Evaluation of Phosphate Ion-Selective Membranes and Cobalt-Based Electrodes for Soil Nutrient Sensing

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ABSTRACT. *A real-time soil nutrient sensor would allow efficient collection of data with a fine spatial resolution to accurately characterize within-field variability for site-specific nutrient application. Ion-selective electrodes are promising candidates because they have rapid response, directly measure the analyte, and are small and portable. Our goal was to investigate the ability of three different phosphate ion-selective electrodes (two fabricated with organotin compound-based PVC membranes, and one fabricated from a cobalt rod) used in conjunction with Kelowna soil extractant to determine phosphorus over the typical range of soil concentrations. Electrodes using organotin compound-based PVC membranes containing bis(p-chlorobenzyl)tin dichloride as an ionophore exhibited sensitive responses to $HPO_4{}^{2-}$ over a range of 10^{-4} to 10^{-1} mol/L in Tris buffer at pH 7. They were nearly insensitive to phosphate when using Kelowna soil extractant as the base solution, perhaps because of the high concentration of fluoride (0.015 mol/L) in the Kelowna solution. In addition, the life of the membranes was less than 14 days. Electrodes using another tin-compound-based PVC membrane containing tributyltin chloride as an ionophore also provided unsatisfactory results, showing much less sensitivity to $H_2PO_4{}^-$ than previously reported. The cobalt rod-based electrodes exhibited sensitive responses to $H_2PO_4{}^-$ over a range from 10^{-5} to 10^{-1} mol/L total phosphate concentration with a detection limit of 10^{-5} mol/L in the Kelowna solution. This detection range would encompass the typical range of soil phosphorus concentrations measured in agricultural fields. The selectivity of the cobalt electrodes was satisfactory for measuring phosphates in the presence of each of six interfering ions, i.e., $HCO_3{}^-$, Cl^-, Br^-, $NO_3{}^-$, Ac^-, and F^-, with the electrodes being 47 to 1072 times more responsive to phosphate than to the tested interfering ions.*

Keywords. Cobalt electrode, Ion-selective electrode, Ion-selective membranes, ISE, Kelowna extractant, Phosphate, Selectivity, Sensitivity, Soil extractant, Soil testing.

Phosphates have been extensively used in agricultural fertilizers and detergents. The measurement of phosphorus concentration levels is important in many areas of science and technology, such as environmental monitoring, clinical chemistry, and biomedical research (Glazier and Arnold, 1988).

The plant-available phosphorus in soil, forming monobasic ($H_2PO_4{}^-$) or dibasic ($HPO_4{}^{2-}$) phosphate, is one of the major essential nutrients for crop growth. However, excessive use of commercial NPK fertilizers has been cited as a source of contamination of surface and groundwater (Staver and Brinsfield, 1990). Furthermore, high levels of phosphorus in the soil have been reported to enter aquatic ecosystems through surface runoff and create an imbalance that results in excessive growth of algae in lakes and rivers (Hanson et al., 2002).

There is high spatial variability of soil phosphorus within individual agricultural fields (Page et al., 2005). Monitoring of phosphorus in soil, using real-time, on-site methods, could allow accurate estimation of required rates for variable fertilizer application within fields, thereby increasing the efficiency of variable-rate application of fertilizers and reducing the potential for environmental pollution in water and soil.

Various analytical methods have been routinely used for phosphorus quantification in soil testing laboratories. These techniques, based mostly on colorimetric or atomic emission spectroscopy, provide fairly accurate results due to their good linear sensitivity and relatively low interference from other ions (Watson and Isaac, 1990; Brown, 1998). However, such analytical methods cannot be adapted for field use because the instruments are expensive, and they require complex sample pre-treatment, which increases the time and cost of sample analysis and thereby limits the number of samples analyzed in the field (Artigas et al., 2001).

The need for fast, on-site monitoring methods allowing the analysis of a large number of samples has led to the investigation of ion-selective electrode (ISE) technology for phosphate measurement. This technology offers several advantages over current analytical spectroscopy methods, e.g., simple methodology, direct measurement of analytes, sensitivity over a wide concentration range, low cost, and portability (Carey and Riggan, 1994). However, for several reasons, the design of a sensing material (i.e., ionophore) for selective recognition of phosphate is especially challenging.

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Due to the very high hydration energy of phosphate, ion-selective membranes have a very poor selectivity for phosphate (Liu et al., 1997; Buhlmann et al., 1998; Engblom, 1998; Fibbioli et al., 2000). According to the characterization by the Hofmeister series ($ClO_4^- > SCN^- > I^- > NO_3^- > Br^- > Cl^- > HCO_3^- > SO_4^{2-} > HPO_4^{2-}$), phosphate, being at the end of the series, shows the lowest selectivity response toward the anions (Ammann, 1986; Liu et al., 1997) because the free energy of the phosphate species is very small and the large size of orthophosphate ions prohibits the use of size-exclusion principles for increased selectivity (Tsagatakis et al., 1994).

In the late 1980s and early 1990s, several researchers reported on the development of phosphate ISEs using PVC-based membranes to detect phosphates in biological samples (Glazier and Arnold, 1988, 1991; Carey and Riggan, 1994). Their ionophores, including tin compounds and cyclic polyamine, provided good selectivity and favorable sensitivity, with a detection limit of 10^{-5} mol/L dibasic phosphate in a solution at pH 7.2. More recently, new ionophores have been reported to enhance selectivity and durability (Tsagatakis et al., 1994; Liu et al., 1997; Fibbioli et al., 2000; Tsagkatakis et al., 2001; Wroblewski et al., 2001). While all these ionophores were synthesized in research laboratories, Sasaki et al. (2004) used a commercially available chemical as an ionophore. The electrode membrane used by Sasaki et al. (2004) contained tributyltin chloride as the ionophore and 25 mol% NaTFPB, and exhibited high selectivity for $H_2PO_4^-$ with a slope of −60 mV/decade.

Xiao et al. (1995) introduced cobalt metal as a phosphate ion-selective electrode material. They reported that oxidized cobalt metal electrodes showed potentiometric sensitivity to phosphate in the concentration range of 10^{-5} to 10^{-2} mol/L in 0.025 mol/L potassium hydrogen phthalate (KHP) solution at pH 4.0. The detailed response mechanism of the cobalt electrodes toward phosphate was examined by Meruva and Meyerhoff (1996). Cobalt electrodes have been applied to the determination of phosphate levels in wastewater, fertilizers, hydroponic nutrient solutions, and soil extract samples (Chen et al., 1997; Chen et al., 1998; De Marco et al., 1998; Engblom, 1999; De Marco and Phan, 2003). In particular, Engblom (1999) studied the applicability of a cobalt rod-based electrode to the measurement of phosphate in soil extracts obtained with the ammonium lactate-acetic acid (AL) solution commonly used in Sweden.

The capabilities of ion-selective electrodes for sensing macronutrients in soil extracts can be limited by the presence of other ions in the soil itself as well as by high concentrations of ions in soil extractants (Kim, 2006). For the simultaneous detection of soil macronutrients in automated on-the-go sensing, as discussed in previous studies (Kim et al., 2006), a universal solution capable of extracting multiple ions from soil would be advantageous because its use could reduce the time and cost involved in the analysis. The Kelowna extractant (0.25 mol/L CH_3COOH + 0.015 mol/L NH_4F), which is used as a multiple-ion extractant in British Columbia, Canada, can simultaneously extract phosphorus, potassium, and nitrate from soils (Van Lierop, 1986, 1988; Van Lierop and Gough, 1989).

The overall goal of this study was to identify an ISE capable of determining phosphorus content in soil extracts. Toward that goal, we investigated the response characteristics of three different phosphate sensors, two electrodes based on

tin-compound-based PVC membranes (Glazier and Arnold, 1988, 1991; Sasaki et al., 2004) and a cobalt-based metal electrode (Xiao et al., 1995), to monobasic or dibasic phosphates. Specific objectives were to:
- Fabricate and validate the performance of three types of phosphate ISEs: two fabricated with tin-compound-based PVC membranes, and one fabricated from cobalt rods.
- Investigate the sensitivity and selectivity responses of the electrodes when using the Kelowna soil extractant as a base solution and select the most promising candidate for soil phosphorus measurement.

MATERIALS AND METHODS

REAGENTS AND SOLUTIONS

Based on previous studies (Glazier and Arnold, 1988, 1991; Sasaki et al., 2004), phosphate ion-selective membranes were prepared using two different tin compounds, bis(p-chlorobenzyl)tin dichloride and tributyltin chloride, as ionophores. The bis(p-chlorobenzyl)tin dichloride was synthesized at the University of Missouri Chemistry Department according to the procedures outlined in Glazier (1988), whereas the tributyltin chloride was commercially available from Sigma-Aldrich Corp. (St. Louis, Mo.)

Dibutyl sebacate and nitrophenyl octyl ether (NPOE) as plasticizers, N, N-dimethylformamide as a solvent for organic compounds, and sodium tetrakis [3,5-bis(trifluoromethyl)-phenyl]borate (NaTFPB) used as an anionic additive, were purchased from Sigma-Aldrich Corp. Two 5 mm diameter cobalt rods (99.95% and 99.99% purities) were also obtained from Sigma-Aldrich Corp. (St. Louis, Mo.).

All base solutions were prepared using distilled and deionized water with a specific resistance of 18.0 MΩ cm^{-1} produced by a distilled water system (model MP-6A, Corning Inc., Corning, N.Y.). The Tris buffer solution (pH 7) consisted of 0.01 mol/L tris(hydroxymethyl) aminomethane (Tris, Fisher Scientific, Cincinnati, Ohio) with 0.0045 mol/L H_2SO_4 (sulfuric acid, Sigma-Aldrich). The KHP buffer solution (pH 4) was prepared using 0.025 mol/L potassium acid phthalate (KHP, Sigma-Aldrich). The Kelowna extractant solution contained 0.25 mol/L CH_3COOH (acetic acid, Sigma-Aldrich) and 0.015 mol/L NH_4F (ammonium fluoride, Sigma-Aldrich). All other chemicals used were of analytical reagent grade and purchased from Sigma-Aldrich Corp. (St. Louis, Mo.) and Fisher Scientific (Cincinnati, Ohio).

PREPARATION OF ION-SELECTIVE MEMBRANES AND ELECTRODES

A phosphate membrane-casting solution containing bis(p-chlorobenzyl)tin dichloride was prepared with a mixture of 70.2 mg (18% wt) of bis(p-chlorobenzyl)tin dichloride, 133.5 mg (34% wt) of polyvinyl chloride (PVC), 141.9 mg (36% wt) of dibutyl sebacate, and 48.3 mg (12% wt) of N,N-dimethylformamide in 3 mL of tetrahydrofuran (THF), as reported in previous studies (Glazier and Arnold, 1991). The phosphate membranes were formed by dipping the free ends of ISE electrode bodies (Hitachi Ltd., Tokyo, Japan) in the casting solution three times. Membranes were allowed to dry overnight after the first two dips. Following the final dip, the membranes were again allowed to dry and then were stored in a blank buffer solution. The tin dichloride-

based membrane ISEs were constructed using 0.1 mol/L KCl as an internal filling solution in the electrode body and inserting an Ag/AgCl reference electrode (1 mm in diameter) into the top.

According to the methods described by Sasaki et al.(2004), the tributyltin-based PVC membranes were prepared from a mixture of 1.5 mg (1 wt %) of tributyltin chloride, 99 mg (66 wt %) of NPOE, 49.5 mg (33 wt %) of PVC, and 1.0 mg (0.25 mol, relative to the ionophore) of NaTFPB in 1.5 mL of THF. The cocktail was poured into a 23 mm glass ring resting on a temperature-controlled (40°C) polished glass plate and allowed to evaporate. Three disks with a diameter of 2.5 mm were cut from each membrane. The membrane disks were attached to the ends of the ISE electrode bodies using THF solvent. The tributyltin-based electrode was filled with 0.1 mol/L NaCl.

The cobalt-based electrodes with 99.95% and 99.99% purities (5 mm in diameter) were prepared according to the following procedures. The cobalt rods were cut into 6 mm long segments and soldered to copper wires with a diameter of 1 mm. A rubber O-ring (6 mm outer diameter, 1 mm thickness) was inserted into a hole (6 mm diameter and 7 mm depth) drilled in a Hitachi ISE body. The cobalt rod was then pressed into the hole of the electrode. To make sure that there was no electrical contact between the brass shield of the ISE body and the rod and to provide a liquid-tight seal, the gap between the electrode body and the rod was filled with silicone and allowed to cure overnight. A double-junction Ag/AgCl electrode (model PHE 3211, Omega Engineering, Stamford, Conn.) was used as the reference electrode containing 1 mol/L LiAc as the outer reference solution.

ELECTRODE CONDITIONING AND PRETREATMENT

As described by Glazier and Arnold (1991), the two PVC-based phosphate ISEs (bis(p-chlorobenzyl)tin dichloride and tributyltin chloride) were conditioned overnight in blank buffer solutions (Tris at pH 7.00 ±0.01 and KHP at pH 4.00 ±0.01, respectively). Prior to testing, the electrodes were immersed in the 0.01 mol/L phosphate solutions (KH_2PO_4 or K_2HPO_4) three times for about 10 min each so that steady electrical potentials could be obtained in the presence of phosphate.

In accordance with the methods prescribed by Xiao et al. (1995), each cobalt electrode was pretreated using the following steps. Prior to testing, the surface of the cobalt electrode was polished using 400 grit and then 1500 grit emery sheets. The cobalt electrode was then immersed in DI water for about 20 min. After a steady-state potential was obtained,

the electrode was immersed in a blank base solution containing no phosphate (potassium hydrogen phthalate (KHP) or Kelowna) for about 20 min. After a new steady-state potential was established as an indication of completion of the pretreatment process, the electrode was ready for use.

TEST EQUIPMENT

Electrode tests were carried out using a previously designed automated test stand. This test stand included a sample solution holder into which the electrodes were inserted, containers to hold test and rinse solutions, and a computer-based data acquisition and control system (Kim et al., 2006). Test electrode EMF values (mV) relative to a double-junction reference electrode were measured and recorded at 15 s and 60 s after each test solution was automatically introduced into the sample holder. At each of the two data collection times, three measurements, each consisting of the mean of a 0.1 s burst of 600 Hz data, were obtained on a 3 s interval and averaged. The solution was stirred by rotation of the sample holder at 37 rpm during data collection. Three rinses were used at each solution exchange to completely remove any residue of the previous solution. Test and rinse solutions were expelled by increasing the rotational speed of the sample holder to 290 rpm. In each test, three replications of each sequence were conducted.

TEST PROCEDURES

Different test sequences were required to evaluate the three different candidate electrodes (table 1). According to information reported in previous studies (Glazier and Arnold, 1991; Xiao et al., 1995; Sasaki et al., 2004), the PVC-based membranes containing bis(p-chlorobenzyl)tin dichloride respond to dibasic phosphate (HPO_4^{2-}), whereas the tributyltin-based PVC membranes and cobalt-based electrodes are sensitive to monobasic phosphate ($H_2PO_4^-$). Since the phosphate species in solution is a function of pH (fig. 1; Lindsay, 1979), separate test sequences were needed for (1) the tin dichloride-based membrane ISEs responding to dibasic phosphate, and (2) the tributyltin-based membrane and cobalt ISEs responding to monobasic phosphate. For each test sequence, sample pH levels were adjusted to ensure that sufficient concentrations of the appropriate phosphate species were present, as detailed below.

Dibasic Phosphate Sensitivity Tests

In the dibasic phosphate tests (table 1), phosphate ISEs based on bis(p-chlorobenzyl)tin dichloride were evaluated. The response characteristics of the electrodes were examined

Table 1. Key parameters of sensitivity and selectivity tests for the three ISE types evaluated in this study.

| ISE Type	Sensed Ion	Test Type	Number of Electrodes	Base Solution
Bis(p-chlorobenzyl)tin dichloride	HPO_4^{2-}	Sensitivity	Six	Tris buffer (pH = 7.0) and Kelowna (pH = 8.5)
		Membrane age	Six (age 4 days), plus one each of age 14, 20, and 33 days	Tris buffer (pH = 7.0)
Tributyltin chloride	$H_2PO_4^-$	Sensitivity	Three	KHP buffer (pH = 4) and Kelowna (pH = 4)
Cobalt	$H_2PO_4^-$	Sensitivity	Three of 99.99% purity Three of 99.95% purity	KHP buffer (pH = 4) and Kelowna (pH = 4)
		Selectivity and rate of response	Three of 99.99% purity Three of 99.95% purity	KHP buffer (pH = 4) and Kelowna (pH = 4)
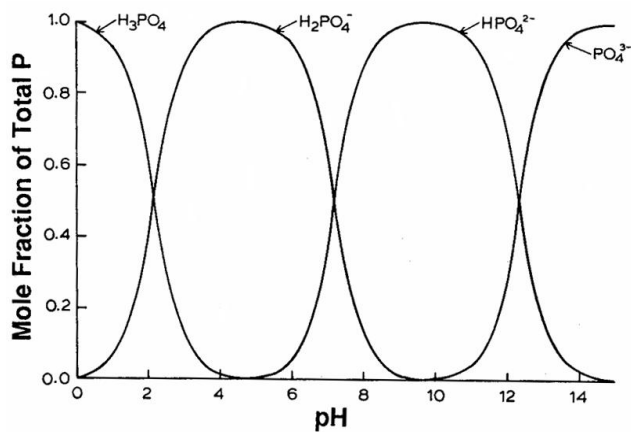
Figure 1. Distribution of orthophosphate ions depending on pH level (Lindsay, 1979).

by measuring the EMFs of each ISE in six standard solutions of K_2HPO_4 containing from 10^{-6} to 10^{-1} mol/L concentrations. The standard solutions were prepared by successive 10:1 dilutions of the 0.1 mol/L concentration using each of two different base solutions (i.e., Tris buffer and Kelowna). We used the Tris buffer to provide results comparable to previous research by Glazier and Arnold (1991). The separate tests using the Kelowna solution provided results applicable to a multiple-nutrient analysis system incorporating our previously identified nitrate and potassium-sensitive membranes (Kim et al., 2006).

To remove any pH effect, the pH levels of the tested solutions were adjusted to be constant across a range of tested phosphate concentrations, i.e., Tris buffer pH = 7.0 and Kelowna solution pH = 8.5. The pH adjustment was monitored with a combination a pH electrode (model 81-72, Orion, Cambridge, Mass.) and a pH meter (model SA-720, Orion, Cambridge, Mass.) while adding either 0.05 mol/L H_2SO_4 or 0.1 mol/L NH_4OH.

Duplicating the pH level (pH = 7.00 ±0.01) for the Tris buffer solutions used by Glazier and Arnold (1991) was done to allow a comparison with those results; however, at this pH level, a portion of the phosphate is not in the dibasic form detected by the ISE (fig. 1). When using the Kelowna extractant as the base solution, the pH of the standard solutions (original pH = 3.2) was readjusted to 8.5 ±0.01, where the predominant form is dibasic phosphate (fig. 1). Another advantage was that pH 8.5 was above the range of pH where small additions of a base solution produced rapid pH changes.

An auxiliary test conducted with the Tris buffer solution investigated the effect of membrane age on response to phosphate. Membranes prepared on four different dates were included in the test: the six membranes of age 4 days used in the primary sensitivity test, and one each of 14, 20, and 33 days age. When they were new, responses of the older (≥14 days) membranes had been similar to those of the 4-day-old membranes. Therefore, we assumed that response differences seen in this test were attributable to aging of the membranes.

Monobasic Phosphate Sensitivity and Selectivity Tests

In the monobasic tests, two different types of phosphate ISEs (i.e., three tributyltin-based PVC membranes and six cobalt-based electrodes) were evaluated with the KHP buffer and Kelowna solutions (table 1). Two sets of three cobalt-based electrodes with purities of 99.99% and 99.95%, respec-

tively, were tested to investigate how cobalt purity affected sensing performance in terms of sensitivity and selectivity. If its performance was acceptable, then using the lower purity (and considerably less expensive) form of cobalt could provide a significant cost advantage.

The sensitivity responses of all electrodes were characterized by measuring the EMFs of each ISE in seven standard solutions of monobasic potassium phosphate (KH_2PO_4) containing 10^{-7} to 10^{-1} mol/L concentrations. The standard solutions were prepared by successive 10:1 dilutions of the 0.1 mol/L concentration using each of the two different base solutions (i.e., KHP buffer and Kelowna).

The 0.025 mol/L potassium hydrogen phthalate (KHP) solution at pH 4 was used as a base solution to determine if the responses of the cobalt electrodes to phosphate would be comparable to results previously reported by Xiao et al. (1995), who used the same solution. As in the dibasic tests, the Kelowna solution provided results applicable as a part of our multiple-nutrient analysis system (Kim et al., 2006). The seven standard solutions prepared using the Kelowna extractant were titrated with 1 mol/L NH_4OH to pH 4.00 ±0.01, where the predominant form is monobasic phosphate (fig. 1).

Using each of the two different base solutions, the selectivity of the cobalt electrodes for phosphate over six interference ions with single ion molecules was investigated in the following order using potassium salts: bicarbonate ($KHCO_3$), chloride (KCl), bromide (KBr), nitrate (KNO_3), acetate (CH_3COOK), and fluoride (KF). The selectivity coefficient (K_{ij}) of the phosphate electrode for an interfering ion (j) relative to phosphate (i) was calculated based on the separate solution method (SSM) (IUPAC, 1994) by using EMF values obtained with pure single electrolyte solutions of the primary ion (0.01M) and interference ion (0.1 M), as follows:

$$K_{i,j} = 10^{(E_j - E_i)/S} \frac{a_i}{a_j^{Z_i/Z_j}} \qquad (1)$$

where
E_i = electrode potential measured with a solution of 0.01 mol/L phosphate ion
E_j = electrode potential measured with a solution of 0.1 mol/L interfering ion
S = Nernstian slope obtained with 0.01 and 0.1 mol/L phosphate solutions
a_i = activity of 0.01 mol/L phosphate ion
a_j = activity of 0.1 mol/L interfering ion
Z_i = charge of primary ion
Z_j = charge of interference ion.

As in a previous study with nitrate-sensitive membranes (Birrell and Hummel, 2000), the EMFs in 0.1 and 0.01 mol/L phosphate solutions were measured to determine Nernstian slopes (S) for each phosphate electrode. The effect of each interfering ion (i.e., $E_j - E_i$) was measured based on the EMF difference between the responses of the 0.01 mol/L phosphate ion and the 0.1 mol/L interfering ion. Thus, in the test sequence, initially the electrode response in the 0.01 mol/L KH_2PO_4 solution was recorded, followed by the response in the 0.1 mol/L KH_2PO_4 solution to calculate the Nernstian slope of each electrode. Then, the response in the 0.01 mol/L KH_2PO_4 solution was re-measured to calculate E_i, followed by the response in the 0.1 mol/L solution of an interfering ion to calculate E_j. The latter two steps were repeated until the responses of the electrodes to all six interference solutions were recorded. The initial two steps were then repeated to check

for any significant change in the phosphate response of the electrodes during each replication.

CALCULATION OF PHOSPHATE SPECIES ACTIVITIES

To calculate sensitivity slopes for monobasic or dibasic phosphate-selective electrodes in the tested concentration ranges, the activity of dibasic and monobasic phosphate species in solution was calculated using an iterative method. The approach considers change in ionic strength and uses known equilibrium constants for the reaction of phosphates in solution, because the ionic strength is a function of the solution pH due to phosphate species equilibrium with the hydrogen ion activity (Lindsay, 1979; Carey and Riggan, 1994).

The total orthophosphate concentration can be calculated as:

$$[PO_4]_{total} = [H_3PO_4] + [H_2PO_4^-]$$
$$+ [HPO_4^{2-}] + [PO_4^{3-}] \qquad (2)$$

where
$[PO_4]_{total}$ = total orthophosphate concentration
$[H_3PO_4]$ = concentration of phosphoric acid
$[H_2PO_4^-]$ = concentration of monobasic (dihydrogen) phosphate
$[HPO_4^{2-}]$ = concentration of dibasic (hydrogen) phosphate
$[PO_4^{3-}]$ = concentration of phosphate.

The equilibrium constant between monobasic and dibasic phosphates in the pH range of 5 to 10 can be represented as:

$$\log \frac{[H_2PO_4^-]}{[HPO_4^{2-}]} = 7.20 - pH \qquad (3)$$

The equilibrium constants in the pH range of 0 to 5 and 10 to 14, respectively, can be described as:

$$\log \frac{[H_3PO_4]}{[H_2PO_4^-]} = 2.15 - pH \qquad (4)$$

$$\log \frac{[HPO_4^{2-}]}{[PO_4^{3-}]} = 12.35 - pH \qquad (5)$$

The ionic strength was calculated using the concentrations estimated by the equations for equilibrium constants described above, and activity coefficients for dibasic or monobasic phosphate species were then determined using the Debye-Hückel formula (Lindsay, 1979; Eggins, 2002).

RESULTS AND DISCUSSION

TIN DICHLORIDE ISE SENSITIVITY

The response (EMF) curves of the dibasic-selective phosphate ISEs to varying potassium phosphate (K_2HPO_4) concentration in pH 7 Tris buffer and Kelowna solution (pH = 8.5) are shown in figure 2. In each of the three replicates of the test sequence, successively more concentrated test solutions were presented to the ISEs. The bis(p-chlorobenzyl)tin dichloride-based phosphate membranes in the Tris buffer solution (fig. 2a) were sensitive to different phosphate concentrations, and the responses were repeatable during three replicate measurements. The use of Kelowna solution reduced the responses of the phosphate ISEs considerably (fig. 2b), thereby resulting in little change in EMF in the range of 10^{-6} to 10^{-2} mol/L total phosphate concentration. The responses of five of the six ISEs in the Kelowna solution were relatively consistent during three replicate measurements, while one electrode (P-06) produced higher EMF values in the first part of the first replication (fig. 2b). A possible reason for this variation in response might be that electrode P-06 was not completely conditioned prior to testing.

Since variations in standard potential among electrodes occur due to differences in internal resistance and thickness of the membrane (Carey and Riggan, 1994), the electric potential was normalized by setting the EMF values obtained at 10^{-1} mol/L total phosphate concentration in the first replication to 0 mV. This procedure removed variability between electrodes in terms of standard potential, while allowing differences between replications to be evaluated.

The sensitivity curves of each membrane type to varying phosphate concentrations were obtained when using the Tris buffer (fig. 3a) and the Kelowna solution (fig. 3b) as base
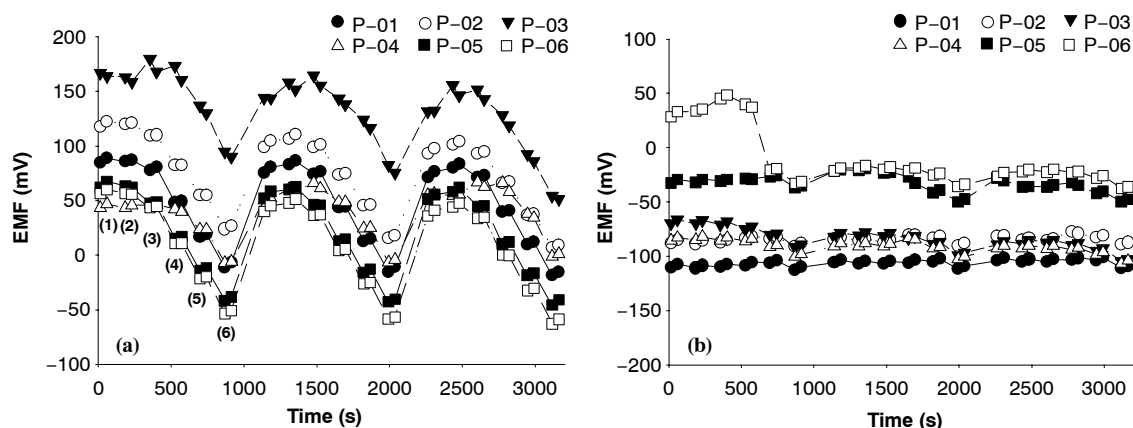

Figure 2. Response test profiles of six ISEs using dibasic phosphate membranes with bis(p-chlorobenzyl)tin dichloride for different K_2HPO_4 concentrations in (a) Tris buffer and (b) Kelowna extractant, The numbers in (a) identify the different K_2HPO_4 concentrations: (1) 10^{-6}, (2) 10^{-5}, (3) 10^{-4}, (4) 10^{-3}, (5) 10^{-2}, and (6) 10^{-1} mol/L.
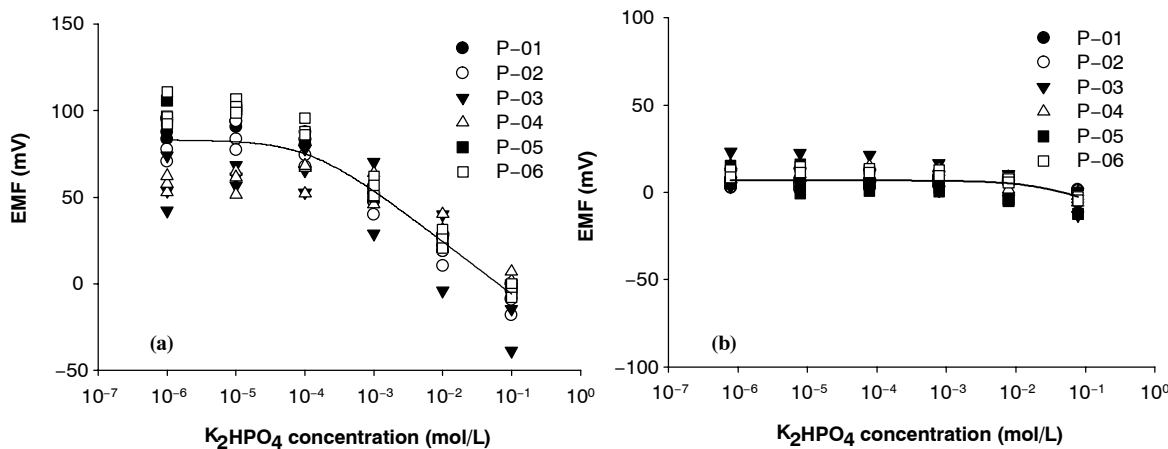
Figure 3. Response of each ISE using bis(p-chlorobenzyl)tin dichloride membranes to different K_2HPO_4 concentrations: dibasic phosphate membrane response in (a) Tris buffer and (b) Kelowna extracting solution.

solutions. In the Tris buffer solution (pH = 7.00 ±0.01), the EMF values obtained with the phosphate membranes were nearly linearly proportional to the logarithm of total phosphate concentration in the range of 10^{-4} to 10^{-1} mol/L with a mean sensitivity slope of −30.9 ±4.5 mV per activity decade of HPO_4^{2-}, which was comparable to the sensitivities reported in previous studies (Glazier and Arnold, 1988). However, as shown in figure 3a, the variability in response among electrodes was high. Analysis of variance showed that the sensitivity of two electrodes (P-03 and P-04) was significantly lower than that of the other electrodes (fig. 4). Additionally, the responses of these two electrodes were considerably more variable between replications (data not shown).

In the Kelowna solution (pH = 8.5 ±0.01), the variability among electrodes was less, but all six electrodes were almost insensitive to phosphate below 0.1 mol/L total phosphate concentration (fig. 3b). Therefore, the bis(p-chlorobenzyl)tin dichloride-based ISEs would not be suitable for sensing phosphate when using the Kelowna solution as a base solution.

This decrease in sensitivity may be associated with the presence of a high concentration of fluoride (0.015 mol/L) in the Kelowna solution. Previous studies by Glazier and

Arnold (1991) showed that the selectivity coefficient of the membrane for fluoride is 0.279, which means that the tin-compound-based phosphate membrane is only about 3.58 times more sensitive to dibasic phosphate than to fluoride. When fluoride and dibasic phosphate having the same concentration are dissolved in solution, the ionic activities for fluoride are larger than those for dibasic phosphate, since there is a greater decrease in ionic activity coefficient for dibasic phosphate than for fluoride. For example, at 0.1 mol/L total phosphate concentration, the ionic activity of dibasic phosphate in the pH 8.5 Kelowna solution was approximately 0.01, which is nearly the same as that of 0.015 mol/L fluoride concentration. This means the sensitivity in the 0.1 mol/L phosphate standard may be reduced by about 8 mV (27.9% of 28.2 mV/decade in a range of 10^{-5} to 10^{-1} mol/L) due to interference by the fluoride ion. The reduced sensitivity of about 20 mV for the phosphate concentration change from 0.01 to 0.1 mol/L is of similar magnitude to the sensitivity of −15 to −18 mV/decade obtained in this experiment.

Effect of Membrane Age on Sensitivity

The change in response to phosphate due to membrane age is shown in figure 5. Electrodes of different ages were stored in the pH 7 Tris buffer at room temperature (22.5°C to 23.5°C) between measurements, and then tested simultaneously. SAS GLM (SAS Institute, Cary, N.C.) analysis (table 2) indicated that the sensitivity of the 14-day-old membrane was significantly reduced in comparison to the 4-day-old membrane. These results are comparable to those of Glazier (1988), who reported that the limits of detection increased during a 28-day period as the membranes aged. Possible causes of the deterioration of electrode response might be rapid leaching of the tin compound ionophore from the membrane or a rapid breakdown of the tin compound structure.

TRIBUTYLTIN ISE SENSITIVITY

Figure 6 shows the EMF responses for the three tributyltin-based phosphate ISEs to seven varying potassium phosphate (KH_2PO_4) concentrations ranging from 10^{-7} to 10^{-1} mol/L in the KHP buffer (fig. 6a) and Kelowna solutions (fig. 6b) at pH 4. The responses of two of the three tributyltin-based phosphate ISEs (tributyltin-1 and tributyltin-3)
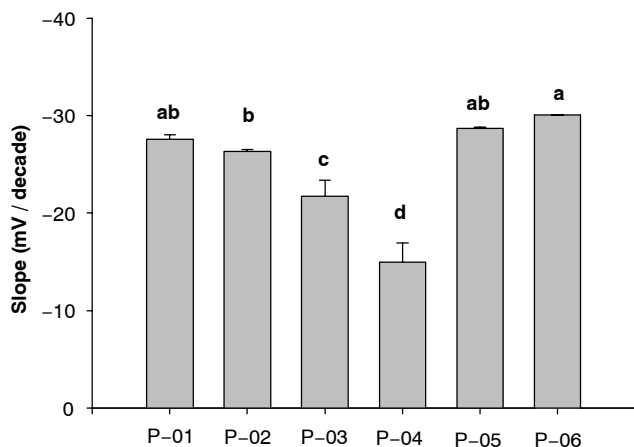

Figure 4. Comparison of sensitivity of six 4-day-old tin dichloride-based electrodes to phosphate in a concentration range of 10^{-5} to 10^{-1} mol/L in Tris buffer. Sensitivity bars with the same letter are not significantly different at the 5% level, based on Duncan's multiple range test.
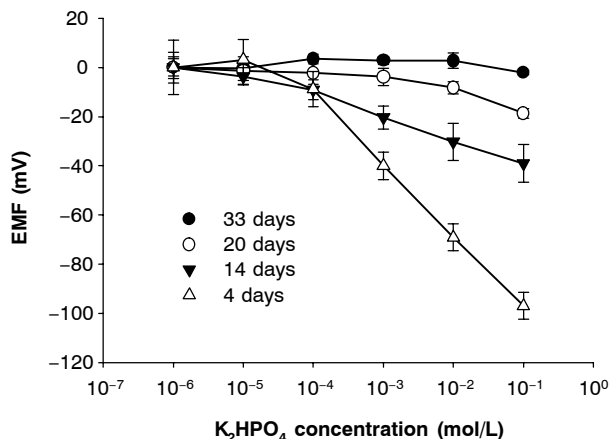
Figure 5. Effect of membrane age on sensitivity of the tin dichloride-based electrodes.

were positive, while the third was relatively insensitive. The positive sensitivity slopes were unexpected because typical anion-selective electrodes, including phosphate ISEs, exhibit negative sensitivity to increases in test solution concentrations (Ammann, 1986). In addition, these test results show a much lower sensitivity, 60 mV over the tested total phosphate range, as compared to a maximum EMF difference of about 160 mV reported in a previous study (Sasaki et al., 2004).

Table 2. Effect of membrane age on the sensitivity response (mV/decade) of bis(p-chlorobenzyl)tin dichloride-based ISEs to phosphate in Tris buffer.

Membrane Age	Total Phosphate Concentration Range[a]		
	10^{-5} to 10^{-1} M	10^{-4} to 10^{-1} M	10^{-3} to 10^{-1} M
4 days	−24.9 a	−30.9 a	−34.8 a
14 days	−9.2 b	−10.2 b	−9.9 b
20 days	−4.3 b	−5.9 bc	−8.8 b
33 days	−3.1 b	−2.8 c	−3.7 c

[a] Membrane sensitivities in the same phosphate concentration range and followed by the same letter are not significantly different at the 5% level, based on Duncan's multiple range test.

Therefore, it was concluded that the tributyltin-based PVC membranes would not be usable for sensing phosphate. The reasons behind the decreased sensitivity of the membrane and opposite slope are unknown. However, a possible cause might be the use of different base solutions. The 0.025 mol/L KHP solution at pH 4.0 was used in this test, whereas in previous work (Sasaki et al., 2004) the sensitivity of the membrane to $H_2PO_4^-$ was investigated using 0.1 mol/L Tris buffer solution at pH 7.0.

COBALT ISE SENSITIVITY

Figure 7 shows the EMF responses for the six cobalt electrodes with two different purities (three each of 99.95%
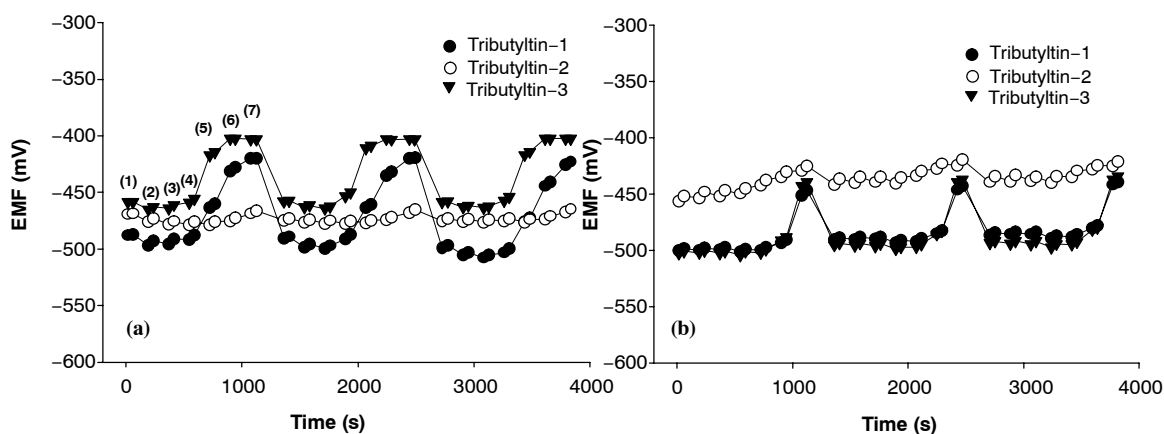

Figure 6. Response test profiles of three PVC membranes containing tributyltin chloride for different KH₂PO₄ concentrations in (a) KHP buffer and (b) Kelowna extractant of pH 4. The numbers in (a) identify the different KH₂PO₄ concentrations: (1) 10^{-7}, (2) 10^{-6}, (3) 10^{-5}, (4) 10^{-4}, (5) 10^{-3}, (6) 10^{-2}, and (7) 10^{-1} mol/L.
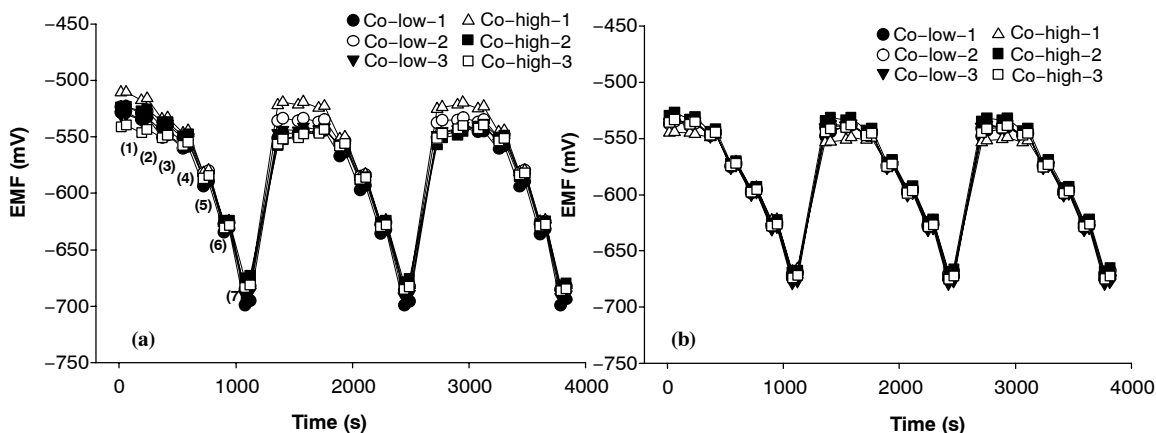

Figure 7. Response test profiles of six cobalt rod electrodes for different KH₂PO₄ concentrations in (a) KHP buffer and (b) Kelowna extractant of pH 4. The numbers in (a) identify the different KH₂PO₄ concentrations: (1) 10^{-7}, (2) 10^{-6}, (3) 10^{-5}, (4) 10^{-4}, (5) 10^{-3}, (6) 10^{-2}, and (7) 10^{-1} mol/L.
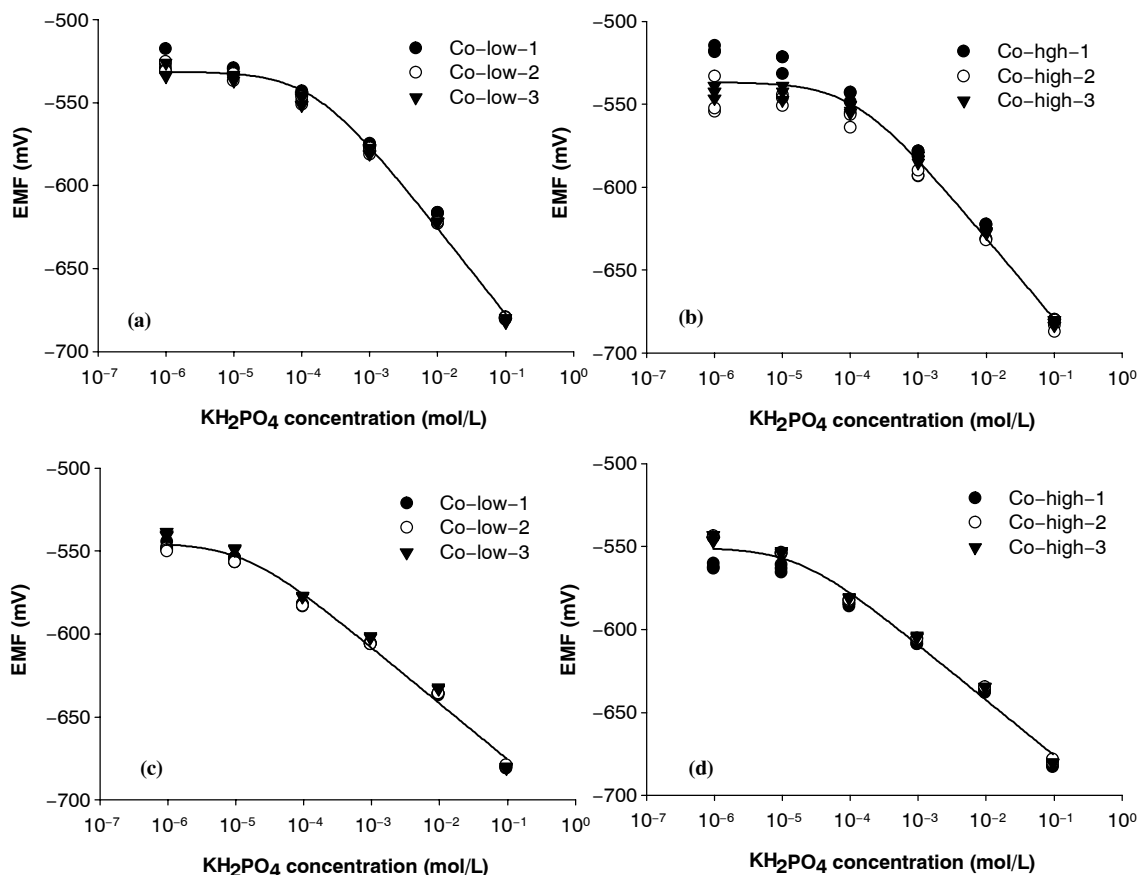
Figure 8. Response of cobalt electrodes with (a, c) low purity of 99.95% and (b, d) high purity of 99.99% to different KH$_2$PO$_4$ concentrations in (a, b) KHP solution and (c, d) Kelowna extracting solution.

and 99.99% purity) to seven varying potassium phosphate (KH$_2$PO$_4$) concentrations ranging from 10^{-7} to 10^{-1} mol/L in the KHP buffer (fig. 7a) and Kelowna extracting solution (fig. 7b) at pH 4. The electrodes responded to phosphate over a wide range (10^{-5} to 10^{-1} mol/L) of total phosphate concentrations, yielding negative sensitivity slopes and repeatable responses during three replicate measurements.

As previously done for comparing sensitivity curves within an electrode type, the electric potential was normalized by setting the EMF values obtained with the cobalt electrodes at 10^{-1} mol/L total phosphate concentration in the first replication to a constant value of −680 mV. Sensitivity curves to varying concentrations of potassium monobasic phosphate (KH$_2$PO$_4$) in the KHP buffer and the Kelowna solution are shown in figure 8. In each of the two different solutions titrated to pH 4, the cobalt electrodes showed sensitive responses to phosphate over a range of 10^{-5} to 10^{-1} mol/L total phosphate concentration, with a linear range of 10^{-4} to 10^{-1} mol/L and a detection limit of about 10^{-5} mol/L total phosphate concentration.

As shown in table 3, the sensitivity of the cobalt electrodes over different total phosphate concentration ranges was strongly affected by the base solution. In general, the sensitivity slopes in the Kelowna solution were decreased by 6 to 14 mV per activity decade of H$_2$PO$_4^-$ as compared to those obtained in the KHP buffer. However, when tested in the Kelowna solution, the usable portion of the phosphate concentration:EMF curve appeared to be from 10^{-5} to 10^{-1} mol/L total phosphate (figs. 8c and 8d), which encompasses

the range of interest (3.2×10^{-5} mol/L to 9.7×10^{-5} mol/L). This range of interest corresponds to 10 to 30 mg L^{-1} P in soil at a dilution ratio (solution:soil) of 10:1 for soil P sensing (Buchholz et al., 1983).

Analysis of variance conducted with SAS PROC GLM (SAS Institute, Inc., Cary, N.C.) showed that there was generally no significant difference in sensitivity between electrodes of different purities of cobalt (99.95% and 99.99%) in the tested phosphate concentration range (table 3). However, the standard deviations of the sensitivity of the low-purity cobalt electrodes were lower than those measured with the high-purity electrodes. This potential for superior repeatability with the lower-purity electrodes, coupled with a significantly

Table 3. Means and standard deviations of sensitivity slopes (mV/decade) of cobalt ISEs with low (99.95%) and high (99.99%) purities.

Base Solution and Electrode	Total Phosphate Concentration Range[a]		
	10^{-5} to 10^{-1} M	10^{-4} to 10^{-1} M	10^{-3} to 10^{-1} M
KHP Buffer			
Cobalt-low	−37.2 ±0.4 a	−45.0 ±0.9 a	−52.7 ±1.0 a
Cobalt-high	−36.5 ±2.3 a	−43.8 ±2.0 a	−49.7 ±2.8 b
Kelowna Solution			
Cobalt-low	−30.9 ±0.9 a	−32.9 ±0.9 a	−38.0 ±1.1 a
Cobalt-high	−30.9 ±2.5 a	−32.6 ±1.8 a	−36.2 ±2.6 a

[a] Mean membrane sensitivities within the same phosphate concentration range and base solution combination and followed by the same letter are not significantly different at the 5% level.
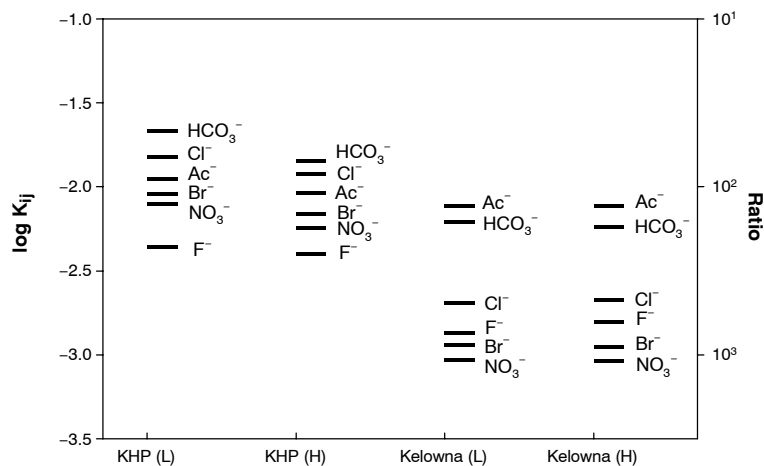
Figure 9. Selectivity coefficients of cobalt electrodes with purities of 99.95% (L) and 99.99% (H) measured in KHP and Kelowna solutions.

lower cost, makes them a better choice for phosphate sensing as compared to the higher-purity electrodes.

COBALT ISE SELECTIVITY

As only the cobalt ISEs were able to provide acceptable sensitivity to phosphate in the Kelowna solution, selectivity was only evaluated for these electrodes. A comparison of potentiometric selectivity coefficients (log K) with respect to the six interference anions, obtained using the separate solution method, is summarized in figure 9 and table 4. As shown in figure 9, the selectivity for monobasic phosphate over the tested anions was enhanced when the cobalt electrodes were tested in the Kelowna solution compared to the KHP solution.

The selectivity patterns for these electrodes tested in different base solutions can be described in the following order:

$H_2PO_4^-$ >> HCO_3^- > Cl^- > Ac^- > Br^- > NO_3^- > F^-
in KHP buffer

$H_2PO_4^-$ >> Ac^- > HCO_3^- > Cl^- > F^- > Br^- > NO_3^-
in Kelowna solution.

In the KHP buffer, the cobalt ISEs with high purity (99.99%) exhibited higher selectivity for monobasic phosphate over four anions (i.e., bicarbonate, chloride, bromide, and nitrate) than did the 99.95% purity cobalt electrodes (table 4). However, in the Kelowna solution, there were no significant differences in selectivity coefficients between the different purities of cobalt electrodes.

RATE OF COBALT ISE RESPONSE

The response rate of the six cobalt electrodes was investigated by relating the EMF values taken at 15 s (premeasure) to those obtained at 60 s (measure) after each test solution was introduced. As shown in table 5, the measure EMF (Y) values were strongly related to the premeasure EMFs (X), yielding an almost 1:1 relationship and a very small y-intercept. Therefore, it was expected that the cobalt ISEs could reach an equilibrium response within 15 s after immersion in a test solution.

CONCLUSIONS

Two types of phosphate sensors, tin-compound-based PVC membranes and cobalt rods with different purities, were evaluated for sensitivity and selectivity to different phosphate concentrations in standard buffer solutions (pH 7 Tris and pH 4 KHP) and Kelowna soil extractant.

The PVC-based phosphate membranes containing an organotin compound, bis(p-chlorobenzyl)tin dichloride, exhibited sensitive responses over a range of 10^{-4} to 10^{-1} mol/L total phosphate concentrations in the Tris buffer of pH 7 with an average slope of -30.9 ± 4.5 mV per activity decade of HPO_4^{2-}, which is comparable to results obtained in previous studies (Glazier and Arnold, 1988, 1991). However, the membrane was not usable for determination of phosphates when using the Kelowna solution because of its insensitivity to phosphate over the 10^{-6} to 10^{-2} mol/L concentration range in the Kelowna extractant. In addition, the functional lifetime of this membrane (<14 days) was less than expected. Moreover, the results of another tin-compound-based PVC membrane containing tributyltin chloride as the ionophore were not satisfactory, showing much less sensitivity, and with a positive sensitivity slope rather than a negative slope as reported in previous studies (Sasaki et al., 2004).

Table 4. Comparison of selectivity coefficients
(log K_{ij}) of cobalt ISEs by extractant type.

Base Solution and Electrode	Interference Ion[a]					
	HCO_3^-	Cl^-	Br^-	NO_3^-	Ac^-	F^-
KHP Buffer						
Cobalt-low	−1.67 a	−1.82 a	−2.04 a	−2.10 a	−1.96 a	−2.36 a
Cobalt-high	−1.85 b	−1.92 b	−2.16 b	−2.24 b	−2.04 a	−2.40 a
Kelowna Solution						
Cobalt-low	−2.21 a	−2.69 a	−2.94 a	−3.03 a	−2.11 a	−2.87 a
Cobalt-high	−2.24 a	2.68 a	−2.95 a	−3.04 a	−2.11 a	−2.80 a

[a] Membrane selectivity coefficients within the same ion species and base solution combination and followed by the same letter are not significantly different at the 5% level.

Table 5. Relationship between EMF values (mV) measured
at 15 s (premeasure, X) and 60 s (measure, Y) after
the injection of test solutions for six cobalt ISEs.

Base Solution	Electrode	Equation	R^2
KHP buffer	Cobalt-low	$Y = 0.99X - 0.96$	0.99
	Cobalt-high	$Y = 0.99X + 1.91$	0.99
Kelowna solution	Cobalt-low	$Y = 0.99X + 0.94$	0.99
	Cobalt-high	$Y = 0.99X + 1.58$	0.99

The cobalt rod-based electrodes with purities of 99.95% and 99.99% exhibited sensitive responses over a range of 10^{-5} to 10^{-1} mol/L total phosphate concentration with a detection limit of 10^{-5} mol/L and a rapid response time of ≤ 15 s when tested in the Kelowna solution. This range encompasses the range of phosphorus concentrations typically found in agricultural soil, assuming a 10:1 dilution ratio. The selectivity of the cobalt electrodes was satisfactory for measuring phosphates in the presence of each of six possible interfering ions, i.e., HCO_3^-, Cl^-, Br^-, NO_3^-, Ac^-, and F^-. The electrodes were 47 to 1,072 times more selective to phosphate than to the tested ions. Although the cobalt electrodes with different purities (99.95% and 99.99%) showed similar sensitivity and selectivity performance, the lower-purity cobalt rod is a better choice, due to a significantly lower cost and potentially more reproducible responses than electrodes fabricated using the higher-purity cobalt rod.

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