VALIDATION TESTING OF A SOIL MACRONUTRIENT SENSING SYSTEM

H. J. Kim, K. A. Sudduth, J. W. Hummel, S. T. Drummond

ABSTRACT. *Rapid on-site measurements of soil macronutrients, i.e., nitrogen (N), phosphorus (P), and potassium (K), are needed for site-specific crop management, where fertilizer nutrient application rates are adjusted spatially based on local requirements. This study reports on validation testing of a previously developed ion-selective electrode (ISE) based soil macronutrient sensing system using 36 soil samples from a single site, the Northern Illinois Agronomy Research Center (NIARC), and previously developed calibration models. Objectives were to (1) validate calibration models with a new array of membranes and electrodes, and (2) evaluate the ability of the system to estimate variations in soil NO_3-N, P, and K within a single test site. Soil extract samples were obtained using the Kelowna extractant. Electrode responses were measured with five ISEs for each of NO_3-N, P, and K and were normalized using the baseline correction and two-point normalization methods developed in our previous work. The array of ISEs fabricated with new membranes and cobalt rod, in conjunction with the previously developed normalization methods and calibration models, accurately estimated NO_3-N, P, and K in solution without need to recalibrate the ISE system through standard laboratory analysis of soil samples from the new test site. ISE-measured NO_3-N, P, and K concentrations in Kelowna-based soil extracts were similar to those determined by standard instruments, validating the ability of the system to identify within-field macronutrient differences. The use of a calibration factor to adjust ISE measurements for the difference in extraction efficiency between Kelowna and standard extractants resulted in a slope near unity between soil NO_3-N, P, and K concentrations determined by ISEs and standard methods. However, a relatively large offset in soil P concentration between calibrated ISEs and standard methods will require further investigation to identify the cause. This study showed that it was possible to transfer existing calibration equations to new membranes and electrodes through application of the baseline correction and two-point normalization methods and an adjustment for differences in extraction efficiency. This finding enhances the applicability of the ISE-based soil macronutrient sensing system and methodology for rapid soil analysis.*

Keywords. *Calibration transfer, Ion-selective electrodes, Kelowna extractant, Soil nutrients, Two-point normalization, Validation.*

The soil macronutrients nitrogen (N), phosphorus (P), and potassium (K) are essential elements for crop growth. These nutrients in the soil solution are taken into plants in various ionic forms, such as nitrate (NO_3^-), orthophosphates ($H_2PO_4^-$ or HPO_4^{2-}), and potassium (K^+), through a combination of root interception, mass flow, and diffusion processes (Havlin et al., 1999).

The application of commercial N, P, and K fertilizers has contributed to a tremendous increase in yields of agricultural crops that feed the world's population. However, excessive use of these fertilizers has been cited as a source of contamination of surface and groundwater. Ideally, application rates should be adjusted based on estimates of the requirements for optimum production at each location because there is high spatial variability of N, P, and K within individual agricultural fields (Page et al., 2005).

Monitoring nutrient levels in soils is necessary to efficiently use fertilizers and minimize the environmental impact of fertilization practices. However, conventional soil testing methods, which combine soil sampling in the field and chemical analysis in the laboratory, are costly and time consuming, thereby limiting the number of samples analyzed in the field and making it difficult to characterize spatial variability in soil nutrient concentrations within fields (Artigas et al., 2001; Schepers and Schlemmer, 1998). In this situation, on-the-go real-time sensors could allow the collection of geographically referenced data on a much finer spatial resolution than is currently feasible with manual and/or laboratory methods (Schirrmann et al., 2011; Sibley et al., 2009). These automated sensor measurements can provide increased density of measurements at a relatively low cost (Adamchuk et al., 2004).

The need for such on-the-go in-field monitoring has led to the investigation of ion-selective electrode (ISE) and ion-selective field effect transistor (ISFET) technology for determining soil chemical properties. Among the advantages of ISE and ISFET technology over current analytical methods (e.g., spectroscopic techniques) are simplicity of use, direct measurement of the analyte, sensitivity over a

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wide concentration range, low cost, and portability (Carey and Riggan, 1994).

The ISEs and ISFETs require recognition elements, i.e., ion-selective membranes, which are integrated with a reference electrode and enable the chemical response (ion concentration) to be converted into a signal (electric potential). Due to an increased demand for measurement of new ions, and advances in MEMS (micro-electro-mechanical systems) technology, significant progress has been made recently in the development of various ion-selective membranes for analytical chemistry. Ion-selective membranes are currently available for most of the important soil nutrients, including NO_3^-, K^+, Na^+, and Ca^{2+} (Bedlechowicz-Sliwakowska et al., 2006; Gallardo et al., 2004; Knoll et al., 1994; Levitchev et al., 1998; Nielson and Hansen, 1976; Tsukada et al., 1989). Furthermore, due to the importance of phosphorus in biological systems and living organisms, many researchers have tried to develop phosphate ion-selective electrodes (Carey and Riggan, 1994; Chen et al., 1997; Glazier and Arnold, 1991; Wroblewski et al., 2000; Xiao et al., 1995).

Several researchers have investigated the use of ISFETs and ISEs in soil analysis with the overall goal of developing an on-the-go soil macronutrient sensing system. Thottan et al. (1994) reported on a nitrate ISE that could estimate actual nitrate level with 95% accuracy after 6 s of measurement. A complete system was developed, including the ISE, an on-the-go sampler and a deionized-water extraction unit (Adsett et al., 1999). Additionally, field tests of the system (Sibley et al., 2009) showed good agreement to NO_3-N measured by standard laboratory methods. Adamchuk et al. (2005) investigated the use of NO_3-N, K, Na, and pH electrodes in direct contact with moist soil. Although they obtained good accuracy for pH, results for NO_3-N, K, and Na were not as accurate ($r^2 \leq 0.62$). In a follow-up study, Sethuramasamyraja et al. (2007, 2008) developed a system that placed the ISEs into an agitated suspension of soil and water. They reported that a 1:1 soil:water ratio and tap water for electrode rinsing were usable for simultaneous measurement of pH, K, and NO_3-N with the ISEs.

Birrell and Hummel (2000, 2001) investigated the use of a multi-ISFET sensor chip to measure soil nitrate in a flow injection analysis (FIA) system using low flow rates, short injection times, and rapid rinsing. The multi-ISFET/FIA system successfully estimated soil NO_3-N content in manually prepared soil extracts ($r^2 > 0.90$). The rapid response of the system allowed samples to be analyzed within 1.25 s with sample flow rates less than 0.2 mL s^{-1}. Price et al. (2003) developed a rapid extraction system to complement the ISFET/FIA system. They reported that nitrate concentration could be determined within 2 to 5 s after injection of the extracting solution.

In our previous studies (Kim et al., 2006a, 2007a), the sensitivity and selectivity of polyvinyl chloride (PVC) membrane-based ISEs with tetradodecylammonium nitrate (TDDA) and valinomycin for sensing nitrate and potassium, respectively, and of cobalt-rod-based phosphate ISEs were satisfactory for measuring NO_3-N, P, and K ions over typical ranges of soil concentrations.

A universal solution for extracting multiple ions from soils would be advantageous for simultaneous analysis of soil macronutrients because its use could reduce the time and cost required for analysis. The Mehlich III extractant (0.2 M CH_3COOH + 0.015 M NH_4F + 0.25 M NH_4NO_3 + 0.013 M HNO_3 + 0.001 M EDTA) is used to extract P, K, and other ions from soil (Fixen and Grove, 1990; Mehlich, 1984). The use of the Mehlich III solution has expanded because of increased adoption of the inductively coupled argon plasma (ICP) analyzer that simultaneously measures P, K, and other ions. However, the Mehlich III solution is not useful for nitrate extraction because the solution itself contains a high concentration of nitrate. The Kelowna extractant (0.25 M CH_3COOH + 0.015 M NH_4F), which is used as a multiple-ion extractant in British Columbia, Canada, can simultaneously extract P, K, and nitrate from soils (Kim et al., 2006b; Van Lierop, 1986, 1988; Van Lierop and Gough, 1989).

In a previous study (Kim et al., 2007b), we established the ability of ISEs and Kelowna extractant to simultaneously quantify NO_3-N, P, and K ions in a set of 37 soils obtained from diverse soil associations across Illinois and Missouri. We developed methods (baseline correction and two-point normalization) that standardized ISE response and allowed application of a single calibration equation across multiple ISEs fabricated simultaneously. Three calibration equations based on the Nikolskii-Eisenman equation were developed using mixed solutions of known concentrations and applied to estimate the concentrations of NO_3-N, P, and K ions in soil extracts. Strong linear relationships were obtained between ion concentrations determined by the ISE and Kelowna method and by standard methods.

Although Kim et al. (2007b) established the feasibility of the ISE-Kelowna methodology, several additional issues needed to be investigated. One issue was related to the durability of ISE membranes, which have a finite life and require replacement on a regular basis. Although replacement membranes would be fabricated according to the same standard procedure, there could be differences in membrane response due to laboratory technique differences or other issues. Our methods worked to standardize response across membranes fabricated in the same batch, but the ability of the baseline correction and two-point normalization methods to standardize responses across different batches of membranes was not known. Another issue was related to the ability of the soil macronutrient sensing system to quantify within-field variations, which would be the primary application of such a system. This was not directly tested in the original study that used a regional calibration dataset where no two soil samples were obtained from the same site. Thus, the overall goal of this research was to further validate the ISE-based macronutrient sensing of Kim et al. (2007b) by addressing two questions related to calibration transfer and the ability of the system to quantify macronutrient variability within a single site. Specific objectives were to: (1) investigate transferability of the calibration models of Kim et al. (2007b) to a new array of membranes and electrodes, and (2) evaluate the ability of this array of ion-selective electrodes to estimate NO_3-N, P, and K concentration variations seen within a 65 ha test site.

Materials and Methods

Preparation of Ion-Selective Electrodes

Ion-selective membranes and electrodes were prepared following the detailed procedures reported in our previous studies (Kim et al., 2006a, 2007a, 2007b). The PVC-based NO_3-N (hereafter shortened to N) ion-selective membrane was prepared with a mixture of 30 mg (15% wt) of ligand (TDDA, tetradodecylammonium nitrate), 80 mg (40% wt) of plasticizer (NPOE, nitrophenyl octyl ether), and 90 mg (45% wt) of high-molecular-weight polyvinyl chloride (PVC) dissolved in 2 mL of THF (tetrahydrofuran). The composition of the K ion-selective membrane was 4 mg (2% wt) of ionophore (valinomycin), 1 mg (0.5% wt) of lipophilic additive (KTpClPB), 129.4 mg (64.70% wt) of plasticizer (DOS, bis(2-ethylhexyl sebacate), and 65.6 mg (32.80% wt) of PVC in 2 mL of THF. The N and K membrane disks were attached to the ends of Hitachi ISE electrode bodies using THF solvent. Each N ISE electrode was filled with an internal solution consisting of 0.01 M $NaNO_3$ and 0.01 M NaCl. Potassium chloride (0.01 M) was employed as the internal reference solution of the potassium electrodes. An Ag/AgCl electrode was immersed as the inner reference electrode. For sensing phosphorus, cobalt electrodes with a purity of 99.95% were prepared according to procedures reported by Xiao et al. (1995). A double-junction Ag/AgCl electrode (Orion 90-02, Orion Research, Inc., Cambridge, Mass.) was used as the reference electrode. The test array consisted of 16 electrodes: five each for NPK ions and one reference electrode.

Test Equipment and Procedures

Electrode tests were conducted with a previously designed automated test stand (fig. 1) that allowed simultaneous sampling of electromotive force (EMF) data from the 15 ISE electrodes measured relative to the reference electrode. A 16-channel circuit board equipped with LF356N operational amplifiers (National Semiconductor, Santa Clara, Cal.) was used for buffering the impedance of each sensor. A Daqbook 200 data acquisition system (IOTech, Cleveland, Ohio) was used to perform analog-to-digital conversion and transfer data to a 400 MHz Pentium II computer. Additional details on the test stand components are given by Kim (2006) and Kim et al. (2006a).

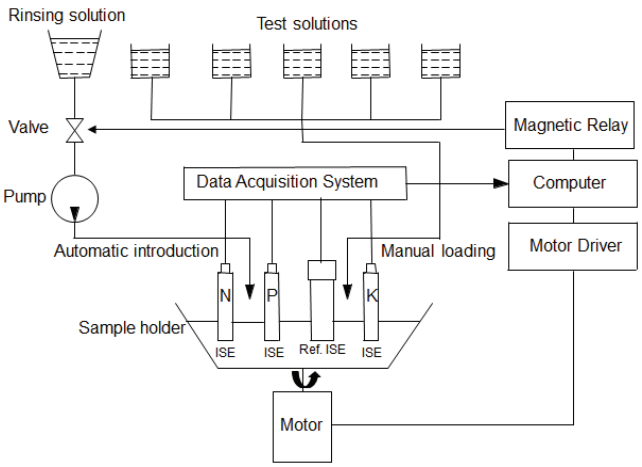

Figure 1. Schematic of the automated test stand for multiple ion-selective electrodes (modified from Kim et al., 2007b).

As shown in figure 1, each test sequence began with automatic triple-rinsing of the electrodes using a solution of 10^{-6} M KNO_3 prior to sample measurement. Under the control of a computer program developed with Access 2000 and Visual Basic 6.0 (Microsoft Corp., Seattle, Wash.), the rotational speed of the Teflon sample holder was increased during each rinse to expel the rinse solution and then slowed while fresh rinsing solution was being introduced. After the rinse sequence, the sample holder was rotated to provide a stirring action while 110 mL of sample solution was manually loaded. The operator pressed a key after sample loading to accurately reference the data collection time to introduction of the new test solution. Thus, each individual test began when the desired volume of test solution had been delivered to the sample holder. The sample holder rotated at a constant speed of 37 rpm from sample injection through data collection.

For each sample, baseline and sample EMF data were collected for the third rinse solution and the test solution, respectively, at 60 s after injection of each solution into the test stand. Three EMF readings were obtained 3 s apart (i.e., at 60, 63, and 66 s) and averaged. Three replications of ISE data were obtained, where each replication consisted of all soil extract samples along with normalization and validation samples.

Application of Baseline Correction and Two-Point Normalization

Two data processing procedures developed in our previous study (Kim et al., 2007b), i.e., baseline correction and two-point normalization methods, were used (1) to compensate for potential drift and (2) to standardize responses of multiple electrodes for each ion, respectively. Baseline-corrected EMF measurements were calculated by subtraction of the preceding baseline (rinse solution) EMF from each sensor reading. Two-point normalization consisted of a sensitivity adjustment followed by an offset adjustment calculated and applied separately to each replication-electrode combination. Standardized EMF values were determined by averaging across all five electrodes and all three replications for each sensor type, and then individual sensitivity slopes for each electrode within each replication were normalized with respect to the standardized values. Finally, an offset normalization adjustment was applied. Additional details are given by Kim et al. (2007b).

Ion concentrations were chosen as reference points for normalization to be consistent with Kim et al. (2007b). A solution containing a mixture of NPK ions at concentration levels of 0.1-0.1-1 mg L^{-1}, respectively, was chosen as the low-concentration normalization point for all NPK sensors, and three other solutions containing a mixture of NPK ions at concentration levels of 20-0.1-1 mg L^{-1}, 0.1-20-1 mg L^{-1}, and 0.1-0.1-50 mg L^{-1} were prepared to provide high concentration levels for the N, P, and K sensors, respectively. The EMF outputs measured with all five sensors for each ion were normalized by first applying the baseline correction to each reading and then by applying the two-point normalization approach separately to each replication. After

Table 1. N, P, and K calibration equations (from Kim et al., 2007b).[a]

Equation	R^2	RMSE (mg L^{-1})	No. of Samples
$C_N = 10^{\frac{EMF_N - 33.8}{-72.5}} - 4.58$	0.99	0.664	960
$C_P = 10^{\frac{EMF_P + 40.14}{-33.3 + 0.0973 C_N}} + 0.04 C_N - 1.2$	0.95	1.610	890
$C_K = 10^{\frac{EMF_K + 102.3}{83.3}} - 15.4$	0.99	1.527	960

[a] C_N, C_P, and C_K = concentrations of N, P, and K, respectively; EMF = electromotive forces after application of baseline correction and 2-point normalization methods.

this normalization, calibration equations for each sensor type (table 1), developed by Kim et al. (2007b), were applied to the ISEs prepared for this study to estimate the concentrations of NPK ions in solution and soil extracts without additional electrode calibration.

SOIL EXTRACT TESTS

Soil Samples and Nutrient Extraction

In contrast to Kim et al. (2007b), where samples were selected to evaluate the ability of the ISEs to estimate nutrients over a wide range of soil types and concentrations, samples in this study were chosen to investigate the ability of the ISEs to estimate nutrient variations within a single field. Thirty-six samples (pH = 6.12 ±0.17, organic matter = 37.0 ±7.13 g kg^{-1}, and CEC = 20.68 ±1.94 cmol kg^{-1}) were obtained from fertility trial test plots located at the Northern Illinois Agronomy Research Center (NIARC) near Shabbona, Illinois. These plots had been used to investigate different NPK application rates and had been in existence for over 20 years when the samples were obtained (Lyle Paul, agronomist, NIARC, personal communication). The NIARC sample sites were all located on a Flanagan silt loam (fine, smectitic, mesic Aquic Argiudolls) within 1 km of one another.

Soil extracts were obtained with the Kelowna multiple-element extractant (Van Lierop, 1986, 1988; Van Lierop and Gough, 1989) because of the documented ability of the Kelowna extractant to extract macronutrients from U.S. Corn Belt soils (Kim et al., 2006b). According to the standard procedures for use in soil testing (Brown, 1998), a nominal 30 g soil sample was obtained by 15 fillings of an NCR-13 standard 2 g soil scoop (Peck, 1998). The measured soil was then transferred into a 500 mL glass bottle, and Kelowna extractant (300 mL) was added to obtain a nominal 10:1 solution-to-soil ratio. The mixtures of soil and solution were shaken on a reciprocating shaker at ~140 cycles min^{-1} for 5 min and then filtered through Whatman No. 42 filter paper. All soil extracts were titrated to pH 4.0 with 1 M NaOH to remove any effect of pH differences on the equilibrium concentration of the orthophosphate ionic forms (Lindsay, 1979).

Estimation of NPK Concentrations in Soil Extracts

Consistent with Kim et al. (2007b), the test sequence within each replication was split into three groups, each including normalization solutions, soil extract samples, and validation solution samples. The 36 soil extracts were randomized and then divided into three groups of 12 samples each. At the beginning of the test sequence, the four normalization solutions of known concentration combinations were tested in a random order. Five mixed solutions containing different NPK concentrations (0.1, 20, 3 mg L^{-1}; 20, 20, 3 mg L^{-1}; 20, 1, 3 mg L^{-1}; 1, 0.1, 50 mg L^{-1}; and 0.1, 5, 50 mg L^{-1} of NO_3-N, P, and K ions, respectively) were inserted into each group of the randomized soil extracts as validation samples. The validation samples, with concentrations that were a subset of the solutions used in the calibration procedure of Kim et al. (2007b), were selected to span the range of expected concentrations of each ion.

For a comparison of the ISE sensor array method to standard soil testing methods, subsamples of the 36 extracted solutions were analyzed in a commercial soil testing laboratory (A&L Great Lakes Laboratories, Fort Wayne, Ind.), using an automated ion analyzer (Lachat QuickChem Analyzer, Lachat Instruments, Loveland, Colo.) for NO_3-N and an ICP spectrometer (Fixons ARL Accuris, Ecublens, Switzerland) for P and K measurements, to determine the actual concentrations present. Subsamples of the 36 tested soils were provided to the same soil testing laboratory for extraction and analysis to determine the actual concentrations based on standard testing methods. Soil NO_3-N was extracted with 1 M KCl and analyzed with the Lachat analyzer based on a 5:1 (25 mL of solution to 5 g of soil v/v) solution-to-soil ratio and a 30 min extraction time. Soil P and K were extracted using the Mehlich III solution based on a 10:1 (20 mL of solution to 2 g of soil v/v) solution-to-soil ratio and a 5 min extraction time, and the concentrations were determined by ICP analyzer.

Transferability of the calibration models developed by Kim et al. (2007b) to the new membranes and electrodes of this study was assessed through comparison of ISE-measured and known validation sample concentrations. ISE-measured concentrations of NPK ions for each soil extract were compared with concentrations obtained with standard instruments using linear regression analysis. Similarly, ISE-measured concentrations in soils were compared with concentrations by standard instruments and extraction methods. Further, because of differences in extraction efficiency between Kelowna and standard extractants (Kim et al., 2007b), another comparison was made between ISE-measured concentrations adjusted for extraction efficiency (table 2) and concentrations by standard methods.

Accuracy was assessed using the error statistics of root mean square error (RMSE), bias, and standard error of prediction (SEP):

$$\text{RMSE} = \sqrt{\frac{\sum (\hat{x}_i - x_i)^2}{N}} \quad (1)$$

Table 2. Relationships between soil NPK ions extracted with Kelowna (*Y*) and with standard extractants (*X*), i.e., 1 M KCl for N and Mehlich III for P and K, using an automated ion analyzer for N and ICP spectrometer for P and K (from Kim et al., 2007b).

Tested Ion	Kelowna (*Y*) vs. Standard Extractants (*X*)	R^2
NO_3-N	$Y = 1.03X + 0.72$	0.99
P	$Y = 0.74X + 0.14$	0.98
K	$Y = 0.49X + 2.14$	0.94

$$\text{Bias} = \frac{\sum_{i=1}^{N}(\hat{x}_i - x_i)}{N} \quad (2)$$

$$\text{SEP} = \sqrt{\frac{\sum_{i=1}^{N}(\hat{x}_i - x_i - \text{Bias})^2}{N-1}} \quad (3)$$

where $\hat{x}_i$ is the predicted concentration obtained by regression from ISE results, x_i is the actual concentration determined by standard instruments, and N is the number of samples.

Results and Discussion

Validation Tests with Known Samples

The validation results using the five mixtures containing known NPK concentrations are shown in figure 2. The values determined by the ISEs were in good agreement with the actual values, yielding almost 1:1 relationships between the predicted (Y) and actual (X) values, with $R^2 \geq 0.90$ in all cases. This shows that the array of N, P, and K electrodes fabricated with new membranes and cobalt rod, in conjunction with baseline correction and two-point normalization procedures, could provide results comparable to those obtained with standard instruments when using a set of previously developed calibrations (Kim et al., 2007b). This finding, that a prior calibration can be transferred to new membranes of the same type, is important because it greatly enhances the usability of this sensor array and methodology.

At NPK ion concentrations of <10 mg L^{-1}, mean estimation errors between the measured and actual values were 1 to 3 mg L^{-1}, whereas at high concentrations (i.e., 20 mg L^{-1} NO_3-N and PO_4-P, and 50 mg L^{-1} K) the estimation errors increased to 4 to 7 mg L^{-1}. This observation is likely due to the fact that the EMF responses of ISEs are linearly proportional to the logarithm of ionic concentration rather than the concentration itself. If measurement errors were quantified on the basis of log(concentration), they would be more nearly uniform across the concentration range studied. Note that the ISEs, on average, as well as the standard instruments underpredicted the actual concentrations of the samples at the high concentration levels. This was particularly evident for K, where the 50 mg L^{-1} concentration was estimated at less than 40 mg L^{-1} by both methods.

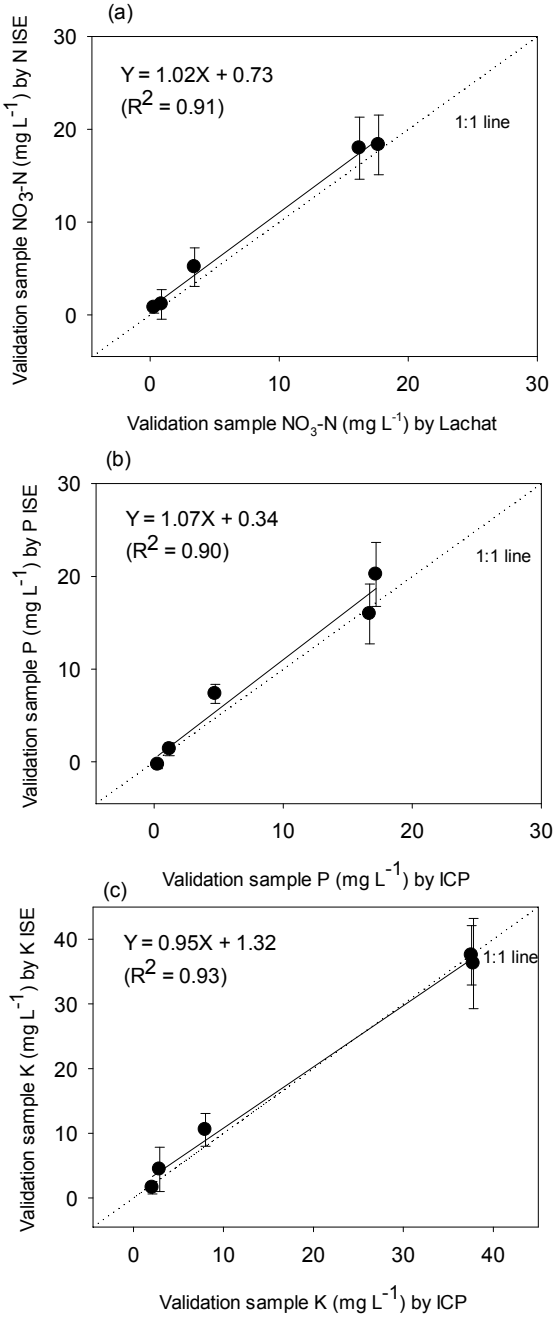

Figure 2. Relationships between validation sample (a) NO_3-N, (b) P, and (c) K determined by ion-selective electrode (ISE) and by standard instruments, i.e., automated ion analyzer (Lachat) for N and inductively coupled argon plasma (ICP) spectrometer for P and K. For ease of visualization, data are presented as means and standard deviations; however, regression equations and statistics were calculated using individual data points.

Solution Ion Concentrations Measured by ISE Sensor Array and Standard Methods

Figure 3 shows the relationships between NPK concentrations in Kelowna-based soil extracts determined by individual ISEs and by standard instruments, i.e., the automated analyzer for N and the ICP spectrometer for P and K measuremeasurements. In all cases, data are close to the 1:1 line, showing that the ISE array and standard instruments behaved similarly in quantifying the concentrations present in the Kelowna-extracted samples. Fit equations for N and K had low intercept values and slopes close to unity (fig. 3). However, the fit equation for P exhibited a high slope of 1.62. Because of the narrow range of P concentration in these samples (2 mg L^{-1}), estimation errors (table 3) were still relatively low. Furthermore, confidence in the value of the regression slope was reduced because of the narrow range.

Estimation error statistics (table 3) were better (NO_3-N and P) or similar (K) to those obtained from the analysis of 37 calibration soil extract samples as described by Kim et al.

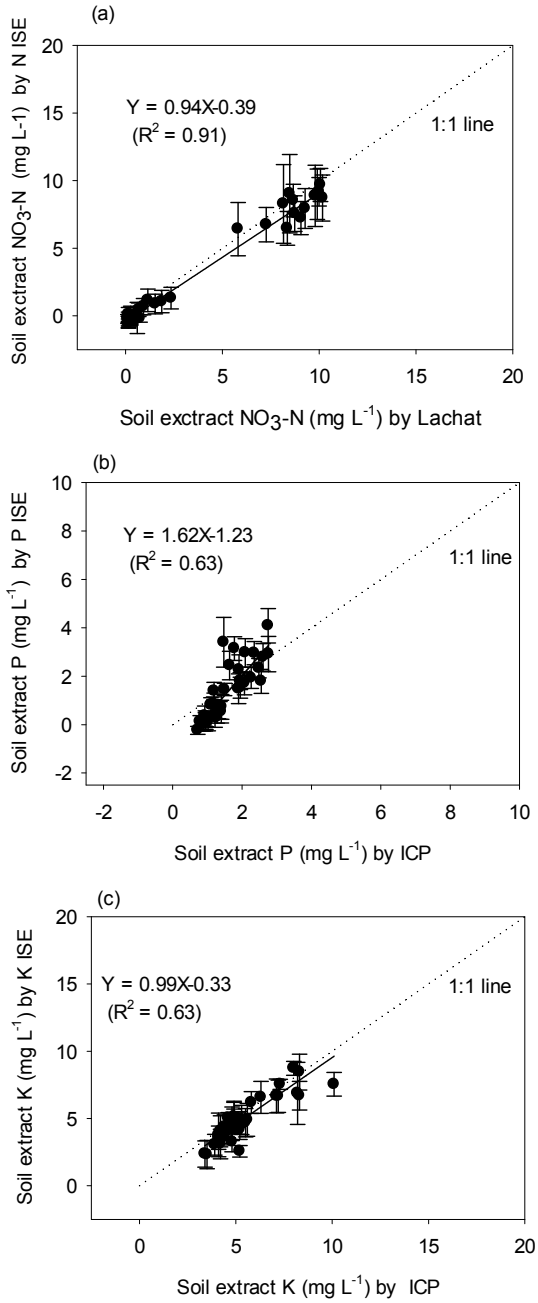

Figure 3. Relationships between Kelowna-based soil extract (a) NO_3-N, (b) P, and (c) K determined by ISE sensor and by standard instruments, i.e., automated ion analyzer (Lachat) for N and inductively coupled argon plasma (ICP) spectrometer for P and K. For ease of visualization, data are presented as means and standard deviations; however, regression equations and statistics were calculated using individual data points.

Table 3. Error statistics for tests of NO_3-N, P, and K concentrations in Kelowna soil extracts, comparing results with the 36 soil samples of this study from the Northern Illinois Agronomy Research Center (NIARC) to those with the 37 Missouri and Illinois soil samples of Kim et al. (2007b) (MO-IL).

Tested Ion	Samples	RMSE (mg L^{-1})	Bias (mg L^{-1})	SEP (mg L^{-1})	Conc. Range (mg L^{-1})
NO_3-N	NIARC	1.30	-0.59	1.15	0.11 to 10.2
	MO-IL	2.95	2.43	1.67	0.18 to 17.1
P	NIARC	0.87	-0.24	0.84	0.72 to 2.8
	MO-IL	3.95	-3.19	2.32	1.2 to 16.9
K	NIARC	1.29	-0.55	1.26	3.4 to 10.1
	MO-IL	1.32	0.74	1.10	3.2 to 25.7

(2007b). Some error reduction, particularly in RMSE and SEP, might be attributed to the narrower concentration range of the NIARC samples in this study. The largest improvement (table 3) was for P, where the data of Kim et al. (2007b) showed a regression slope of only 0.46 between ISE-measured and ICP-measured concentration. Although similar behavior was not seen in the current study, the much narrower range of P concentration (2 vs. 16 mg L^{-1}) could have masked a low regression slope if it were indeed present.

Soil Ion Concentrations Measured by ISE Sensor Array and Standard Methods

Estimation error statistics for soil ion concentration measurements (table 4) were better for the NIARC samples of this study than for the samples of Kim et al. (2007b). As with the solution samples, some portion of this reduction might be attributed to the narrower range of concentrations in the current study. For improved soil concentration estimates, we applied a calibration factor (table 2) to adjust ISE measurements for the difference in extraction efficiency between Kelowna and KCl (for N) or Mehlich III (for P and K). Error statistics for these "calibrated NIARC" data are shown in table 4.

There was little improvement in accuracy statistics for N, as the calibration applied for that ion was very close to unity slope (table 2). Bias and RMSE for P estimation decreased moderately due to the moderate slope adjustment of 1.35 (1/0.74). Bias and RMSE for K estimation decreased considerably due to application of a large slope adjustment (2.04 = 1/0.49). Based on Midwest U.S. corn fertility recommendations reviewed by Kim et al. (2009), and the assumption of applying fertilizer at five discrete rates over the critical range of soil test values, approximate accuracy requirements for N, P, and K sensing would be 5, 7, and 36 mg L^{-1}, respectively. Comparison of these approximate values with the calibrated NIARC data in table 4 shows that accuracy would need to be improved by a factor of 3 for both N and P, while K estimates were of sufficient accuracy. Note, however, that this assessment is based on a comparison of single-sample accuracy. Because the ISE system is intended for mobile collection of spatially dense data, many sensor data points could be obtained to represent the same spatial area as a single laboratory sample. Thus, an on-the-go system can provide improved overall accuracy while tolerating high single-sample analysis errors.

Table 4. Error statistics for tests of NO_3-N, P, and K concentrations in soil, comparing results with the 36 soil samples of this study from the Northern Illinois Agronomy Research Center (NIARC), the NIARC samples after calibration to compensate for efficiency differences between extraction solutions (cal. NIARC), and the 37 Missouri and Illinois soil samples of Kim et al. (2007b) (MO-IL).

Tested Ion	Samples	RMSE (mg L^{-1})	Bias (mg L^{-1})	SEP (mg L^{-1})	Conc. Range (mg L^{-1})
NO_3-N	NIARC	15.0	-8.59	12.4	1.8 to 109.8
	Cal. NIARC	16.1	-10.1	12.5	1.8 to 109.8
	MO-IL	29.7	22.3	19.5	5.1 to 206.6
P	NIARC	25.62	-24.51	7.57	16.6 to 71.9
	Cal. NIARC	21.82	-20.04	8.75	16.6 to 71.9
	MO-IL	64.6	-54.6	34.5	21.3 to 235.8
K	NIARC	54.91	-53.09	15.12	59.1 to 157
	Cal. NIARC	23.39	-5.99	22.66	59.1 to 157
	MO-IL	88.7	-68.2	56.8	51.9 to 445.5

Figure 4 compares soil ion concentrations by extractant-calibrated ISE measurement with standard methods. Although regression slopes for soil N and K measurements were not exactly unity, most N and K estimates were near the 1:1 line, with RMSE values of 16.1 mg L^{-1} for N and 23.4 mg L^{-1} for K (table 4). These errors were similar (for N) or much less (for K) compared to the uncalibrated NIARC data (table 4), suggesting that the calibration applied for extraction efficiency differences was appropriate.

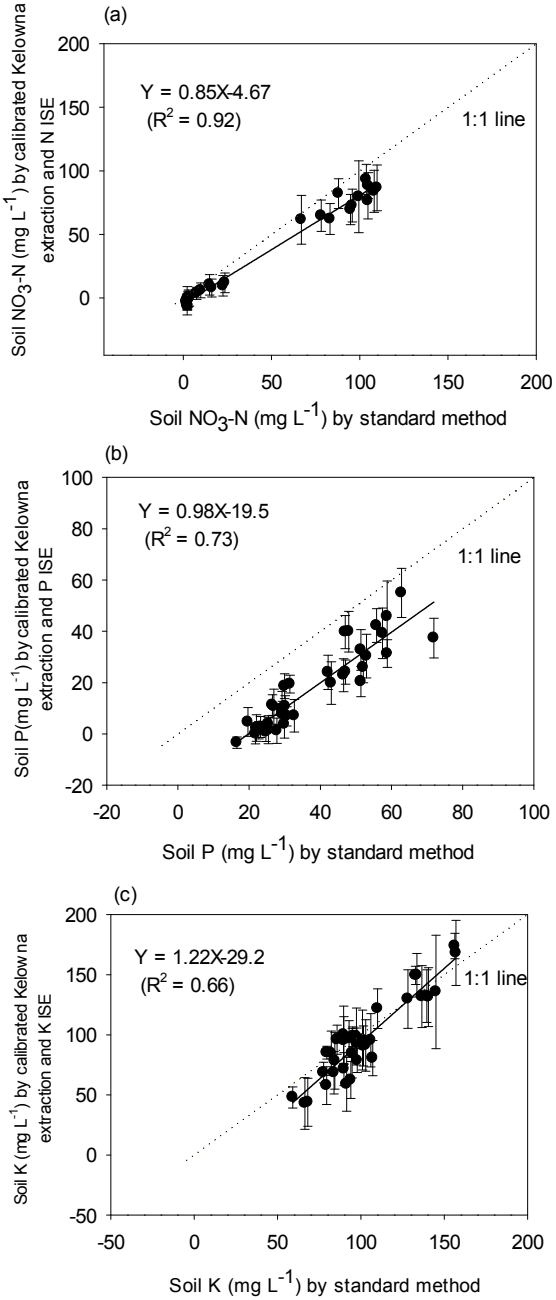

Figure 4. Relationships between soil (a) NO_3-N, (b) P, and (c) K determined by Kelowna extractant and ISE sensor and by standard extractants and an automated ion analyzer (Lachat) for N and inductively coupled argon plasma (ICP) spectrometer for P and K. For ease of visualization, data are presented as means and standard deviations; however, regression equations and statistics were calculated using individual data points.

On the other hand, P estimates retained a relatively large offset between calibrated ISE and standard concentrations (fig. 4) in a concentration range of 18 to 78 mg L^{-1}. It seems plausible that this may have been due to a difference in Kelowna P extraction efficiencies between the NIARC and MO-IL soils. Although extraction efficiency data were not obtained for the NIARC soils, some individual soils analyzed by Kim et al. (2007b) were not as well fit by the extraction efficiency regression (table 2). Similar to figures 2 and 3, figure 4 shows increasing error with increasing concentration. This could be expected, as ISE EMF readings are proportional to the logarithm of the ion concentration, and measurement error could be assumed constant when expressed in EMF. Other researchers (Adamchuk et al., 2005; Sethuramasamyraja et al., 2008) have dealt with this issue by developing calibration equations between sensor reading and the logarithm of the concentration. Although that approach was not feasible here, where the calibration equations were already established in our previous research (Kim et al., 2007b), application of logarithmic calibration to data from this ISE sensor array would be an important future research topic.

Taken together, these soil extract and soil results showed that the ISE sensor array, in conjunction with calibration transfer using standard solutions, and an adjustment for differences in extraction efficiency, was able to estimate N, P, and K concentrations with similar or improved accuracies compared to those obtained in the original calibration dataset of Kim et al. (2007b). This enhances the applicability of this instrumentation system and methodology for rapid soil analysis by showing that system recalibration would not be needed when replacing ISEs. Further, these results show that the system was able to discriminate NPK differences in soils obtained from a single farm, albeit with a lower accuracy than might be desired, as required for control of variable-rate fertilizer application. Although the tested ISEs successfully estimated N, P, and K in soil extracts, additional research leading to successful automation of the soil sampling and extraction process will be needed before the ISEs can be integrated into a real-time on-the-go soil nutrient measurement system. One potential area for improvement is compensation for differences in sensitivity and/or extraction efficiency across different soils. Similar to the two-point normalization already used to compensate for differences in membrane sensitivity, a field-specific normalization that uses soil extracts from the test field as the two-point normalization samples should be investigated. An additional issue to address in future research is correlation of ISE-determined, Kelowna-extracted N, P, and K concentrations to plant uptake or crop yield. This basic soil fertility research will be important to support development and implementation of the on-the-go ISE sensing system.

CONCLUSIONS

In this study, a validation test of an ISE-based macronutrient sensing system developed in our previous study (Kim et al., 2007b) was conducted using 36 soil samples from an Illinois research farm and a set of previously developed cal-

ibration equations. The transferability of the calibration equations to a new array of membranes and electrodes was investigated. The ISE array was evaluated for accuracy in simultaneously analyzing NPK ions in soils within a single test site. The EMF data measured with the ISEs were normalized using the baseline correction and two-point normalization methods, and the normalized EMF data were then used to estimate the concentrations of NPK ions in soil extracts by application of previously developed calibration equations. The relationships between the concentrations obtained with the ISEs using Kelowna solution and standard soil tests were investigated by comparison to results previously obtained from the MO-IL soils. The following conclusions can be drawn from these tests:

- The validation results using five mixtures containing known NPK concentrations showed that the array of N, P, and K electrodes fabricated with new membranes and cobalt rod, in conjunction with baseline correction and two-point normalization methods, provided accurate results using a set of previously developed calibrations (Kim et al., 2007b).
- The NPK ISEs measured NPK ions in Kelowna-based soil extracts independently, with approximately 1:1 relationships between the concentrations determined by ISEs and by standard laboratory instruments. Estimation error statistics were better for these samples than for the 37 MO-IL soil samples of Kim et al. (2007b). However, some of this error reduction might be attributed to the narrower concentration range of the NIARC samples compared to the MO-IL samples.
- When ISE measurements were adjusted for the difference in extraction efficiency between Kelowna and KCl (for N) or Mehlich III (for P and K), soil N and K estimates were similar to actual N and K values, suggesting that the calibration for extraction efficiency differences was appropriate. On the other hand, although bias and RMSE for P estimation were somewhat lower after calibration, P estimates retained a relatively large offset between calibrated ISE and standard method concentrations. This might be attributed to a difference between P amounts extracted from the NIARC and MO-IL soils with the Kelowna extractant.
- The N and P estimates obtained in this study would likely not be of sufficient accuracy for control of variable-rate fertilizer application, while the K estimates could be used for application control. Averaging of multiple sensor readings, as would be feasible with a mobile system, would have potential to provide further accuracy improvements.

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REFERENCES

Adamchuk, V. I., J. W. Hummel, M. T. Morgan, and S. K. Upadhyaya. 2004. On-the-go soil sensors for precision agriculture. *Comp. Elect. Agric.* 44(1): 71-91.

Adamchuk, V. I., E. D. Lund, B. Sethuramasamyraja, M. T. Morgan, A. Dobermann, and D. B. Marx. 2005. Direct measurement of soil chemical properties on-the-go using ion-selective electrodes. *Comp. Elect. Agric.* 48(3): 272-294.

Adsett, J. F., J. A. Thottan, and K. J. Sibley. 1999. Development of an automatic on-the-go soil nitrate monitoring system. *Applied Eng. in Agric.* 15(4): 351-356.

Artigas, J., A. Beltran, C. Jimenez, A. Baldi, R. Mas, C. Dominguez, and J. Alonso. 2001. Application of ion-selective field effect transistor based sensors to soil analysis. *Comp. Elect. Agric.* 31(3): 281-293.

Bedlechowicz-Sliwakowska, I., P. Lingenfelter, T. Sokalski, A. Lewenstam, and M. Maj-Zurawsk. 2006. Ion-selective electrode for measuring low Ca_2^+ concentrations in the presence of high K, Na, and Mg background. *Anal. Bioanal. Chem*. 385(8): 1477-1482.

Birrell, S. J., and J. W. Hummel. 2000. Membrane selection and ISFET configuration evaluation for soil nitrate sensing. *Trans. ASAE* 43(2): 197-206.

Birrell, S. J., and J. W. Hummel. 2001. Real-time multi-ISFET/FIA soil analysis system with automatic sample extraction. *Comp. Elect. Agric.* 32(1): 45-67.

Brown, J. R. 1998. *Recommended Chemical Soil Test Procedures for the North Central Region*. Extension Circular No. 923. Columbia, Mo.: University of Missouri, Missouri Agricultural Experiment Station.

Carey, C. M., and W. B. Riggan. 1994. Cyclic polyamine ionophores for use in a dibasic-phosphate-selective electrode. *Anal. Chem.* 66(21): 3587-3591.

Chen, Z. L., R. De Marco, and P. W. Alexander. 1997. Flow-injection potentiometric detection of phosphates using a metallic cobalt wire ion-selective electrode. *Anal. Commun.* 34(3): 93-95.

Fixen, P. E., and J. H. Grove. 1990. Testing soils for phosphorus. In *Soil Testing and Plant Analysis*, 141-172. 3rd ed. R. L. Westerman, ed. Madison, Wisc.: SSSA.

Gallardo, J., S. Alegret, and M. D. Valle. 2004. A flow-injection electronic tongue based on potentiometric sensors for the determination of nitrate in the presence of chloride. *Sensors Actuators B* 101(1-2): 72-80.

Glazier, S. A., and M. A. Arnold. 1991. Selectivity of membrane electrodes based on derivatives of dibenzyltin dichloride. *Anal. Chem.* 63(8): 754-759.

Havlin, J. L., J. D. Beaton, S. L. Tisdale, and W. L. Nelson. 1999. *Soil Fertility and Fertilizers: An Introduction to Nutrient Management*. Upper Saddle River, N.J.: Prentice Hall.

Kim, H. J. 2006. Ion-selective electrodes for simultaneous real-time analysis of soil macronutrients. Unpublished PhD diss. Columbia, Mo.: University of Missouri, Department of

Biological Engineering.
Kim, H. J., J. W. Hummel, and S. J. Birrell. 2006a. Evaluation of nitrate and potassium ion-selective membranes for soil macronutrient sensing. *Trans. ASABE* 49(3): 597-606.
Kim, H. J., J. W. Hummel, and K. A. Sudduth. 2006b. Sensing nitrate and potassium ions in soil extracts using ion-selective electrodes. *J. Biosystems Eng.* 31(6): 463-473.
Kim, H. J., J. W. Hummel, K. A. Sudduth, and S. J. Birrell. 2007a. Evaluation of phosphate ion-selective membranes and cobalt-based electrodes for soil nutrient sensing. *Trans. ASABE* 50(2): 215-225.
Kim, H. J., J. W. Hummel, K. A. Sudduth, and P. P. Motavalli. 2007b. Simultaneous analysis of soil macronutrients using ion-selective electrodes. *SSSA J.* 71(6): 1867-1877.
Kim, H. J., K. A. Sudduth, and J. W. Hummel. 2009. Soil macronutrient sensing for precision agriculture. *J. Environ. Monit.* 11(10): 1810-1824.
Knoll, M., K. Cammann, C. Dumschat, M. Borchardt, and G. Hogg. 1994. Microfibre matrix-supported ion-selective PVC membranes. *Sensors Actuators B* 20(1): 1-5.
Levitchev, S., A. Smirnova, A. Bratov, and Y. Vlasov. 1998. Electrochemical properties of photocurable membranes for all-solid-state chemical sensors. *Fresenius J. Anal. Chem.* 361(3): 252-254.
Lindsay, W. L. 1979. *Chemical Equilibria in Soils.* New York, N.Y.: John Wiley and Sons.
Mehlich, A. 1984. Mehlich III soil test extractant: A modification of Mehlich II extractant. *Commun. Soil Sci. Plant Anal.* 15(12): 1409-1416.
Nielson, H. J., and E. H. Hansen. 1976. New nitrate ion-selective electrodes based on quaternary ammonium compounds in nonporous polymer membranes. *Anal. Chim. Acta* 85(1): 1-16.
Page, T., P. M. Haygarth, K. J. Beven, A. Joynes, T. Butler, C. Keeler, J. Freer, P. N. Owens, and G. A. Wood. 2005. Spatial variability of soil phosphorus in relation to the topographic index and critical source areas: Sampling for assessing risk to water quality. *J. Environ. Qual.* 34(6): 2263-2277.
Peck, T. R. 1998. Standard soil scoop. In *Recommended Chemical Soil Test Procedures for the North Central Region*, 7-9. J. R. Brown, ed. Columbia, Mo.: University of Missouri, Missouri Agricultural Experiment Station.
Price, R. R., J. W. Hummel, S. J. Birrell, and I. S. Ahmad. 2003. Rapid nitrate analysis of soil cores using ISFETs. *Trans. ASAE* 46(3): 601-610.
Schepers, J. S., and M. R. Schlemmer. 1998. Influence of grid sampling points on fertilizer recommendations. In *Proc. 1st Intl. Conf. on Geospatial Information in Agriculture and Forestry*. Ann Arbor, Mich.: ERIM International.
Schirrmann, M., R. Gebbers, E. Kramer, and J. Seidel. 2011. Soil pH mapping with an on-the-go sensor. *Sensors* 11(1): 573-598.
Sethuramasamyraja, B., V. I. Adamchuk, D. B. Marx, A. Dobermann, G. E. Meyer, and D. D. Jones. 2007. Analysis of an ion-selective electrode based methodology for integrated on-the-go mapping of soil pH, potassium, and nitrate contents. *Trans. ASABE* 50(6): 1927-1935.
Sethuramasamyraja, B., V. I. Adamchuk, A. Dobermann, D. B. Marx, D. D. Jones, and G. E. Meyer. 2008. Agitated soil measurement method for integrated on-the-go mapping of soil pH, potassium, and nitrate contents. *Comp. Elect. Agric.* 60(2): 212-225.
Sibley, K. J., T. Astatkie, G. Brewster, P. C. Struik, J. F. Adsett, and K. Pruski. 2009. Field-scale validation of an automated soil nitrate extraction and measurement system. *Prec. Agric.* 10(2): 162-174.
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. *Commun. 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 Actuators* 18(3-4): 329-336.
Van Lierop, W. 1986. Soil nitrate determination using the Kelowna multiple-element extractant. *Commun. Soil Sci. Plant Anal.* 17(12): 1311-1329.
Van Lierop, W. 1988. Determination of available phosphorus in acid and calcareous soils with the Kelowna multiple-element extractant. *Soil Sci.* 146(4): 284-291.
Van Lierop, W., and N. A. Gough. 1989. Extraction of potassium and sodium from acid and calcareous soils with the Kelowna multiple-element extractant. *Canadian J. Soil Sci*. 69(2): 235-242.
Wroblewski, W., K. Wojciechowski, A. Dybko, Z. Brzozka, R. J. M. Egberink, B. H. M. Snellink-Ruel, and D. N. Reinhoudt. 2000. Uranyl salophenes as ionophores for phosphate-selective electrodes. *Sensors Actuators B* 68(1-3): 313-318.
Xiao, D., H. Y. Yuan, J. Li, and R. Q. Yu. 1995. Surface-modified cobalt-based sensor as a phosphate-sensitive electrode. *Anal. Chem.* 67(2): 288-291.