Real-time multi ISFET/FIA soil analysis system with automatic sample extraction

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Abstract

Successful implementation of site-specific crop management relies on accurate quantification of spatial variation of important factors. Therefore, there is a tremendous need for the development of sensing technologies that will allow automated collection of soil, crop and pest data, to more accurately characterize within-field variability. The objective of this work was to develop an integrated multi-sensor soil analysis system. Ion-selective field effect transistor (ISFET) technology was coupled with flow injection analysis (FIA) to produce a real-time soil analysis system. Testing of the ISFET/ FIA system for soil analysis was carried out in two stages: (1) using manually extracted samples, and (2) the soil to be analysed was placed in the automated soil extraction system, and the extracted solution fed directly into the FIA system. The sensor was successful in measuring soil nitrates in manually extracted soil solutions ($r^2 > 0.9$). The rapid response of the system allowed a sample to be analysed in 1.25 s, which is satisfactory for real-time soil sensing. Precision and accuracy of the system were highly dependent on maintaining precise, repetitive injection times and maintaining constant flow parameters during the calibration and testing cycle. The progress toward an automated soil extraction system was notable, but considerable effort will be necessary before commercialization can be realized. However, the concept of using ISFETs for the real-time analysis of soil nitrates is sound. The rapid response and low sample volumes required by the multi-sensor ISFET/FIA system make it a viable candidate for use in real-time soil nutrient sensing. © Published by Elsevier Science B.V.

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1. Introduction

Site-specific management (SSM) is a management strategy which addresses within-field variability by optimizing inputs such as pesticides and fertilizers on a point-by-point basis within a field rather than applying them according to the field average. The full benefit of SSM will only be realized if the spatial variation of important production factors across the field is accurately determined. Accurate estimation of spatial variation will require the development of sensing technologies that will allow automated collection of soil, crop, and pest information, since data collection is required on a finer spatial resolution than is economically feasible with manual and/or laboratory methods. Real-time sensors can provide a sampling intensity several orders of magnitude greater than traditional methods, resulting in significant reductions in sampling errors.

Ion-selective field effect transistors (ISFETs) have inherent features such as small dimensions, low output impedance, high signal-to-noise ratio, low sample volume, and the potential for mass production, all of which are required for a real-time soil sensor. However, ISFETs have the disadvantages of greater long-term drift and hysteresis than ion-selective electrodes (ISEs). Although these disadvantages are potential problems in static measurements, the use of a dynamic measurement system such as flow injection analysis (FIA) minimizes or eliminates their effect. In fact, FIA and ISFETs are complementary since the small sample volume and rapid response of ISFETs allow the miniaturization of the FIA system, dramatically decreasing the sample dispersion and thereby increasing both sample resolution and sample frequency (Bergveld, 1991). This study involved the production and testing of nitrate ISFETs and the development of an FIA system using ISFETs as detectors. The ability to use a small sample volume and sense multiple species makes ISFET and FIA use an attractive technique for the development of a real-time soil nutrient sensing system.

Ruzicka and Hansen (1975) reported on the development of the FIA system, which is based on the injection of a sample slug into a carrier stream. An excellent overview of the principles and practice of FIA is provided by two Analytica Chimica Acta monographs (Ruzicka and Hansen, 1978, 1988). Much of the work on FIA involved the analysis of agricultural samples using several different sensing technologies including: spectrophotometric methods (Stewart and Ruzicka, 1976; Stewart et al., 1976; Hansen et al., 1977b; Anderson, 1979), atomic absorption and flame emission spectrometry (Reis et al., 1979; Zagatto et al., 1979a,b) and ion-selective electrodes (Hansen et al., 1977a; Ruzicka et al., 1977; Slanina et al., 1978, 1979, 1980; Alegret et al., 1984; Cardwell et al., 1988; Alegret et al., 1989).

A dramatic change in the miniaturization of ion-selective membrane technol-

ogy occurred when Bergveld reported on ion-sensitive field effect transistors (Bergveld, 1970, 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 surface of the field effect transistor. Subsequent research was reported on the development of ISFETs to measure different ionic species including calcium, potassium, sodium, and ammonium ions (Matsuo and Wise, 1974; Moss et al., 1975; Janata and Moss, 1976; Moss et al., 1978; McBride 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 long-term drift and hysteresis characteristics of ISFETs are significantly inferior to those of ion-selective electrodes (Janata and Huber, 1979; Cattrall and Hamilton, 1984; Miyahara and Simon, 1991). However, use of dynamic measurement systems alleviates the problems associated with long-term drift of ISFETs. Miniaturization of the FIA system, which is possible with ISFETs, has the potential to decrease dispersion which improves the detection limit, and to increase sample frequency (van der Schoot and Bergveld, 1985; Covington and Whalley, 1986; Alegret et al., 1989; Chandler et al., 1990). Haemmerli et al. (1982) used an FIA system to measure the chemical response time of ISFETs, and their system had a total response time of less than 500 ms. Significant advantages, as compared to ISEs, result from the use of ISFETs in FIA systems due to their ability to measure ion fluxes occurring within milliseconds, multiple ion sensor capability and improved signal-to-noise ratio with integral signal conditioning (Bergveld, 1991). Tsukada et al. (1989) reported on the development of long-life multi-ISFETs that might be appropriate for measurement of soil nitrate. Each multi-ISFET chip, 5×5 mm in dimension, included four independent ISFET sensors. These chips were used by Birrell and Hummel (2000) to show that the selectivity and sensitivity of the ion-selective nitrate membranes were within acceptable limits for soil nitrate analysis over the range of soil nitrate concentrations where varying levels of nitrogen fertilizer might be applied for maize production.

The overall objective of this research was to investigate the use of multi-IS-FET sensors in a flow injection analysis system for real-time analysis of soil extracts and to investigate the potential for developing a real-time automated soil extraction system. Results are presented on the effect of different calibration solution ranges and chloride ion interferences on the nitrate prediction capability of a multi-ISFET/FIA system and the potential of using the multi-ISFET sensors for multi-component analysis. An evaluation of the capability of the multi-ISFET/FIA system to predict the nitrate concentration of manually extracted samples representing a range of Illinois soil types is presented and discussed. A prototype automatic sample extraction apparatus is also discussed, but will require further research and development before accurate on-the-go soil nitrate analysis can be realized.

2. Equipment

2.1. ISFETs

The ISFET chips, which were obtained from Hitachi, Japan, had been bonded to the ceramic carrier and the polyamide layer already applied, with a sensor well (250 × 700 μm) over the gate area of each of the four ISFETs, and were ready for membrane application (Tsukada et al., 1989). A thin layer of an epoxy resin was carefully applied over the thin bonding wire and the bonding pads to protect the bonding wires. After the epoxy had cured, ion-selective nitrate membranes were applied on each ISFET sensor and the flow cell was attached to the ISFET chip, exposing the ISFET gate areas to the solution (Birrell and Hummel, 2000).

The ISFET chip's integrated circuits, as designed by Hitachi engineers, consisted of four ISFET sensors operating with constant drain current, source follower circuits, buffer amplifier circuits, analog switches, and a decoder to multiplex the outputs (Fig. 1). The outputs of the four ISFETs were multiplexed to permit sequential access to the four output signals on a single conductor. The multiplex connections were made after impedance conversion to avoid switching delay during multiplexing from one ISFET signal to another ISFET signal (Tsukada et al., 1989). The non multiplexed, amplified ISFET's outputs were available and used in this study instead of the multiplexed output, so that the output of all four ISFETs could be monitored simultaneously.

Since the source-follower circuits maintained a constant drain current through the FET, the output of the buffer amplifier followed the gate potential of the FET, which is the sum of the potential of the sample solution, the junction potentials, and the ion-selective membrane potential. If the total potential was below a certain threshold voltage, the buffer amplifier remained in negative saturation (ground potential); when this threshold potential was exceeded, the amplifier output had a linear response until the output was forced into positive saturation. During this
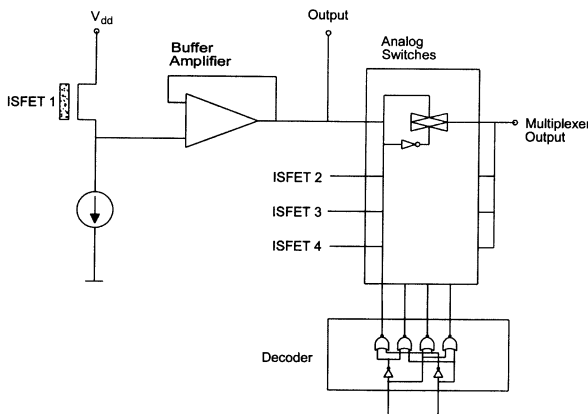

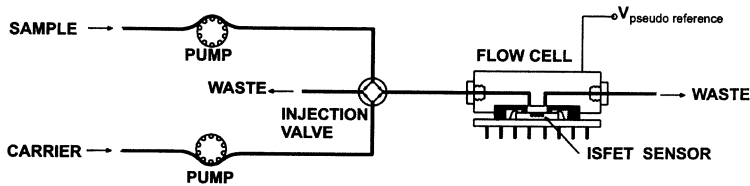Fig. 1. Circuit diagram of the integrated multi-ISFET chip.

Fig. 2. Schematic of flow injection analysis system and ISFET flow cell.

study, a pseudo reference was used to adjust the potential of the sample solution to maintain the amplifier within the linear response region. Although theoretical gains of the amplifiers were designed to be one, the actual gains of individual ISFETs were lower, and most exhibited a gain between 0.6 and 0.7 (Birrell and Hummel, 2000).

2.2. FIA

FIA is based on the injection of a liquid sample into a moving non-segmented, continuous carrier stream of a suitable liquid (Ruzicka and Hansen, 1978). The injected sample forms a zone in the carrier stream, which is then transported to a detector whose output continuously changes due to the passage of the sample through the flow cell. FIA is founded on a combination of three principles: (1) precise sample injection, (2) reproducible timing, and (3) controlled dispersion of the injected sample zone (Ruzicka and Hansen, 1978). The resolution and cycle time of an FIA system depend on the degree that the original sample solution is diluted as it travels to the detector and how much time has elapsed between a sample injection and detector readout. The dispersion coefficient D has been defined as the ratio of the concentration of the sample C° before dispersion, to the concentration of the sample C after dispersion (Ruzicka and Hansen, 1988). An FIA system for real-time soil nutrient sensing requires high resolution with minimum sample time. When direct detectors such as ISFETs are used, the FIA system should be designed with limited dispersion ($D \cong 1-2$) at detector maximum response, minimum residence time, and the smallest possible injection volume while maintaining adequate peak height and negligible carryover between sample injections.

The FIA system (Fig. 2) consisted of a variable-speed, multi-channel peristaltic pump to propel the carrier and sample solution to the two-position Valco model EF60 injection valve with a Valco Cheminert C1-417-2460 electric actuator controlled using an electronic control circuit to set duty cycle and cycle frequency. During the washout 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 an acrylic spacer attached to the top of the multi-ISFET chip and holder, which directed the flow stream over the ISFET ion-selective membranes, and over the $40 \times 25 \times 12.5$ mm stainless steel top half of

the cell, which was machined to match the acrylic spacer while minimizing 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.

2.3. Automated soil extraction system

A prototype automated, soil extraction system was developed for proof of concept testing under laboratory conditions (Fig. 3). The system utilized a slide mechanism and soil plunger to move a 'constant' volume of soil from the soil inlet into one of eight chambers in a rotating extraction cylinder (Birrell, 1995). Sequencing of the slide mechanism and soil plunger was controlled by using analog delay elements and digital logic to operate air valves (Atkomatic, model M28C-115v). The system's extraction cylinder rotated three times/s to move the soil sample from station to station. The sequences of events in the extraction cylinder were: the chamber was filled with soil at the first station, then rotated 45° to the second station where the extracting solution was sprayed into the chamber. After two more 45° increments, the initial chamber charged with soil was positioned above a filter disk with a small collection channel below the filter. The suction of the peristaltic pump transferred the filtered extract from the collection chamber to the FIA injection valve. After the fifth, sixth and seventh increments of the cylinder,
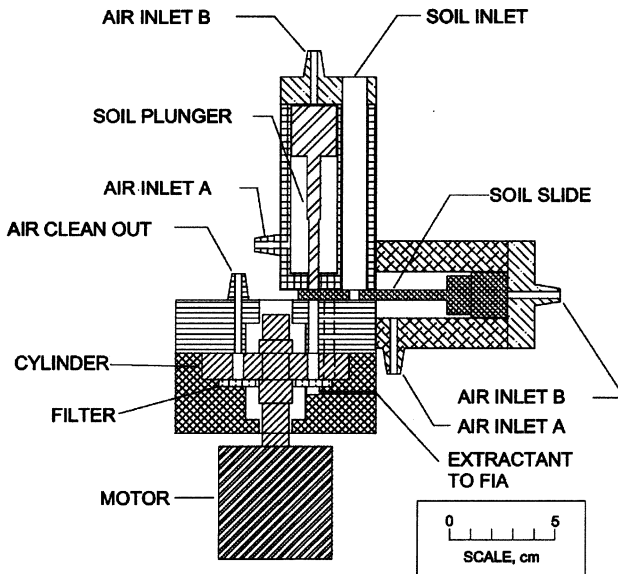

Fig. 3. Modified cross-section of automated extraction system.

the initial chamber was positioned below an air nozzle which forced the soil and liquid out of the chamber into a waste drainage channel below. The sequence of events was automatically controlled by a 486 personal computer using a servo motor controller card (Omnitech, model MC-3000) and permanent magnet DC servomotor (Matsushita, model GMX-6MP013A) with a rotary encoder.

3. Methods and procedure

3.1. Optimization of FIA parameters

The optimal flow rate, injection time, and washout time were investigated using the ISFET/FIA system to minimize the sampling period while maintaining a reasonable detection limit and without substantial loss of accuracy. The first set of tests provided a preliminary evaluation of the effects of FIA parameters, and the second set of tests was carried out for the parameters that seemed to be optimal.

During the preliminary evaluation, four flow rates (0.06, 0.12, 0.18, and 0.24 ml/s), three washout times (2, 1, and 0.75 s), and five injection times (2, 1, 0.75, 0.5, and 0.25 s) were used in a randomized block design. Due to operational constraints, these tests were blocked for a single combination of washout and injection time, with each combination tested in a random order. The FIA system was tested using five standard solutions, 0.2, 0.14, 0.10, 0.07, and 0.05 mM $NaNO_3$ in 0.01 M $CuSO_4$, respectively; spanning the nitrate concentration range which was considered significant when the pre-sidedress nitrate test is used (Blackmer et al., 1989).

The second set of tests used two injection flow rates (0.12 and 0.17 ml/s), two washout times (0.5 and 0.75 s), and one injection time (0.5 s). The different combinations of washout time, flow rate, and sample solution were then tested in a completely random order within each replication with a total of three replications.

During these tests, the responses of all four ISFETs were collected at 2 kHz, digitally filtered using a 50-point running average of the raw points followed by a fifth-order, low-pass digital Butterworth filter. The local maxima and minima of the smoothed data were obtained using a peak-finding algorithm similar to those used in gas chromatography data processing. For analysis, ten peaks were selected for each test by repeatedly discarding the first and then the last peak until only ten peaks remained. The SAS (GLM) procedure was used to obtain a linear regression of the peak height against the logarithm of the concentration for each combination of flow rate, injection time, and washout time. The slopes of the regressions, and the coefficients of correlation for the tests were then compared.

3.2. System calibration

The multi-ISFET/FIA system was calibrated before the analysis of manually extracted samples using two sets of standard solutions. The first set, which spanned a larger concentration range consisted of 10.0, 1.0, and 0.1 mM $NaNO_3$ in a 0.01 M $CuSO_4$ solution. The second set spanned a limited concentration range, with fifteen different standard solutions, five consisting only of nitrates (0.20, 0.14, 0.10,

0.07, and 0.05 mM $NaNO_3$ in 0.01 M $CuSO_4$ solution), five which included 0.1 mM NaCl in addition to the nitrates, and five with 0.2 mM NaCl added.

The peak heights for all four ISFETs were determined for each calibration solution as described previously. The non-linear regression procedure (NLIN) of the statistical program SAS was used to estimate the standard potential E_i^o, Nernst slope S, and the selectivity factor K (Nikolskii, 1937; Eisenman, 1967; Birrell and Hummel, 2000) used in the extended form of the Nernst equation for each ISFET. (The Nernst equation relates the potential across the membrane with the respective activities of the solutions on either side of the membrane. The Nikolskii–Eisenman (extended) form of the Nernst equation includes the effects of any interference ions that may be present in the soil solution.) The parameters were estimated using the following subsets of the original calibration tests: (1) all solutions with nitrate concentrations less than 1.0 mM; (2) all solutions with nitrate concentrations less than 1.0 mM and greater than 0.05 mM; (3) all solutions with nitrate concentrations less than 10.0 mM and greater than 0.05 mM; and (4) all solutions with nitrate concentrations greater than 0.05 mM, 5–8 (subsets 1–4), respectively, with solutions containing chlorides excluded. However, for subsets 5–8, the selectivity factor K was assumed to be zero, and it was excluded from the nonlinear model.

3.3. Analysis of manual soil extractions

Soil extracts from ten different types of soils representing a broad range of Illinois soils were tested in the multi-ISFET/FIA analysis system (Table 1). Since organic matter is a significant source of mineralizable nitrogen, the organic carbon content of the soils was determined using dry combustion of duplicate samples of each soil in an LECO model HF10 induction furnace. The textural classification and textural properties in terms of sand, silt, and clay content were determined in a previous study (Worner, 1989). Two different samples of each of the original soils were tested — an original air-dried sample, and a second sample that was leached, air-dried and stored separately from the original sample. All soils were screened using a 2 mm sieve before testing. The loamy sand and sandy loam soils were not included in the automated extraction tests since the large sand particles jammed the soil metering mechanism, making the automated extraction procedure inoperable.

A manual extract of each soil was obtained by placing 20 ml of soil into an Erlenmeyer flask, adding 200 ml 0.01 M $CuSO_4$ extracting solution and shaking by hand for 2 min. The mixture was then filtered through Whatman No. 42 filter paper to obtain the clear soil extract. Sub-samples of the soil extracts were then analysed using a Lachat flow injection system using the cadmium reduction method for comparison with the ISFET predicted extract concentration.

Immediately after the system had been calibrated using the standard solutions, sub-samples of the 20 soil extracts were analysed in a random order using the multi-ISFET/FIA with a 0.75 s washout time and a 2.5 s washout time, with three replications for each set of soil samples. The Nernst slope and standard potential determined during the calibration analysis were used to estimate the nitrate concentration for individual peaks for each soil sample.

Table 1
Organic carbon and textural properties of 14 Illinois surface soils used in the study

Textural class[a]/soil name	ID	Organic carbon	Textural properties		
			Sand (%)	Silt (%)	Clay (%)
Loamy sand					
Ade	1	0.34	86.5	7.3	6.2
Plainfield	2	1.24	83.7	12.7	3.6
Sparta	3	0.46	85.4	10.4	4.2
Sandy loam					
Carmi	5	1.10	67.2	21.7	11.1
Clay loam					
Proctor	8	2.23	25.6	47.1	27.3
Silt loam					
Birkbeck	12	1.43	5.4	77.5	17.1
Saybrook	16	2.95	12.7	62.8	24.5
Catlin	17	2.21	5.2	70.6	24.2
Saybrook	18	1.83	4.8	72.3	26.9
Piopolis	20	2.77	4.1	68.8	27.1
Silty clay loam					
Drummer	24	1.42	9.0	63.4	27.6
Drummer	26	1.53	8.7	61.0	30.3
Flanagan	28	1.90	6.2	66.4	27.4
Drummer	29	2.24	12.6	55.9	31.5

[a] Textural classification and properties from Worner (1989).

The SAS linear regression (REG) procedure was used to determine the regression between the ISFET predicted concentration and the actual concentration for each sensor within each replication. During analysis, any soil extract with an actual nitrate concentration greater than 0.5 mM was excluded since this level was outside the range of the calibration solutions and because approximately 0.2 mM (30 μg/g soil N–NO$_3$) is an upper limit, beyond which additional nitrogen fertilizer is usually not recommended.

3.4. Soil analysis using automated soil extractions

Prior to conducting the automatically extracted soil solution tests, the soil delivery system was calibrated. The bottom section and rotating cylinder were removed from the automated soil extraction system. A small beaker was then positioned under the soil plunger. The soil delivery system was operated for ten cycles and the mass of soil collected in the beaker measured. Each soil was tested ten times to estimate the mean and standard deviation of the soil metered by the soil delivery system. The average mass of soil delivered was approximately 0.04 g per cycle, which would be equivalent to 0.12 g/s. The measurements showed a reasonable amount of variability within each soil and between different soils.

Generally, the mass for each test was within 20% of the mean for that particular soil. Assuming a bulk density of 1.4, the amount of extraction solution required (1:10 ratio by volume) would be 1.8 ml/s. The actual flow rate from the peristaltic pump was 1.67 ml/s. Therefore, the theoretical soil to extractant ratio was slightly less than 1:10.

Manual extractions of the soils were obtained as previously described. Sub-samples of the manually extracted solutions were analysed using the Lachat FIA system to determine the actual concentration in the soil extract. Immediately after calibration using the standard solutions, different soils were analysed using the automated extraction system connected to the ISFET/FIA system.

Prior to testing for each soil, the automated extraction system was cleaned, reassembled, and connected to the FIA system. Then the test of the first automatically extracted soil solution was conducted using a 0.75 s washout time followed by a test using a 2.50 s washout time. If the automated soil extraction system malfunctioned during a test, it was disassembled, cleaned, reassembled, and the test was repeated. The whole testing sequence was then repeated for each soil tested.

After the response peak heights were determined for each test, the Nernst slope and standard potential determined by the calibration analysis were used to estimate the soil's nitrate concentration from individual response peaks.

4. Results and discussion

4.1. Optimization of FIA parameters

The optimal flow injection parameters were identical for each ISFET assuming ideal mixing in the ISFET flow cell. In theory, the actual concentration profile associated with ISFET will be different due to geometry and non-ideal mixing in the flow cell. However, the actual effects of the differences on the optimal flow injection parameters were insignificant, and the response of only one ISFET sensor will be discussed.

The responses of the ISFET in the FIA system to changes in injection time (0.25, 0.5, 1.0, and 2.0 s) with a 2.0 s washout time are shown in Fig. 4 for four different flow rates (0.06, 0.12, 0.18, and 0.24 ml/s) for one nitrate standard solution (0.2 mM). The rising edges of the ISFET response curves coincide for all curves at a particular flow rate, as the theory predicts, with the peak height and peak width at the baseline being affected by the injection time. A decrease in injection time increased the dispersion factor which resulted in a lower peak response, provided the injection time was shorter than that required for a dispersion factor of one. The ISFET response curves when the injection time was 0.5 s were different from the other curves in that a higher than expected response maximum peak height was exhibited. Complete washout of the flowcell, as indicated by a zero gradient of the ISFET response prior to injection of the next sample, occurred for all except the lowest flow rate (0.06 ml/s).

At the highest flow rate (0.24 ml/s), the response peak height was not substantially decreased by a decrease in injection time; therefore, the dispersion factor at peak maxima was approximately one, and the concentration of the solution in the detector flowcell at peak maxima was the same as the original sample concentration for all injection times. At the two intermediate flow rates (0.12 and 0.18 ml/s), response peak heights for the 0.25 s injection time were lower than for the longer injection times, showing that the maximum dispersion factor was decreasing and less than one under these conditions, although the response peak heights were only reduced by 25% of the maximum. These response curves demonstrate that, even with an injection time of only 0.5 s, the response is satisfactory, provided that the flow rate is high enough for adequate washout of the flowcell. An increase in injection time beyond 0.5 s does not significantly affect the peak height but does increase the peak width at the baseline which increases the minimum washout time required. An increase of flow rate from 0.12 to 0.24 ml/s did not significantly increase the peak height but did decrease the peak width.

Plots of a linear regression of the response peak height against the logarithm of the concentration, with different flow rates (0.06, 0.12, 0.18, and 0.24 ml/s) and injection times (0.25, 0.5, 1.0, and 2.0 s) with 2.0 s washout (Fig. 5) show that a 2.0 s washout time is sufficient for complete washout in most cases. At the lowest flow rate (0.06 ml/s), the slopes of the regression lines of peak height versus concentra-
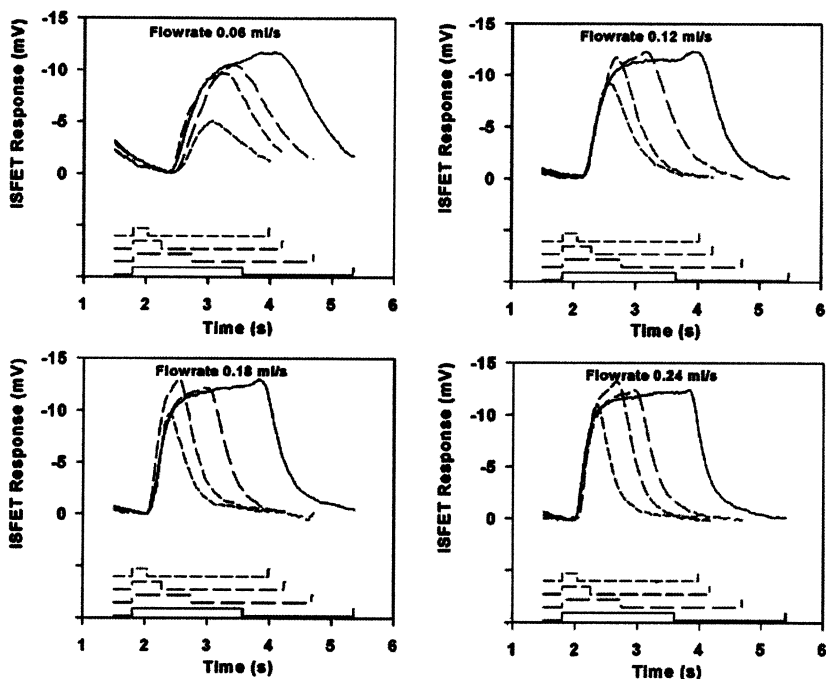

Fig. 4. ISFET response for four injection times (0.25, 0.5, 1.0, and 2.0 s) with a 2.0 s washout time, for four flow rates (0.06, 0.12, 0.18, and 0.24 ml/s) using 0.2 mM nitrate sample.
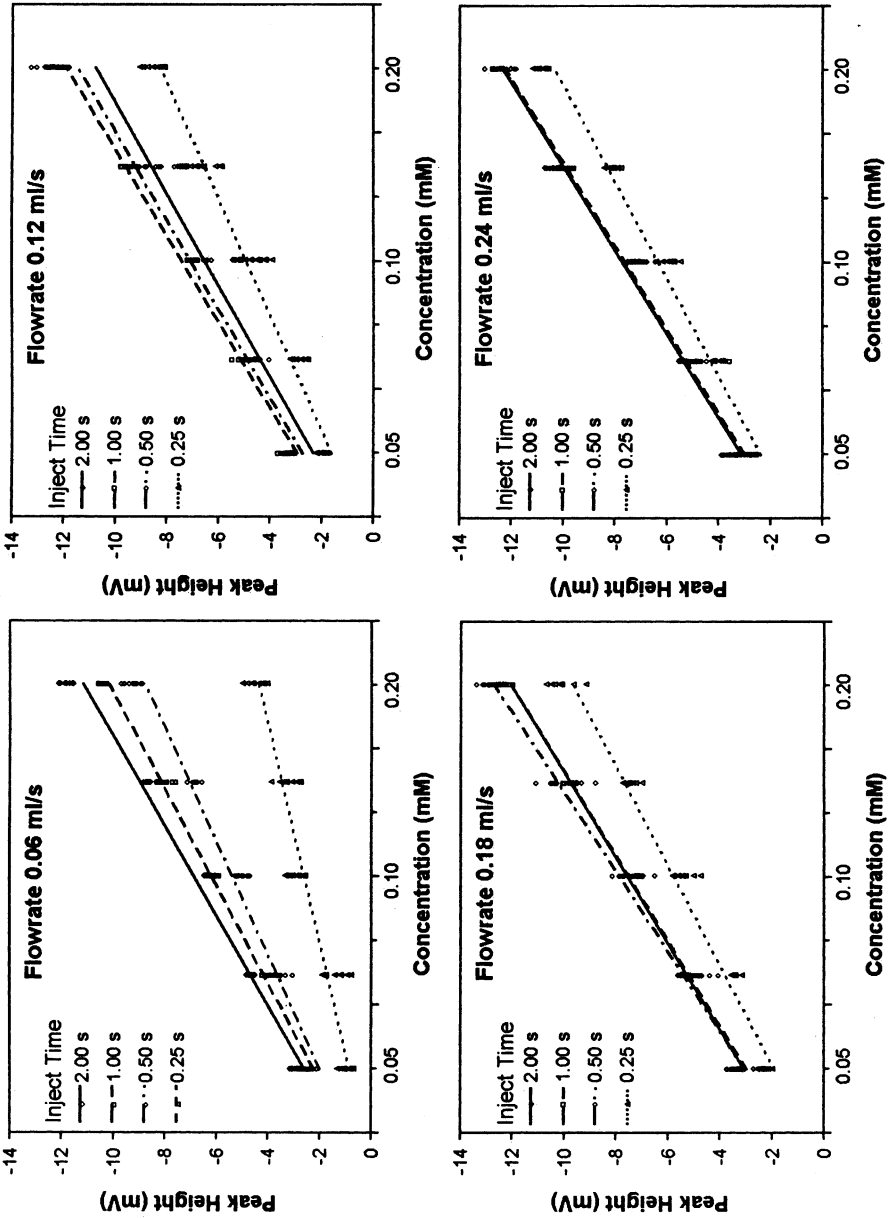
Fig. 5. Signal peak height vs. concentration of nitrate for different flowrates (0.06, 0.12, 0.18, and 0.24 ml/s) and injection times (0.25, 0.5, 1.0, and 2.0 s) with 2.0 s washout time.

tion were significantly different for all injection times, and the dispersion factor was greater than 1 in all cases except the 2.0 s injection time. As the flow rate increased the regression lines for the three longest injection times become increasingly similar and for the highest flow rate, the regression lines for the three longest injection times were identical. The washout time of 2 s was sufficient to ensure complete washout, except possibly at the lowest flow rate. However, even at this flow rate, dilution of the sample slug was the dominant effect and the effects of carryover were not apparent. Results of additional tests with washout times of 1 and 0.75 s (data not shown) differed little from those with 2-s washout times, except for slight increases in the slopes of the regression lines with increasing flow rate and injection volume.

To minimize cycle time, further investigations concentrated on the response of the system at two flow rates (0.12 and 0.18 ml/s), with 0.75 s and 1.0 s washout times and a 0.5 s injection time. Injection times of less than 0.5 s were not evaluated since the lower limit of the valve actuator cycle time would be approached and a small change in injection time would have caused unreliable operation of the valve, introducing significant errors.

The individual peak heights and the linear regressions of the peak height against the logarithm of the sample solution concentration (Fig. 6) for the different combinations of flow rate and washout times show that the regression lines for replications two and three are very similar, and in one case (flow rate 0.17 ml/s, washout time 1.0 s) cannot be distinguished from each other. However, the regression line for replication one clearly has a different slope and intercept. The reason for this difference is not certain, but the most likely cause is a change in the flow parameters during the test.

The washout time and flow rate did not affect the sensitivity and resolution of the FIA system except when the washout time was 0.75 s and the flow rate was 0.12 ml/s. The decrease in sensitivity was a result of incomplete washout of the previous sample from the detector flowcell before the next sample arrived. Increasing either the flow rate or the washout time alleviated this problem. The overall response of the ISFET/FIA system was determined by the volume of the injected sample and the washout volume between samples. Increasing the volume of sample injection decreased the dispersion factor, while increasing the washout volume decreased the likelihood of carryover between samples. These tests confirmed the results from the preliminary tests that an injection time and washout time of 0.5 and 0.75 s, respectively, were possible, provided that the flow rate was 0.17 ml/s or greater.

4.2. System calibration

Nernst slope S, standard potential E, and selectivity factor K were estimated using the SAS non-linear regression (NLIN) procedure (Tables 2 and 3). The mean response peak heights for ISFET 1 were greater than those for ISFETs 2, 3, and 4, particularly for the second set of calibration solutions (0.05–0.2 mM $NaNO_3$) which spanned the lower concentration range, resulting in a higher sensitivity for ISFET 1. In general, at the lower concentrations, the response peak height for
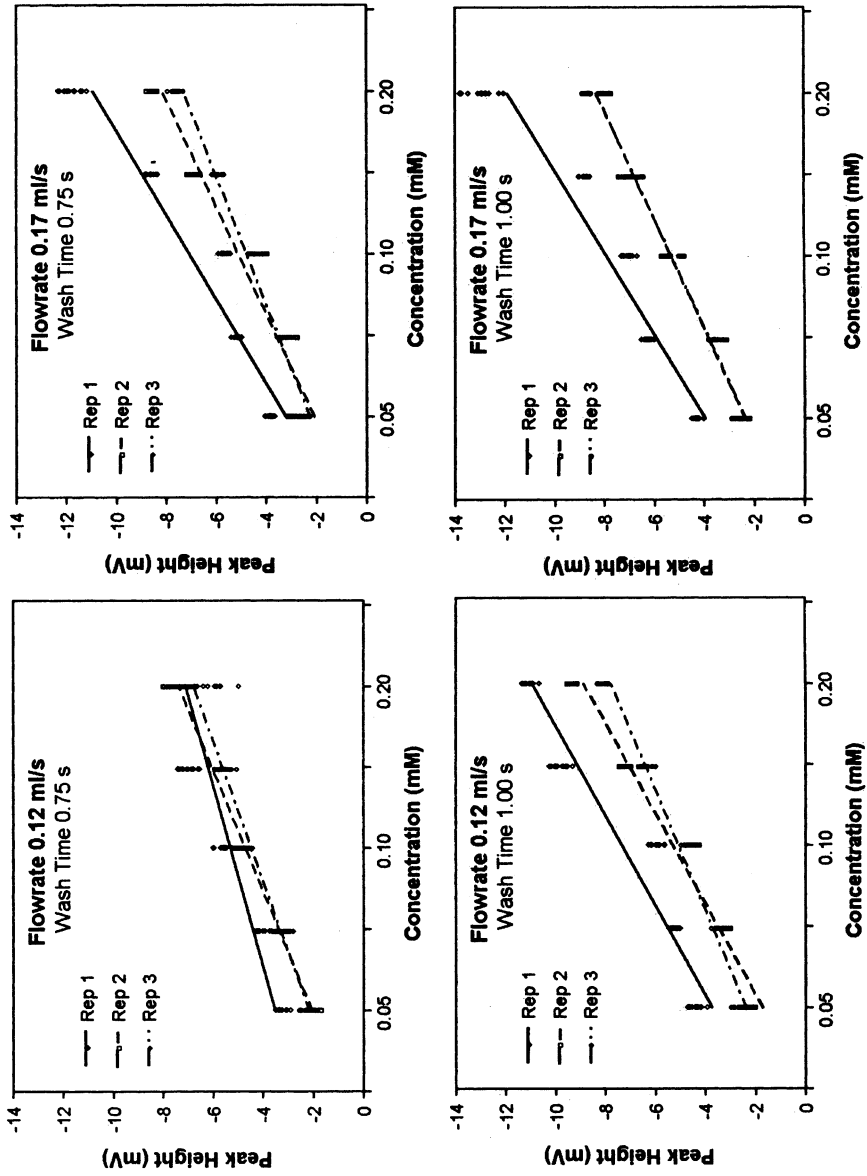
Fig. 6. Signal peak height *vs.* concentration of nitrate for two flowrates (0.12, and 0.17 ml/s) and washout times (0.75 and 1.0 s) with 0.5 s injection time.

Table 2
Estimate of Nernst slope, standard potential, and selectivity factors with a FIA washout time of 0.75 s

	Estimate of Nernst parameters				
	Nernst slope (mV/decade)		Standard potential (mV)		Selectivity factor
	All[a]	Nitrates[b]	All[a]	Nitrates[b]	All[a]
Concentration range 0.05–0.20 mM					
ISFET1	−22.8	−22.5	−104	−103	0.027*
ISFBT2	−12.0	−12.3	−54	−56	0.004
ISFBT3	−12.6	−12.8	−58	−58	0.004
ISFBT4	−14.9	−14.2	−66	−64	0.038*
Concentration range 0.07–0.20 mM					
ISFET1	−24.4	−25.0	−111	−113	0.031*
ISFET2	−11.6	−13.2	−52	−59	0.000
ISFET3	−11.8	−13.1	−54	−59	0.000
ISFET4	−17.5	−15.8	−77	−70	0.003
Concentration range 0.07–1.00 mM					
ISFET1	−26.6	−27.1	−119	−121	0.035*
ISFET2	−23.9	−25.8	−101	−109	0.032
ISFET3	−24.1	−26.0	−102	−111	0.035*
ISFET4	−21.3	−21.9	−92	−94	0.017
Concentration range 0.07–10.0 mM					
ISFET1	−25.2	−25.2	−114	−114	0.025*
ISFET2	−26.6	−26.8	−112	−113	0.050*
ISFET3	−25.1	−25.2	−106	−108	0.042*
ISFET4	−20.7	−20.7	−89	−90	0.011

[a] Estimation of Nernst parameters with all chloride/nitrate solutions included.
[b] Estimation of Nernst parameters with all solutions containing chlorides excluded.
* Selectivity factors are significantly different from zero at the 5% level.

ISFET 1 was approximately 30% higher than the corresponding peak heights for the other ISFETs. At the lowest concentrations, all of the ISFETs began to display a nonlinear response. However, the linear detection limit for ISFET 1 was much lower than for the other three ISFETs.

The estimated regression slopes for ISFET 1 (Table 2) were relatively constant (−22.8, −24.4, −26.6, and −25.2 mV/decade for the concentration ranges 0.05–0.2, 0.07–0.2, 0.07–1.0, and 0.07–10.0 mM $NaNO_3$, respectively, with data from all of the chloride solutions included and while using a 0.75 s washout time). Due to the gain of the ISFET amplifier (0.69), an estimated Nernst slope of −40 mV/decade would reflect the ideal theoretical Nernst response. The estimated Nernst slopes for ISFETs 2, 3, and 4 all showed a dramatic increase as higher nitrate concentrations were included in the calculations (Table 2). The greater differences in the regression slope for ISFETs 2, 3 and 4 as compared to the regression slope for ISFET 1 as the range of solution concentration increased

reflects the decreased linear response range of these ISFETs. The effect of the concentration range on the calculated regression slope showed similar trends for the 2.5 s washout time (Table 3). The difference in the linear detection limit among the ISFETs was most likely due to differences in adhesion of membranes on each sensor.

The calculated selectivity factors, K, were in the range 0.01–0.04, 0.00–0.05, 0.00–0.04, and 0.00–0.04 for ISFETs 1, 2, 3, and 4, respectively (Tables 2 and 3). However, the asymptotic confidence interval ($\alpha = 0.05$) for ISFETs 2, 3, and 4 included zero, in most cases, and the null hypothesis ($K = 0$) could not be rejected. ISFET 1, with a 0.75 s washout time (Table 2), was the only time that chloride interference was generally significant at the 5% level.

The inclusion of the calibration solutions with chloride ions present did not significantly affect the estimation of the slope and standard potential, except for one case for ISFET 2 with a 2.5 s washout time ($\alpha = 0.05$) (Table 3). The maximum concentration included in the calibration range was highly significant in all cases

Table 3
Estimate of Nernst slope, standard potential, and selectivity factors with a FIA washout time of 2.5 s

	Estimate of Nernst parameters				
	Nernst slope (mV/decade)		Standard potential (mV)		Selectivity factor
	All[a]	Nitrates[b]	All[a]	Nitrates[b]	All[a]
Concentration range 0.05–0.20 mM					
ISFET1	−26.6	−26.5	−122	−121	0.012
ISFET2	−14.1	−17.1	−65	−78	0.000
ISFET3	−13.3	−18.7	−59	−84	0.000
ISFET4	−18.2	−18.8	−82	−84	0.012
Concentration range 0.07–0.20 mM					
ISFET1	−28.7	−29.0	−130	−130	0.012
ISFET2	−14.5	−18.6	−66	−83	0.000
ISFET3	−13.4	−20.7	−59	−92	0.000
ISFET4	−20.3	−21.6	−90	−95	0.007
Concentration range 0.07–1.00 mM					
ISFET1	−33.1	−33.7	−147	−150	0.020*
ISFET2	−28.3	−29.8	−121	−128	0.025
ISFET3	−30.6	−32.1	−131	−137	0.003
ISFET4	−27.7	−28.8	−119	−123	0.027
Concentration range 0.07–10.0 mM					
ISFET1	−36.1	−36.2	−158	−159	0.037*
ISFET2	−34.5	−34.7	−145	−147	0.042*
ISFET3	−34.7	−35.0	−147	−148	0.028
ISFET4	−30.4	−30.5	−129	−130	0.045

[a] Estimation of Nernst parameters with all chloride/nitrate solutions included.
[b] Estimation of Nernst parameters with all solutions containing chlorides excluded.
* Selectivity factors are significantly different from zero at the 5% level.

($\alpha = 0.01$) except for ISFET 1 with a 0.75 s washout time (Table 2), while the minimum concentration was not significant except for ISFET 1 with a 2.5 s washout time ($\alpha = 0.05$) (Table 3). The nonlinear response of ISFETs 2, 3, and 4 at the lower concentration range, and the correlation between the estimate of the slope and standard potential makes the selection of the optimum range of calibration concentrations critical. If all solutions are included, the estimation of the slope is mathematically robust; however, the response at lower concentrations will be overestimated, which would result in underestimating the predicted concentration. However, if the higher concentration solutions are excluded, the correlation between the estimation of the slope and standard potential makes the calibration sensitive to the mathematical methods used and to any outliers that may occur.

Multi-component analysis, as described by Otto and Thomas (1986), and a factorial design method described by Virtanen (1980), could not be successfully applied to these data. The use of four sensors, all optimized for nitrate, resulted in four co-linear equations, rather than four independent equations. This result is not surprising, but does offer some reassurance as to the veracity of the ISFET response to the nitrate ion.

4.3. Analysis of manual soil extractions

The multi-sensor ISFET/FIA system was calibrated before the analysis of manually extracted samples using standard solutions. The plots of predicted concentration versus actual concentration show obvious differences in the response between the first replication and last two replications with a 0.75 s washout time (Fig. 7). This response difference among replications could be a result of changes of the ISFET membrane and junction potential. However, the more likely cause was a change in FIA parameters such as flow rate or the injection valve operation. The difference in sensor response between replications was not obvious for the 2.5 s washout time (Fig. 7), which suggests that the injection valve was the probable cause.

The successful prediction of manual soil extractions depended largely on the correct estimation of the sensitivity response of the ISFET using the calibration solutions. The ISFET-predicted concentrations of manually extracted soil solutions were compared to the actual soil concentrations determined using the cadmium reduction method (Lachat analyser) on the identical solutions (Fig. 7). The slope of the regression line of predicted versus actual concentration was approximately one ($r^2 > 0.9$) for all four ISFETs (Birrell, 1995) provided the ISFETs were calibrated correctly before testing.

4.4. Soil analysis using automated soil extractions

The response peak heights for the data collected from the automatically extracted soil solutions were highly variable (Fig. 8). The recording of data began before the soil metering device was started. At this time the 'samples' being analysed were the pure extraction solutions and the peak heights would be zero. After the metering
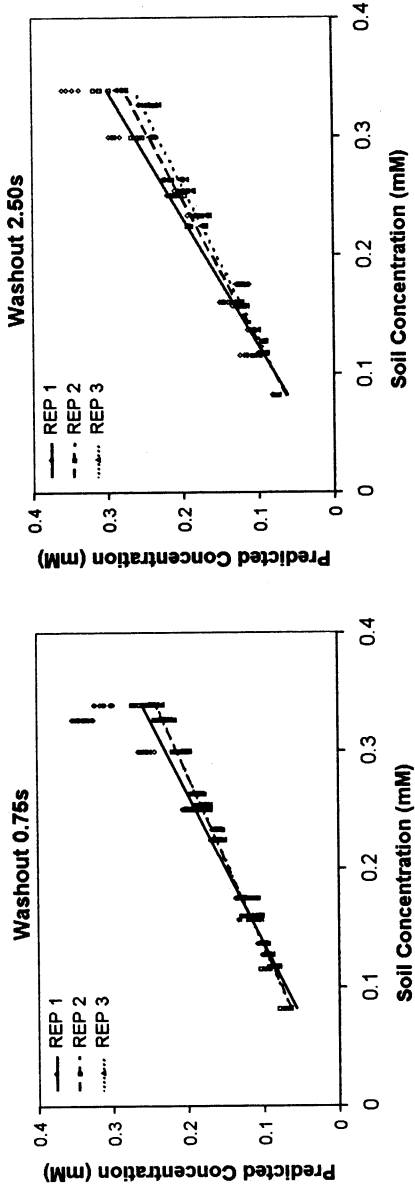
Fig. 7. Predicted *vs.* actual soil nitrate concentration for manually extracted solutions with 0.75 s (left) and 2.5 s (right) washout time.
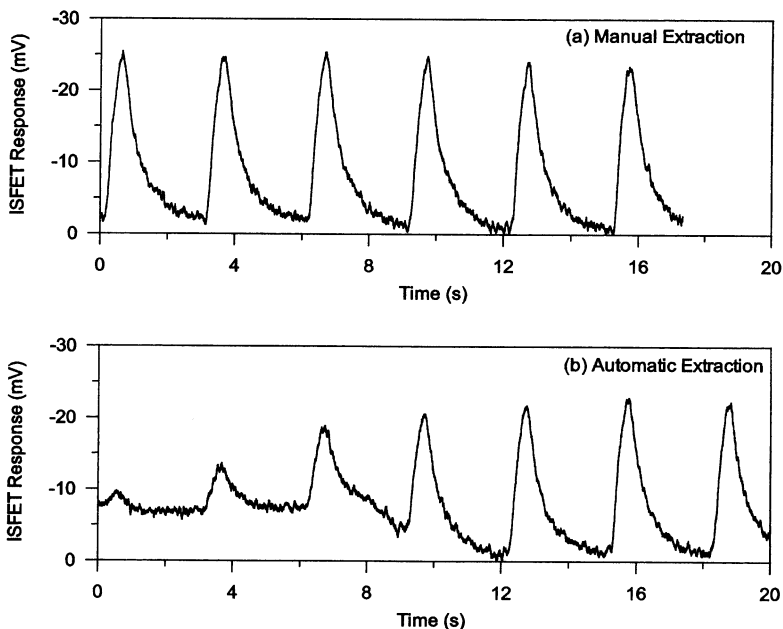
Fig. 8. ISFET response to (a) injection of manually extracted soil solution, and (b) injection of soil solution from the automated soil extraction system.

device was started, the first few injected samples consisting of a mixture of soil extract and the pure extractant solution were analysed. If the automated extraction proceeded successfully, with time, the peaks increased in magnitude as the sample being analysed increasingly consisted of the soil extract only (Fig. 8b). At the end of the data stream for each sample, when the automated soil extraction system began to clog or run out of soil, the response peaks became very erratic. The data were visually inspected, and response peaks at the beginning of the test that resulted from mixed samples and those at the end of the test during the erratic period were discarded. While this method relied on subjective judgment as to when the system was operating correctly, an effort was made to minimize any subjective bias in the acceptance or rejection of response peaks.

The ISFET-predicted concentrations of automatically extracted soil solutions were compared to corresponding manually extracted soil solution concentrations from the same soils (Fig. 9). The slope of the regression line for predicted versus actual concentrations was considerably less than one for all ISFETs (Birrell, 1995). Although the regression slopes were far from ideal, the predicted concentrations were grouped around their respective regression lines. However, the predicted concentration showed much greater variability for the automated analysis than those for the manual extractions.

The automated soil extraction system was unreliable, and at times the sample flow from the automated extraction collection chamber was intermittent, and
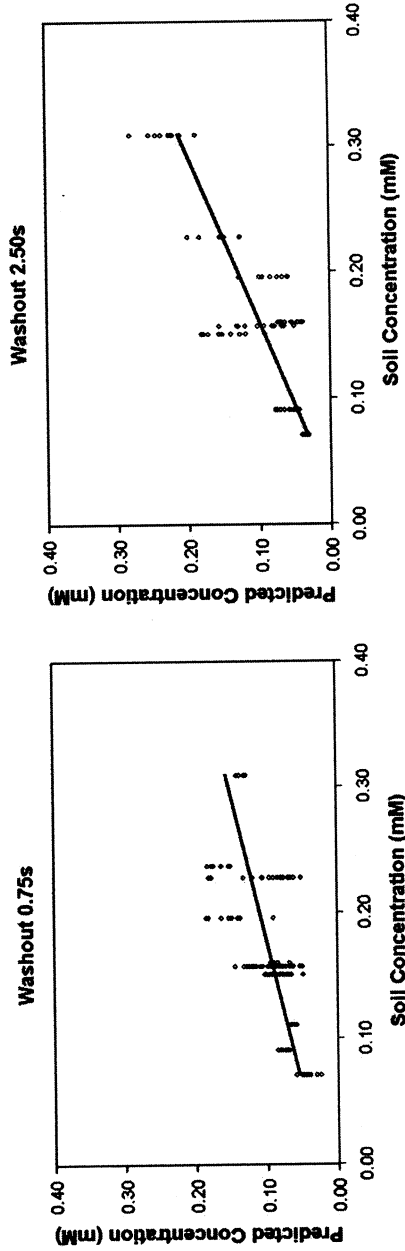
Fig. 9. ISFET/FIA predicted concentration for automatically extracted samples vs. actual soil nitrate concentration from manually extracted solutions, with 0.75 s (left) and 2.5 s (right) washout time.

clogging of the filter reduced the flow rate through the peristaltic pump. The gross underestimation of nitrate levels could be a result of incomplete extraction of the nitrate from the soil and inconsistent flow of the sample stream. The two most likely reasons for the poor recovery using the automated procedure were the variation in the volume of soil metered out for each cycle and the change in sample flow to the injection valve which translates into a decrease in the volume of the sample injected. Although the automated extraction system was not successful at predicting soil nitrate levels, the results showed some promise and warrant further development.

5. Conclusions

The multi-ISFET sensor that was developed and tested for sensing soil nitrate must be operated in the non-linear response range of ion selective membranes. Consequently, the selection of the range of calibration solution concentrations is critical. When appropriate calibration solutions were used, the sensor successfully measured soil nitrate levels in manually extracted soil solutions ($r^2 > 0.9$). The multi-ISFET/FIA system can accurately measure soil solution nitrate levels in real time (ca. 1.25 s). The automated soil solution extraction system, however, was not particularly successful, and considerable additional design and development input will be needed before a real-time continuously-operational sensor can be commercialized. Recent advances in microelectromechanical systems (MEMS) have shown that mechanical devices as well as electronics can be incorporated through microfabrication to produce fully integrated microsystems. This technology can result in batch fabrication of a sensor having all electronic and mechanical components on a single silicon chip, with a great potential for cost reduction, faster response times, and even lower sample volumes than were required by the multi-ISFET/FIA system reported here.

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