quality components will be needed in this portion of a real-time nitrate-N sensor to minimize flow rate variability.

The time required to obtain a data descriptor value, using each data descriptor, is important in the development of a real-time sensor. With the slope data descriptors, the value is obtained at a selected time interval after the start of injection. Similar data were extracted from the tests for the remaining data descriptors (median-filtered peak, regression peak, 90% median-filtered peak, and 90% regression peak). The time to acquire the cumulative area-to-peak value is the same as that for the median-filtered peak. The results of these calculations for ISFET2 (table 6) indicate that, on average, from 2 to 5 s are required to produce a value, while the elapsed time could be as long as 25 s. Similar results (Price, 2000) were obtained for ISFET3. Obviously, one of the slope data descriptors, with an elapsed times of 0.25 to 2 s, would be a better choice if response time is critical, even if the nitrate-N response is less than optimum.

Table 5. Variables showing significant effect on ISFET response at P < 0.01 for ISFET2 regression peak data.

Variable ^[a]	Testing of Fixed Effects			
	NDF ^[b]	DDF ^[c]	F-Value	Pr > F
S	3	231	107.50	0.0001
N	1	231	588.33	0.0001
D	1	231	23.33	0.0001
L	1	231	47.32	0.0001
F	1	231	11.03	0.001
S*N	3	231	23.56	0.0001
N*F	1	231	24.85	0.0001

[a] Table 3 defines the symbols used to represent the variables.

[b] NDF = numerator degrees of freedom.

[c] DDF = denominator degrees of freedom.

Table 6. Time to attain a data descriptor value for ISFET 2 data.

Data Descriptor	Time (s)			
	Mean Time to Peak	Standard Deviation	Minimum	Maximum
Regression peak	4.4	4.8	0.5	24.7
Regression 90% peak	2.3	2.9	0.2	20.8
Raw data peak	5.1	5.3	0.7	23.9
Raw data 90% peak	3.2	3.7	0.4	20.3

NITRATE–N LABORATORY ANALYSIS

The results of the laboratory nitrate–N tests using the diffusion method (Mulvaney and Khan, 1999) were inconsistent among the test dates. The soils were resampled (3 replications) and analyzed using a colorimetric method (flow injection analyzer, Lachat Instruments, Loveland, Colo.). Blind spiked samples and laboratory–prepared solutions included in the sample set verified the accuracy and repeatability of the analyses. Comparison of results of analysis of the samples from the 16 soil lots over several months, using the colorimetric method, indicated that little or no change in nitrate–N concentration was occurring under the sample storage conditions.

NITRATE–N PREDICTION

A logarithmic transformation was applied to the data from the colorimetric laboratory analyses, in preparation for correlation with the data descriptors representing the ISFET–generated nitrate prediction data. Since statistical analysis of these data indicated that ISFET2 and ISFET3 responded similarly, the two datasets were combined. Three additional variables – soil density, organic carbon (Birrell, 1995), and clay content (Worner, 1989) – were included to differentiate among the four soils included in the study. Density, calculated from weights recorded during sample preparation for ISFET analysis, ranged from 0.85 g/cc to 3.63 g/cc. Organic carbon ranged from 0.34% to 2.23%, and clay content ranged from 6.2% to 30.3%.

The PROC CORR procedure in SAS (SAS, 1999) was used to investigate correlation among the variables in the dataset. Except for regression cumulative–area–to–peak (var12), all of the data descriptors developed from the ISFET

response data were positively correlated to each other and to the logarithmically transformed colorimetric laboratory analysis data with correlation coefficients >0.50 (data not shown). Clay content and organic carbon were negatively correlated to the same data descriptors, although some of the regression coefficients were < 0.50.

A multiple linear model (table 7) including all the soil variables, system design variables, and ISFET data descriptors was formed and analyzed using the PROC STEPWISE routine in SAS. The initial analysis included the combined dataset for all four soils in the test. Variables were retained in the model if their contribution was significant ($\alpha = 0.05$). The best statistically valid model (table 7) for the combined dataset (both ISFETs and all soils) included 2 system design variables, 2 soil variables, and 6 data descriptors. Several classes (raw data peak, regression peak, etc.) of data descriptors were significant contributors to the model. When the model was applied to datasets representing each of the four soils, system design variables remained significant in all models, and generally, the significant data descriptors were based on peak and slope of the ISFET response curves. Improved correlations of the data descriptors (derived from the initial portion of the ISFET response curve) with core nitrate concentration might have been expected with coarse–textured soils, which release a larger portion of nitrate more quickly than fine–textured soils (fig. 5 vs. fig. 6). However, higher coefficients of determination (R^2) were found for the finer textured soils than for the combined dataset for all soils, which may have resulted from sample–to–sample variations in the quantity of soil in each core with the coarse–textured Ade soil.

Stepwise multiple linear regression (SMLR) was used to further investigate the system design variables. Combining core diameter and core length formed a new variable, core size, which had four levels. The combined dataset was divided into four subsets, each representing one of the core sizes. The best statistically valid ($P > 0.0001$) models (table 8) for each of the four core sizes typically included extraction solution flowrate and at least four of the data descriptors. The coefficient of determination (R^2) was higher, and the standard error of calibration (SEC) was lower

Table 7. Models selected by stepwise multiple linear regressions of the combined data for ISFET2 and ISFET3.

Model	Dataset	Variables	R^2	SEC ^[a]
$\log_{10}(\text{NO}_3) = f(\text{D, L, F, M, C, } \rho, \text{ all data descriptors})$ ^[b]	All soils	L, F, M, ρ , var1, var3, var6, var8, var9, var12	0.65	0.341
$\log_{10}(\text{NO}_3) = f(\text{D, L, F, M, C, } \rho, \text{ all data descriptors})$	Proctor clay loam (8)	D, L, F, M, var3	0.71	0.368
$\log_{10}(\text{NO}_3) = f(\text{D, L, F, M, C, } \rho, \text{ all data descriptors})$	Ade loamy sand (1)	D, L, F, var3, var7	0.54	0.163
$\log_{10}(\text{NO}_3) = f(\text{D, L, F, M, C, } \rho, \text{ all data descriptors})$	Drummer silty clay loam (24)	L, ρ , F, var1, var3, var7, var10	0.69	0.344
$\log_{10}(\text{NO}_3) = f(\text{D, L, F, M, C, } \rho, \text{ all data descriptors})$	Drummer silty clay loam (26)	D, L, M, var1, var3, var7, var11	0.75	0.294

^[a] SEC (standard error of calibration) is the standard error of the estimate in the calibration data, in $\log_{10}(\text{NO}_3)$.

^[b] Table 3 defines the symbols used to represent the variables.

Table 8. Models selected by stepwise multiple linear regressions of the core size data subsets.

Model	Core Diameter (cm)	Core Length (cm)	Core Volume (cm ³)	Variables	R^2	SEC ^[a]
$\log_{10}(\text{NO}_3) = f(\text{D, L, F, M, C, } \rho, \text{ all data descriptors})$ ^[b]	1.27	1.27	1.61	M, F, var3, var6, var9, var12	0.74	0.298
$\log_{10}(\text{NO}_3) = f(\text{D, L, F, M, C, } \rho, \text{ all data descriptors})$	1.27	0.64	0.80	M, F, var1, var3	0.65	0.342
$\log_{10}(\text{NO}_3) = f(\text{D, L, F, M, C, } \rho, \text{ all data descriptors})$	0.95	1.27	0.91	F, var2, var4, var6, var9, var12	0.69	0.324
$\log_{10}(\text{NO}_3) = f(\text{D, L, F, M, C, } \rho, \text{ all data descriptors})$	0.95	0.64	0.45	F, var1, var4, var8, var12	0.65	0.344

^[a] SEC (standard error of calibration) is the standard error of the estimate in the calibration data, in $\log_{10}(\text{NO}_3)$.

^[b] Table 3 defines the symbols used to represent the variables.

Table 9. Summary of most predictive models selected by stepwise multiple linear regression analysis of data descriptors calculated from ISFET nitrate response curves.

Data Descriptors Included in Calibration Data	Valid Factors ^[a]	R ²	SEC ^[b]	SEP ^[c]
$\log_{10}(\text{NO}_3) = f(\text{F, M, C, } \rho, \text{O, peak data descriptors})$ ^[d]	F, var3	0.779	0.286	0.365
$\log_{10}(\text{NO}_3) = f(\text{peak data descriptors})$	var3	0.696	0.327	0.416
$\log_{10}(\text{NO}_3) = f(\text{F, M, C, } \rho, \text{O, slope data descriptors})$	var8, var9	0.719	0.318	0.443
$\log_{10}(\text{NO}_3) = f(\text{slope data descriptors})$	var8, var9	0.719	0.318	0.443

^[a] Those variables significant at the 0.05 level in the best model.

^[b] SEC (standard error of calibration) is the standard error of the estimate in the calibration data, in $\log_{10}(\text{NO}_3)$.

^[c] SEP (standard error of prediction) is the standard error of the estimate in the validation data, in $\log_{10}(\text{NO}_3)$.

^[d] Table 3 defines the symbols used to represent the variables.

(table 8), for the largest core size (volume). The data descriptors, which are all derived from the initial portion of the ISFET response curve, are more indicative of the core nitrate concentration in the larger core.

Using the data subset for the largest core size, data descriptors calculated from the response data from ISFET2 and ISFET3 were averaged for each individual test. The responses of the two active ISFETs were similar, and using both estimates of nitrate-N concentration should improve prediction capability. The largest core size data subset was randomly divided, with 2/3 of the tests used in a calibration dataset and the remaining 1/3 of the tests in a prediction dataset. SMLR was used to identify the best statistically valid models for each of four different groups of variables (table 9). Permitting process variables and only data descriptors related to response curve peak to be considered for inclusion resulted in a two-variable model that included a design variable (extraction solution flowrate) and a single data descriptor variable – raw data peak (var3). The model had an R² of 0.779, an SEC of 0.286, and an SEP of 0.365. Limiting the variables to be considered to only the peak data descriptors resulted in raw data peak (var3) being the only statistically significant variable included in the model. The associated decrease in the coefficient of determination again underscores the importance of extraction solution flowrate to real-time nitrate-N prediction. The best statistically valid model based on process variables and slope data descriptors included only two data descriptor variables – regression slope – 2.0 s (var8) and raw data slope – 0.5 s (var9). The nitrate-N

predictive capability using the raw data peak descriptor alone was similar to that obtained using the two slope data descriptors (table 9), as indicated by similar coefficients of determination, SEC, and SEP values.

These results suggest that a data descriptor based on peak should be used for nitrate-N prediction whenever a peak in the extraction curve is obtained quickly. Predictions based on slope of the response curve can be used when a response peak is not obtained rapidly, although prediction will be less accurate. The plot of predicted vs. measured nitrate-N concentration (fig. 7) shows that the data are distributed on either side of the 1:1 line. A running average of samples along a transect might give useful predictions of soil nitrate concentration, if the time for collection and analysis of each sample can be held to 3 to 5 s.

CONCLUSIONS

- The nitrate extract collected from an intact soil core was indicative of the nitrate concentration in the soil core. The method of extraction and sample injection into the ISFET/FIA system appeared to be satisfactory.
- Nitrate-N level, soil type, core length, core diameter, and extraction pump flow rate were variables whose effects on the 12 data descriptors were statistically significant.
- The significance of soil type suggests that *a priori* knowledge of soil type might be necessary for the ISFET technology to make accurate real-time measurements of soil nitrate-N.

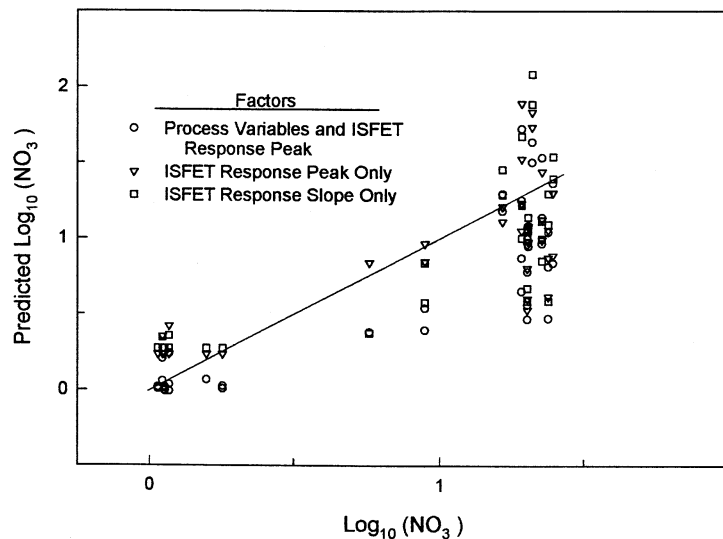


Figure 7. Predicted vs. measured nitrate-N concentration in four Illinois soils, with 1:1 line.

- The significance of soil core length and core diameter indicates that the values of these variables used in the design of a real-time sampler will be critical.
- The highly significant effect of extraction pump flow rate indicated that high-quality components would be needed in this portion of a real-time nitrate-N sensor to minimize flow rate variability.
- Stepwise multiple linear regression on the dataset representing the largest core volume showed that the nitrate-N response curve peak is the best basis for estimating nitrate-N concentration in the soil core, if a peak occurs quickly after injection. Nitrate-N response curve slope can provide a rapid estimate of nitrate-N concentration in the core, but the prediction will be less accurate.
- A real-time implementation of this sensing technology using both peak and slope data descriptors would ensure rapid estimates of nitrate-N concentration from each core extraction. The more accurate predictions based on response curve peaks could be supplemented with predictions based on response curve slope. Both prediction techniques sacrifice some accuracy to achieve rapid prediction, since the later portion of the response is ignored.

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REFERENCES

- Adsett, J. F., and G. C. Zoerb. 1991. Automated field monitoring of soil nitrate levels. In *Automated Agriculture for the 21st Century*, 326–335. ASAE Publication No. 1191. St. Joseph, Mich.: ASAE.
- Adsett, J. F., J. A. Thottan, and K. J. Sibley. 1999. Development of an automated on-the-go soil nitrate monitoring system. *Applied Eng. in Agric.* 15(4): 351–356.
- Anderson, C. D., L. V. Boone, D. W. Graffis, R. G. Hoefft, C. J. Kaiser, E. L. Knake, D. E. Kuhlman, M. D. McGlamery, E. D. Nafziger, T. R. Peak, G. E. Pepper, D. R. Pike, J. C. Siemens, and F. W. Simmons. 1992. Soil testing and fertility: Nitrogen. In *Illinois Agronomy Handbook 1993–1994*, 57–67. Circular 1321. Urbana, Ill.: University of Illinois at Urbana-Champaign, College of Agriculture Cooperative Extension Service.
- Bergveld, P. 1991. Future applications of ISFETs. *Sensors and Actuators B-4*: 125–133.
- Birrell, S. J. 1995. Multi-ISFET sensor system for soil analysis. Unpublished PhD diss. Urbana, Ill.: University of Illinois at Urbana-Champaign.
- Birrell, S. J., and J. W. Hummel. 2000. Membrane selection and ISFET configuration evaluation for soil nitrate sensing. *Trans. ASAE* 43(2): 197–206.
- _____. 2001. Real-time multi ISFET/FIA soil analysis system with automatic sample extraction. *Computers and Electronics in Agric.* 32(1): 45–67.
- Blackmer, A. M., D. Pottker, M. E. Cerrato, and J. Webb. 1989. Correlations between soil nitrate concentrations in late spring and corn yields in Iowa. *J. Prod. Agric.* 2(2): 103–109.
- Clements, C. R., M. J. Hopper, R. J. Canaway, and L. H. P. Jones. 1974. A system for measuring the uptake of ions by plants from flowing solutions of controlled composition. *J. Experimental Botany* 25(84): 81–99.
- Ehsani, M. R., S. K. Upadhyaya, D. Slaughter, S. Shafii, and M. Pelletier. 1999. A NIR technique for rapid determination of soil mineral nitrogen. *J. Precision Agric.* 1(2): 217–234.
- Ehsani, M. R., S. K. Upadhyaya, D. Slaughter, L. V. Portsailo, and W. R. Fawcett. 2000. Quantitative measurement of soil nitrate content using mid-infrared diffuse reflectance spectroscopy. ASAE Paper No. 001046. St. Joseph, Mich.: ASAE.
- Freese, B. 1988. Well aware. *Successful Farming* 86(14): 32–39.
- Fruhling, L. 1986. Please don't drink the water. *The Progressive* 50(10): 31–33.
- Hallberg, G. R. 1986. From hoes to herbicides: Agriculture and groundwater quality. *J. Soil and Water Cons.* 41(6): 357–364.
- Hansen, E. H., A. K. Ghose, and J. Ruzicka. 1977. Flow injection analysis of environmental samples for nitrate using an ion-selective electrode. *Analyst* 102: 705–713.
- Hubbard, R. K., L. E. Asmussen, and H. D. Allison. 1984. Shallow groundwater quality beneath an intensive multiple-cropping system using center pivot irrigation. *J. Environ. Qual.* 13(1): 156–161.
- Hubbard, R. K., R. A. Leonard, and A. W. Johnson. 1991. Nitrate transport on a sandy coastal plain soil underlain by plinthite. *Trans. ASAE* 34(3): 802–808.
- Kaiser, J. 2001. The other global pollutant: Nitrogen proves tough to curb. *Science* 294(5545): 1268–1269.
- Li, S., and K. A. Smith. 1984. The rapid determination of nitrate concentrations at low concentrations in soil extracts: Comparison of ion selective electrode with continuous-flow analysis. *Comm. Soil Sci. and Plant Analysis* 15(12): 1437–1451.
- Magdoff, F. R., D. Ross, and J. Amadon. 1984. A soil test for nitrogen availability to corn. *SSSA J.* 48(6): 1301–1304.
- McGrath, D. E., A. V. Skotnikov, and V. A. Bobrov. 1994. A site-specific expert system with supporting equipment for crop management. In *Site-Specific Management for Agricultural Systems*, 619–635. Madison, Wisc.: ASA-CSSA-SSSA.
- Mulvaney, R. L., and S. A. Khan. 1999. Use of diffusion to determine inorganic nitrogen in a complex organic matrix. *SSSA J.* 63(1): 240–246.
- Parker, L. K. 1996. Nitrate-nitrogen analysis of soils, manures, and saturation media extracts. Standard Operation Procedure ASL-10-003. Fort Wayne, Ind.: A & L Great Lakes Laboratories, Inc.
- Power, J. F., and J. S. Schepers. 1989. Nitrate contamination of groundwater in North America. *Agric. Ecosystems Environ.* 26(6): 165–187.
- Price, R. R. 2000. A real-time core extraction system for soil nitrates. Unpublished PhD diss. Urbana, Ill.: University of Illinois at Urbana-Champaign.
- Ruzicka, J., and E. H. Hansen. 1975. Flow injection analysis: Part I. A new concept of fast continuous flow analysis. *Anal. Chim. Acta* 78: 145–157.
- SAS. 1999. *SAS/STAT User's Guide*. Version 8 edition. Vols. 1, 2, and 3. Cary, N.C.: SAS Institute, Inc.
- Staver, K. W., and R. B. Brinsfield. 1990. Patterns of soil nitrate availability in corn production systems: Implications for reducing groundwater contamination. *J. Soil and Water Cons.* 45(2): 318–323.
- Thottan, J., J. F. Adsett, K. J. Sibley, and C. M. MacLeod. 1994. Laboratory evaluation of the ion selective electrode for use in an automated soil nitrate monitoring system. *Comm. Soil Sci. Plant Anal.* 25(17&18): 3025–3034.
- Tsukada, K., M. Sebata, Y. Miyahara, and H. Miyagi. 1989. Long-life multiple-ISFETs with polymeric gates. *Sensors and Actuators* 18: 329–336.
- Worner, C. R. 1989. Design and construction of a portable spectrophotometer for realtime analysis of soil reflectance properties. Unpublished MS thesis. Urbana, Ill.: University of Illinois at Urbana-Champaign.