

Cation Exchange Capacity and Proton Binding Properties of Pea (*Pisum sativum* L.) Roots

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Received: 6 August 2008 / Accepted: 3 November 2008 / Published online: 30 November 2008
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Abstract Risk assessment of trace-metal contamination in soils requires predictive models that take into account the interaction of metal ions with other cations (e.g., H^+ and Ca^{2+}) that can change the speciation of trace metals in solution and compete for binding sites on plant roots thus affecting metal uptake and toxicity. Acid–base titrations were used to estimate the types and quantity of cation-binding sites on fresh pea (*Pisum sativum* L. cv. Lincoln) roots and their binding strength with protons. The roots were found to have three types of cation-binding sites with site densities of 190, 382, and 347 $\mu\text{mol}_c \text{g}^{-1}$ (dry weight), respectively. The binding strength with H^+ was indicated by the equilibrium formation constants (K_{HLj}). The $\log K_{HLj}$ values under different ionic strengths were determined. At zero ionic strength, the $\log K_{HLj}$ values are estimated to be 2.5, 5.5, and 8.3, respectively. Complementary experiments were used to validate the titration results. These included an ion exchange experiment, an experiment with HCl extractions, and a KOH neutralization method. Estimates from all four methods were consistent under the experimental conditions. The quantification of the

binding capacity and the characteristics of these binding sites will assist in the development of more appropriate solution speciation models that incorporate biotic ligands. The derived parameters will provide the basis on which further development of a biotic ligand model is dependent.

Keywords Bioavailability · Biotic ligand model · Cation exchange capacity · Formation constants · Metals · Titration

1 Introduction

Trace-metal contamination in soils poses potential risks to human and environmental health. These risks need to be assessed using appropriate predictive models. The robustness of a predictive model is sensitive to its accuracy in describing the complex uptake processes in the soil–plant systems, which are usually characterized by the coexistence of, and interaction between, multiple components. One of the possible approaches to model these interactive processes involves using the thermodynamic equilibrium approximations.

Increasing experimental evidence from hydroponic studies with plants suggested that competing components such as Ca^{2+} , H^+ , and dissolved organic matter (DOM) could affect metal availability and subsequent rhizotoxicity (Baker 1987; Cataldo and Wildung 1978; Chaudhry and Longeregan 1972; Checkai et

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al. 1987; Gabara et al. 1995; Garland and Wilkins 1981; Giordano et al. 1974; Göthberg et al. 2004; Grauer and Horst 1992; Hatch et al. 1988; Lexmond and van der Vorm 1981; Parker and Pedler 1997; Parker et al. 2001; Rains et al. 1964; Tyler and McBride 1982). The effect of solution chemistry on metal bioavailability and toxicity depends on the properties of the metal, the properties of the competing components in the solution, and the characteristics of the roots. Therefore, the biotic ligand model (BLM), a thermodynamic equilibrium model that has been widely used in aquatic toxicology studies (Morel 1983; Paquin et al. 2002), could be a promising approach to integrate the interaction of a metal with aqueous phase ligands affecting its speciation and the interaction of that metal with the receptor sites on the plant roots while competing with other cations (Voigt et al. 2006).

When we use the BLM in the study of plants, the cation-binding sites of terrestrial plants can be assumed to be at the surface of the roots; the root cell surface (X-Cell) can be treated as an assemblage of biotic ligands in soil solutions. Metals compete with other cations such as Ca^{2+} and H^+ for binding to the root cell surface and the surface of soil particles. At the same time, negatively charged ligands in a solution such as Cl^- , SO_4^{2-} , and DOM compete with the roots for complexation with metal ions.

Different plant species are thought to have different types of cation-binding sites and varied binding strengths (Grignon and Sentenac 1991). In general, root cell walls possess cation-exchange properties, probably due to the negative charges of galacturonic acid residues within the pectin polysaccharides (Clarkson 1988; Lauchli 1976). Chemical analyses revealed that there were several types of ionogenic groups (amine, carboxyl, phenolic, and probably phosphate groups) in the composition of the root cell wall that can take part in ion-exchange reactions (Meychik and Yermakov 1999). The root cell walls control ion movement, and the root plasma membrane is thought to be exposed to a solute that has been modified by the apoplast (Haynes 1980). From another point of view, the cell wall is considered to be an insignificant barrier to the flux of ions while the plasma membrane is the barrier to ion uptake. The plasma membrane has a basic composition of phospholipids and proteins. Its lipid bilayers are hydro-

phobic and have electrostatic properties. Associated proteins function as receptors, pumps, channels, and transporters of ions at the membrane surfaces. The driving force for cation uptake across the membrane is the large negative electrochemical potential (-100 to -200 mV) inside the membrane created by the hydrolysis reaction of adenosine triphosphate (ATP), which is mediated by membrane H^+ translocating adenosine triphosphatases (ATPases) (Serrano 1990).

No matter what molecular mechanism actually governs the uptake of metal ions in plant roots, the root cell surface can be generalized as one or more negatively charged biotic ligands. There have been various approaches to modeling cation binding to plant roots (Allan and Jarrell 1989; Amory and Dufey 1984; Cheng and Allen 2001; Grauer and Horst 1992; Kinraide et al. 1998; Meychik and Yermakov 2001; Morvan et al. 1979; Sentenac and Grignon 1981; Voigt et al. 2006). Kinraide et al. (1998) developed a root surface chemistry model to compute the sorption of ions by wheat and succeeded in using it to explain metal ion rhizotoxicities. Almost all of these models treated the root cell wall or the whole plant root as hypothetical negatively charged ligands with discrete or continuous distributions of binding constants with cations, or with an explicit electrostatic energy term.

Thermodynamic equilibrium models have long been used for modeling speciation of trace metals in aqueous systems (Bassett and Melchior 1990). If the same concept is to be used to describe the interaction of cations (protons, major cations, and trace metal ions) in the soil solution with plant roots, we need to find a quantitative method to parameterize the heterogeneous nature of the plant root itself as well as its complicated interaction with the cations. In this way, the bioavailable pool of metals can be predicted and related to rhizotoxicity. In particular, there is a need to evaluate the total number of cation-binding sites and the stability constants of various cations with the negatively charged sites.

In this study and succeeding studies, a biotic ligand model is to be developed to describe the acute toxicity of Cd, Cu, Ni, and Zn to pea roots. As the first part of the BLM developmental work, in this study, experiments were designed to estimate the fundamental parameters for describing the ion-binding properties of plant roots, under the conceptual framework of the BLM. These parameters are:

1. The total number of cation-binding sites of each type of functional group on the root cell surface (Q_{Lj}).
2. Their binding strength with cations (H, Ca, Mg, Cd, Cu, Ni, and Zn), which is represented by the equilibrium formation constants (K_{MeLj}).

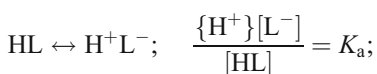
The effect of pH on the availability and toxicity of trace metals needs to be addressed first. Studies with soils revealed that low pH could increase metal toxicity, probably due to speciation effects (Sauvé et al. 1998). Contrasting this, solution studies demonstrate that low pH decreased metal toxicity because H^+ acts as a competing ion for binding to the sites of toxic action (Parker et al. 1998). Further work could be done to investigate the effect of H^+ on both the surface-sorption processes and the plants' physiological processes.

In this paper, estimates of the types and quantity of cation-binding sites on fresh pea roots and their binding strength with protons (H^+) by using acid–base titration techniques are presented. The titrations were conducted using excised fresh pea roots to remove the influence of translocation from the adsorption process. In addition, ion-exchange experiments using relevant metal ion (Cd, Cu, Ni, and Zn) solutions at realistic ranges of pH and ionic strength were used to validate the accuracy of the estimates from the acid–base titrations.

2 Theoretical Background of an Acid–Base Titration

The fresh pea root is considered as a collection of biotic ligands having several types of weak acidic groups (HL_j). The objective of the acid–base titration is to determine the total number of cation-binding sites (Q_{Lj}) of each type of these ionogenic groups (L_j^-) in the root cells and their dissociation constants (K_{aj}). By finding the inflexion point(s) of a titration curve, one can define the equivalence point(s) that can be used to calculate Q_{Lj} . After the values of Q_{Lj} are determined, the modified Henderson–Hasselbach equation can be used to estimate the dissociation constants of each type of the weak acidic groups:

For each type of the weak acidic group HL_j , the dissociation equilibrium of HL is:



Where K_a is the dissociation constant, $\{ \}$ represents equilibrium activity and $[]$ represents equilibrium concentration. The solution pH at equilibrium (pH_e) is related to the degree of dissociation (α) of the weak electrolyte by the following modified Henderson–Hasselbach equation:

$$pH_e = pK_a + n \log(\alpha/(1 - \alpha)) \quad (1)$$

pK_a is equal to $-\log K_a$, α is the fraction of the weak acid that dissociates, $1 - \alpha$ is the remaining fraction of undissociated molecules at equilibrium, $\log X$ represents $\log_{10} X$. n ($0 < n < 1$) allows for an empirical representation of the non-ideal exchange of cations, where each binding site is statistically not always occupied with only one of the cations. The modified Henderson–Hasselbach equation has been successfully used to describe the acid–base properties of synthetic polyfunctional ion exchangers (Leykin et al. 1978; Meychik et al. 1989).

If pH_e is plotted against $\log(\alpha/(1 - \alpha))$, a straight line with a slope of n and the y -intercept of pK_a (Eq. 1) would be generated. The formation constant (K_H) of the weak acidic group with H^+ is equal to K_a^{-1} . In other words, $\log K_H = pK_a$.

Two methods were used to determine the equivalence point. Firstly, dpH/dV against V (volume of strong base added) was plotted and the first-derivative curves were obtained. The local maxima represent the equivalence points. The second method used Gran's linear transformation (Gran 1952). Detailed procedures for finding the equivalence points by Gran's method are presented in the [Appendix](#).

3 Materials and Methods

3.1 Root Sample Preparation

Seeds of pea (*Pisum sativum* L. cv. Lincoln) were surface sterilized for 10 min with 0.3% NaOCl and then germinated in darkness at $22 \pm 1^\circ C$ for 72 h on moist filter paper. Seedlings were placed in open grid polypropylene (PP) floats and transferred to high-density polyethylene (HDPE) pots containing 10 L of continuously aerated 2 mmol L^{-1} $CaCl_2$ solution (60 seedlings per 10 L pot). The seedlings were grown at $22 \pm 1^\circ C$ for 48 h in darkness. The roots were then rinsed with deionized water, excised, and stored

temporarily in PP bottles at room temperature. These samples are designated as the ‘fresh roots’.

For the titration experiments, fresh roots were transformed to the fully protonated form so that a reproducible starting point was established. Fresh roots were soaked in 0.05 mol L^{-1} HCl for 2 h and then rinsed with deionized water until Cl^{-} ions were absent from the effluent (verified by adding a drop of 0.1 mol L^{-1} AgNO_3 into the effluent; no cloudy white precipitate was observed against a black background with a strong side light). After washing, the samples were allowed to drip dry, blotted with micro-fiber cloth, and air dried at room temperature. These samples are designated as ‘standardized roots’. The weak acidic groups on the roots are assumed to have been transformed to H-form.

In order to avoid bacteria and fungi growth on the processed fresh pea root, the standardized acid-form roots should be used in the subsequent experiments as soon as possible. They can be kept in capped PP bottles in the refrigerator for less than 72 h if immediate use is not possible.

3.2 Titration of Standardized H-form Fresh Pea Root with KOH and HCl

All titrant (KOH and HCl) and background electrolyte (KCl) solutions were prepared with low- CO_2 water. The low- CO_2 water was obtained by boiling and stripping the double deionized water with N_2 gas for 1 h and then storing in sealed glass bottles. Continuous titration and pH measurement were conducted in a glove case with its headspace filled with low-pressure N_2 to prevent access to atmospheric CO_2 . Room temperature was stable at $22.0 \pm 0.5^\circ\text{C}$. Titrant (0.085 mol L^{-1} KOH or 0.1 mol L^{-1} HCl) aliquots of $10 \mu\text{L}$ were added using a calibrated pipette. Preliminary experiments indicated that using a magnetic stirrer to agitate the solution caused difficulties in reaching a stable pH reading, especially when the pH was higher than 5.5. It is speculated that the fresh pea root samples might be easily broken down and might disintegrate under higher pH with mechanical force. Therefore, we homogenized the suspension by shaking it on a horizontal reciprocating shaker.

We prepared three series of KCl background electrolyte solutions of 0.001 , 0.01 , and 0.1 mol L^{-1} , respectively. Weighed ($1.016 \pm 0.020 \text{ g}$) duplicate samples of standardized roots were placed in two

bottles that contained 10 mL of KCl solution of one ionic strength (0.001 mol L^{-1} , for example). These duplicate samples were titrated with KOH. Another set of duplicate samples were prepared and titrated with HCl. One set of two bottles containing 10 mL of 0.001 mol L^{-1} KCl only was prepared as the control. The initial pH of these samples was measured before starting the titration. The cycle was repeated for the other two ionic strengths.

Each bottle (sealed) containing the root sample and KCl solution was allowed to equilibrate for 0.5 h before starting the titration. After 0.5 h, the initial pH (pH_i) in each bottle, including the control, was measured. Then 0.01 mL of 0.085 mol L^{-1} KOH or of 0.1 mol L^{-1} HCl was added to each bottle and the bottle was shaken for 15 min. Solutions were measured for final equilibrium pH. Another 0.01 mL of KOH or HCl was added, the solution was shaken for 15 min, and another equilibrium pH was measured. The procedure was continued until the suspension was titrated from the initial pH ($\text{pH}_i \approx 4$) to an equilibrium pH of 2.5 for the HCl titration and 11.8 for the KOH titration.

In each solution, the initial (pH_i) and the equilibrium (pH_e) values of pH were measured by pH meter. The operationally defined equilibrium point was a drift of the pH reading less than 0.01 unit during 30 s after a uniform 15-min shaking.

Concentration of the titrants was verified by performing an acid–base titration of potassium hydrogen phthalate (KHP). It was dried before use in an oven at 50°C for 12 h, rapidly weighed, and titrated with both KOH and HCl.

3.3 Complementary Methods to Validate the pH-dependent Cation Exchange Capacity (CEC) of Fresh Pea Roots

Method 1: Excised fresh roots in exchange with HCl

Fresh roots were excised and rinsed with deionized water, allowed to drip dry, blotted with micro-fiber cloth, weighed ($\approx 3.0 \text{ g}$), and put in 30-mL PP bottles containing 20 mL 0.05 mol L^{-1} HCl. The capped bottles were shaken on a horizontal reciprocating shaker. After 24 h, the HCl solutions were sampled and measured for final pH, for Ca concentration by flame atomic absorption spectrograph (FAAS), and for other cations by inductively coupled plasma-mass

spectrometer (ICP-MS). The total amount of Ca and other divalent cations measured approximates the CEC of fresh roots in exchange with H. Roots were then washed with deionized water, dried at 65°C to constant weight, and accurately weighed. We prepared 16 samples of fresh roots and two controls (0.05 mol L⁻¹ HCl).

Method 2: Standardized roots in exchange with metal chloride solutions

Triplicate samples of weighed (~3.0 g) standardized H-form fresh roots were equilibrated with 3 mmol L⁻¹ MeCl₂ (Me=Cd, Cu, Ni, Zn) solutions. The stock solutions were prepared using analytical grade CdCl₂·21/2H₂O, CuCl₂·2H₂O, NiCl₂·6H₂O, and ZnCl₂ respectively. The capped PP bottles were shaken on a horizontal reciprocating shaker for 24 h. After 24 h, roots were taken out, rinsed with deionized water, dripped dry, blotted with micro-fiber cloth, and then exchanged with 20 mL 0.05 mol L⁻¹ HCl for 24 h as described above. The final concentrations of divalent cations in these HCl solutions were measured by ICP-MS and FAAS. The roots were washed, dried at 65°C to constant weight, and accurately weighed. The same procedure was applied to four samples that contained fresh roots in exchange with a metal mixture solution consisting of 0.6 mM of CaCl₂, CdCl₂, CuCl₂, NiCl₂, and ZnCl₂ simultaneously. All solutions were measured for initial and equilibrium pH.

Method 3: Standardized roots in exchange with KOH solution

Six samples of weighed (~3.0 g) standardized H-form fresh roots were put in 30-mL PP bottles containing 20 mL pH=11 KOH solutions. The capped sample bottles were shaken for 24 h. After that, final solution pH was measured. Roots were dried at 65°C to constant weight and accurately weighed. CEC is calculated with the following equation:

$$\text{CEC} = 10^6 * \left([\text{H}]^e - [\text{H}]^i + [\text{OH}]^i \right) * V/w$$

CEC is in μmol_c g⁻¹ (dry weight, DW), concentration of H⁺ ([H]) and OH⁻ ([OH]) is in mol L⁻¹, volume of the solution (*V*) is in L, dry weight of the sample (*w*) is in g. “i” stands for initial state, “e” stands for equilibrium state. Concentrations were

calculated from measured pH and activity coefficients (γ) derived using the Davies equation.

All plastic containers were rinsed with deionized water, soaked in 10% HNO₃ for at least 2 h, rinsed with double deionized water, and then air dried before being used in the experiments. Subsamples of solutions for metal and Ca analysis were preserved by adding HNO₃ to obtain a final HNO₃ concentration of 0.2% (v/v). Internal and external quality controls were used in ICP-MS and FAAS analysis.

4 Results

4.1 Estimates of Total Number of Cation-Binding Sites and K_H by Titration

Analysis of the data set revealed that there was no significant difference between the duplicate experiments; we thus chose one set of the titration curves to illustrate our analysis, as displayed in Fig. 1. The titration curves indicated that the fresh pea roots showed buffer capacity over a wide range of pH (4 to 10). The titration curves obtained under three ionic strengths of electrolyte background have a similar poly-sigmoid shape but are approximately parallel to each other.

To determine the inflexion point(s) of the titration curves, we plotted dpH/d*V* against *V* and obtained the first-derivative curves. There are two local maxima on

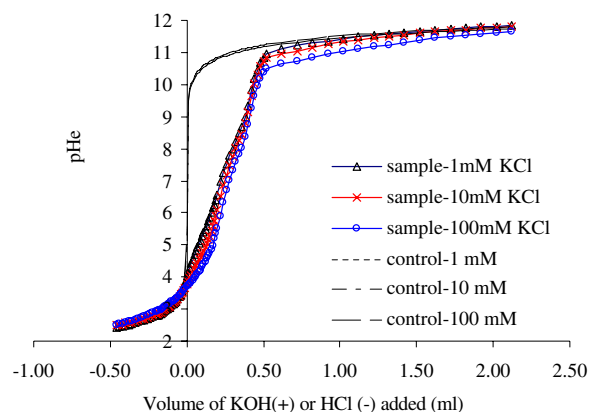


Fig. 1 Titration of fresh pea roots at three ionic strengths of KCl (1, 10, and 100 mM). Measured equilibrium pH (pH_e) is plotted against volume (mL) of KOH (+) or HCl (-) added. Duplicate samples of same fresh weight (1.016±0.020 g) are titrated in 10 mL of KCl solution

the KOH titration side in all three first-derivative curves (see Appendix A2, Fig. 3). These two local maxima, to be identified as 0.22 mL and 0.42 mL of KOH added, respectively, represent mathematically the two inflexion points of the titration curves or physically the two equivalence points of an acid–base titration where two monobasic weak acidic groups were titrated by a strong base (KOH). We could not obtain a full inflexion point at low pH because we only titrated the samples to pH=2.5 and the reading would not be certain at pH lower than 2. However, we were able to estimate the total amount of this type of acidic group (Q_{L1}) using the calculation illustrated in Appendix A4.2.

In addition to the method using first-derivative curves, we used Gran's linear transformation method (Gran 1952) to crosscheck the estimates (see Appendix A3, Fig. 4). This method also confirmed two equivalence points at which V_{e1} =0.22 mL and V_{e2} =0.42 mL of KOH were added. Same results were obtained from independent, duplicate samples.

From the derived equivalence points (V_{e1} and V_{e2}), we calculated the total number of cation exchange sites (Q_{L2}) of the second, relatively weak, type of acidic group (L_2^-) as: $Q_{L2} = C_B V_{e1} / DW = 0.085 (\text{mol L}^{-1}) \times 0.22 (\text{mL}) / 0.049 (\text{g}) = 382 \mu\text{mol}_c \text{g}^{-1}$ (dry wt.), where C_B is the concentration of KOH and V_{e1} is the volume of KOH added to neutralize all the protons binding to the sites of type 2.

The total number of cation exchange sites (Q_{L3}) of the third, very weak, type of acidic group (L_3^-) is: $Q_{L3} = C_B (V_{e2} - V_{e1}) / DW = 0.085 (\text{mol L}^{-1}) \times (0.42 - 0.22) (\text{mL}) / 0.049 (\text{g}) = 347 \mu\text{mol}_c \text{g}^{-1}$ (dry wt.).

As was discussed above, the titration curves had two full inflexion points on the KOH titration side, which suggested the titration of weak to very weak acidic groups (designated as L_2 and L_3) with OH^- . As for the HCl titration side, it represents the titration of a relatively stronger acidic group (designated as L_1)

with H^+ . Equilibrium pH (pH_e) was plotted against $\log ([L_j] / [\text{HL}_j])$ to obtain a straight line intersecting the y -axis at $\text{p}K_{aj}$ (see Appendix A4, Fig. 5 for detail).

In summary, the total number of cation-binding sites and the dissociation constants of all three types of these sites are summarized in Table 1. The titration results suggested that there were three types of proton binding functional groups on the fresh pea root. When solution pH is below 3.5, dissociation equilibrium of one set of relatively strong acidic ligands (L_1^-) dominates. The other two sets of weak acidic ligands (L_2^- and L_3^-) are all in fully protonated forms. When pH is between 3.5 and 6.5, the second type of weak acidic ligands (L_2^-) dissociates. When pH is higher than 6.5, there might be a third type of very weak acidic ligand (L_3^-) participating in the dissociation process. However, we are not sure about the estimate of the third equivalence point because the fresh pea roots might have partially disintegrated under high pH ($\text{pH} > 8$) and the titration at this pH range might overestimate the total amount of cation exchange sites of the third type.

4.2 Complementary Experiments to Validate the pH-dependent CEC

CEC values (mean \pm 95% confidence interval) estimated by complementary method 1 are listed in Table 2. Fresh pea roots grown in 2 mmol L^{-1} CaCl_2 solution for 48 h consist mainly of Ca (97% of total divalent ion species, in molar fractions) and other divalent ions with less abundance (0.2–2 $\mu\text{mol g}^{-1}$ DW) in the order of $\text{Zn} > \text{Cu} > \text{Mn} > \text{Ni}$. The equilibrium pHs of the solutions were in the range of 2.83 to 3.02. Potassium values were not included in the calculation of CEC here because the uptake and transport mechanisms are highly selective for K^+ in plants, involving mass-flow kinetics and specific channels and transporters. They are usually not

Table 1 Total number of cation-binding sites (Q_{Lj}) and the dissociation constants (K_{aj}) of the ionogenic groups in fresh pea roots

Ionic strength mol L^{-1}	Q_{L1} ($\mu\text{mol}_c \text{g}^{-1}$ DW)	$\text{p}K_{a1}$	Q_{L2} ($\mu\text{mol}_c \text{g}^{-1}$ DW)	$\text{p}K_{a2}$	Q_{L3} ($\mu\text{mol}_c \text{g}^{-1}$ DW)	$\text{p}K_{a3}$
0	190	2.52	382	5.46	347	8.29
0.001	190	2.55	382	5.39	347	8.26
0.01	190	2.78	382	4.85	347	7.99
0.1	190	2.79	382	4.38	347	7.55

The $\text{p}K_a$ values at zero ionic strength are estimated by second order polynomial regressions. K_a is the apparent dissociation constant

Table 2 Estimates of CEC of fresh pea root using complementary method 1 (mean±95% CI, dry weight)

[Mn] μmol g ⁻¹	[Ni] μmol g ⁻¹	[Cu] μmol g ⁻¹	[Zn] μmol g ⁻¹	[Ca] μmol g ⁻¹	CEC μmol _c g ⁻¹
0.21±0.01	0.16±0.01	0.34±0.02	2.06±0.07	96.26±3.24	198.21±6.63

Fresh roots were equilibrated with 0.05 mol L⁻¹ HCl for 24 h

considered as ion-exchange processes. Grauer and Horst (1992) excluded the K values from the calculation of effective cation exchange capacity based on a similar argument that the dissolved K could be ascribed to efflux from the cytoplasm and were insensitive to K supply.

CEC values estimated by complementary method 2 are listed in Table 3. The equilibrium pH of the solutions was between 3.40 and 3.54. No attempt was made to adjust the equilibrium pH by adding a third component such as KOH. A pH-edge ion sorption experiment will unavoidably introduce complexity into the estimation because further assumption about the cation-exchange conventions regarding the interaction of the metal ions and the third cation is required. Therefore, experimental pH values were not adjusted and were measured after equilibration.

Method 3, based on the ability of the standardized roots to buffer a KOH solution, gave an estimate of CEC as 187±8 (μmol_c g⁻¹ DW) (mean±95% CI).

From these experiments, it is of note that:

1. In a solution environment of pH 3–4 with moderate concentration of divalent cations (ionic strength ≤ 10 mM), the total CEC of a fresh pea root that was germinated for 3 days and then grown for 2 days is approximately 200 μmol_c g⁻¹ DW.
2. Acid treatment (method 1) gave the highest estimate of CEC in spite of a lower equilibrium

pH. The explanation could be that proton binding with root cell components is covalent in nature and is generally stronger than electrostatic attraction as found in an ion exchange process. The strong acid might have extracted some multivalent cations that are precipitated or irreversibly bound to the root cell ligands. This method would overestimate CEC if there was any leakage of internal cations into the HCl solution hence the value is probably too high.

3. When Ca, Cd, Cu, Ni, and Zn were added simultaneously in the solution, Cu was adsorbed by fresh pea root five times more than Cd, Ni, and Zn and two times more than Ca. The amount of cations adsorbed was in the order of Cu ≫ Ca > Cd ≥ Ni = Zn. Electrostatic interaction can partly explain the competition for sorption on pea roots of different divalent cations, based on different hydrated radii. However, further chemical analysis would help to explain the stronger binding of Cu to the root surface and the differences in binding selectivity between cations with the same valence. Specific binding involving certain functional groups or specific conformation or configuration fits might be possible explanations.

4.3 pH-dependent Cation Exchange Capacity

An acid–base titration experiment was undertaken to estimate the total amount (Q_{Lj}^0) of the cation-binding

Table 3 Estimates of CEC of fresh pea root using complementary method 2 (mean±95% CI, dry weight)

Electrolyte	Ca (μmol g ⁻¹)	Cd (μmol g ⁻¹)	Cu (μmol g ⁻¹)	Ni (μmol g ⁻¹)	Zn (μmol g ⁻¹)	CEC (μmol _c g ⁻¹)
CdCl ₂		76±38				153±76
CuCl ₂			82±5			164±11
NiCl ₂				69±21		139±43
ZnCl ₂					85±21	169±42
Metal mixture	19±1	11±2	45±7	9±2	9±2	185±28

Standardized H-form fresh roots were equilibrated with 3 mM CdCl₂, CuCl₂, NiCl₂, or ZnCl₂ solutions and a metal mixture solution simultaneously containing 0.6 mM of CaCl₂, CdCl₂, CuCl₂, NiCl₂, and ZnCl₂

sites of each type of cation-binding groups and their affinity constants with proton (K_{Hj}) (Table 1). From these estimates, we can simulate the variable negative surface charges present on the roots at a certain solution pH. The total amount of all three types of the functional groups that are in anionic form (ΣQ_{Lj}) at a given pH gives an estimate of the potential CEC at that pH. Figure 2a illustrates the calculated pH-dependent CEC of fresh pea roots. The simulation was carried out in Visual MINTEQ 2.40b (Gustafsson 2006). The experimentally derived $\log K_{Hj}$ values were compiled into an edited thermodynamic database to be used by the program. Ionic strengths were fixed at 0.001 and 0.01 mol L⁻¹, which cover the typical range of ionic strengths found in field soil solutions and are similar to those used in our experiments. Root mass concentration was set as 0.1 g (dry weight) per 20 mL. From Fig. 2a, we can see that, at pH 3 to pH 6, the potential CEC values of excised 5-day-old fresh pea roots are between 140 to 500 $\mu\text{mol}_c \text{g}^{-1} \text{DW}$.

Measurements of CEC from the three complementary methods are compared to the results of the computer simulation. Figure 2b indicates that the measured CECs from these three methods are close to the results from the acid–base titration, within the experimental pH range of 2.8 to 4.

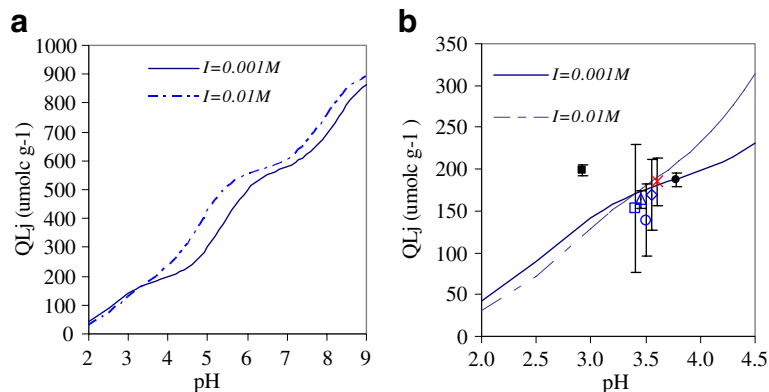


Fig. 2 Simulated pH-dependent cation exchange capacity ($\mu\text{mol g}^{-1} \text{DW}$) using Visual MINTEQ (ver. 2.40b) with parameters derived from acid–base titrations. *Solid line* —: simulated curve for ionic strength (I) of 0.001 M. *Dotted line* - · - · -: simulated curve for $I=0.01$ M. **a** Simulation from pH 2 to pH 9; **b** measured CECs from complementary method 1 through 3 were compared to simulation results at relevant pH. ■

5 Discussion

Different methods have been used by other researchers to estimate the CEC of plant roots. A review of the literature shows that the mean concentrations of the total fixed charges in the cell walls, calculated from CEC and Donnan Free Space (DFS) volume, or from electrical potential, with living tissues or isolated cell walls, range from about 0.1 to 1.5 mol L⁻¹ (Grignon and Sentenac 1991). The highest values have been found in hydrophytes (Ritchie and Larkum 1982; Van Cutsem and Gillet 1982), and the lowest observed in monocotyledons (Pitman et al. 1974; Sentenac and Grignon 1985). Most of the values for terrestrial dicotyledons ranged from 0.2 mol L⁻¹ in parenchyma cells of the bean petiole (Starrach and Mayer 1986) to 1 mol L⁻¹ in legume roots (Sentenac and Grignon 1981) and tomato xylem (Wolterbeek 1987). Some of these values represented charge densities averaged over whole tissues and organs, and might explain the large heterogeneity in the data. When directly measured after blotting or centrifuging samples of isolated cell walls, the water content ranged from 1 to 6 mL g⁻¹ DW, with most of the estimates centered between 1 and 2 mL g⁻¹ DW (Bush and McColl 1987; Ritcher and Dainty 1989; Starrach et al. 1985; Wolterbeek 1987). From this compilation, we can see

Estimates from method 1; ■ estimates from method 2 using CdCl₂; △ estimates from method 2 using CuCl₂; ○ estimates from method 2 using NiCl₂; ◊ estimates from method 2 using ZnCl₂; ж estimates from method 2 using metal mixture solution; ● estimates from method 3. *Vertical bars*: 95% confidence interval

that the reported estimates of total fixed charges ranged from 100 to 9,000 $\mu\text{mol}_c \text{g}^{-1}$ DW. Our estimates are within the reported range.

In an earlier study, Meychik and Yermakov (1999) investigated the acid–base properties of isolated root cell walls of wheat, lupine, and pea using batch titrations. They used processed excised roots that were previously fixed at a high temperature (100°C). They found that in the isolated root cell walls from seedlings and from older plants, there were three ‘cation-exchange’ groups and one ‘anion-exchange’ group in the root cell walls. The ‘anion-exchange’ group they defined is equivalent to the type 1 ligand (L_1) in this study. They showed that the quantity of the ‘anion-exchange’ groups varied within a small range (60–185 $\mu\text{mol}_c \text{g}^{-1}$ DW) in all the species tested. Our estimate (190 $\mu\text{mol}_c \text{g}^{-1}$ DW) is in agreement with their findings. The corresponding pK_a value estimated at an ionic strength of 10 mM for this type of ‘anion-exchange’ group was 3.34 for the root cell walls of 35-day-old pea (*Pisum sativum* L.) plants. Our estimate of the pK_{a1} value is 2.78 at $I=10$ mM for fresh 5-day-old pea roots. The discrepancy might be attributed to the difference in samples studies (fixed versus fresh roots) and differences in experimental methods.

The study by Meychik and Yermakov (1999) found that in the isolated root cell walls of 35-day-old pea plants there were three types of cation-exchange groups with total CEC and pK_a values (Q in $\mu\text{mol}_c \text{g}^{-1}$ DW, pK_a) of (310, 5.32), (310, 7.59), and (680, 10.38), respectively. We did not find a group with a pK_a value near 10, because we suspected an overestimation of the CEC at higher pH in our experiment since partial dissolution of the fresh roots from young pea seedlings was observed at pH higher than 8.

Grauer and Horst (1992) estimated the pH-dependent CEC of excised fresh roots of 8-day-old rye and 10-day-old yellow lupine. Ion exchange experiments were conducted at pH 4.3 and an ionic strength of about 0.06 mol L^{-1} . The estimated total amounts of cation-binding sites were around 320 $\mu\text{mol}_c \text{g}^{-1}$ DW for rye and 360 $\mu\text{mol}_c \text{g}^{-1}$ DW for yellow lupine. Our results are in agreement with theirs.

Further chemical analyses of the root samples might help to reveal the chemical composition of the functional groups in the root cells, although it is still difficult to obtain clear-cut identification of the exact

functional groups present. There might be multiple ionizable groups with overlapping pK_a 's present in the cross-linked polymeric structure of the root cells. Therefore, from the pK_a values obtained above, we can only speculate that there might be some substituted carboxylic groups present on the root cell walls. The substituting side chains linked to the basic acetate backbone might be some alkyl derivatives that shift the pK_a value to the higher end ($pK_a > 4.75$), or some amine and/or pyridine derivatives that shift the pK_a value to both the lower and the higher ends (Hoffman 2004).

The titration curves obtained under three ionic strengths of KCl background are in similar poly-sigmoid shape but are approximately parallel to each other. The equivalence points estimated under three ionic strengths were not significantly different from each other, although the apparent stability constants (K_{HLj}) decreased with increased ionic strength. This could be explained using a simple qualitative Donnan model. The cell walls could be viewed as dissolved “indiffusible” anions restricted to the apoplast-free space by a hypothetical membrane permeable to all other ions and statistically neutralized by diffusible cations. Increased electrolyte concentration in the bulk solution would result in a shielding of the electrostatic field of the fixed charges (ionic strength effect), in a decrease in the accumulation ratio of cations, and consequently in a decreased apparent affinity constant compared to an intrinsic value.

Although electrostatic interaction plays a key role in ion sorption, it is tempting but not easy to give a detailed quantitative description of the macromolecular properties of the root cell walls or the plasma membrane and their relationship with ion uptake physiology. It is still difficult to experimentally measure membrane surface electrostatic potentials at the molecular level. The heterogeneous nature of both the soil and the plant environment poses challenges in developing mechanistic models to describe metal binding to terrestrial plants. Therefore, treating the plant roots as an assemblage of soluble ligands in solution thus facilitating the application of a chemical equilibrium calculation has the same justification as treating them as charged surfaces with defined electrostatic representations, because both approaches need some simplifying assumptions that are still difficult to test experimentally. However, treating the plant roots as biotic ligands has the advantage of

obtaining a relative simple set of parameters that can be used to characterize the heterogeneous nature of plant roots and be further incorporated into chemical speciation programs.

6 Conclusion

Realistic estimate of cation exchange capacity of plant roots is a prerequisite for better modeling of trace metal uptake and toxicity. Estimates of the types and amount of cationic binding groups on the surface of fresh pea roots can be obtained from acid–base titration experiments. The titration approach also provided estimates of the affinity constants (K_H) of these functional groups with protons. In addition, ion exchange experiments with divalent metal ion chloride salts, HCl extractions, and KOH neutralization methods can be used as complementary methods to validate the accuracy of the titration results. The set of parameters derived from this approach can be further incorporated into solution speciation models and toxicity or transport models. In addition, these parameters could be compared among different plant species. The parameters established from this study provide the basic reference on which further determination of the BLM parameters are based.

Acknowledgements The authors gratefully acknowledge the support of the Natural Sciences and Engineering Research Council of Canada (NSERC) Metal in The Human Environment (MITHE) Research Network. Support from NSERC (Discovery) and Le Fonds québécois de la recherche sur la nature et les technologies (FQRNT) is also gratefully acknowledged.

Appendix

A1. Theory Background of Defining the Equivalence Point of an Acid–base Titration

Gunnar Gran [15] proposed a method of transforming the acid–base titration curve into two lines intersecting at the equivalence point by a numerical manipulation

A1.1 Monobasic Weak Acid and Strong Base

If V_0 mL of a weak acid, HA, with initial concentration of C_A (mmol mL^{-1}), is titrated with a strong base, KOH, of concentration C_B (mmol mL^{-1}), after adding V mL of KOH, $C_B V$ mmol of HA will be neutralized by KOH to yield $C_B V$ mmol of K^+A^- . The concentration of HA will decrease to $C_{\text{HA}} = (C_A V_0 - C_B V)/(V_0 + V)$ mmol mL^{-1} and that of A^- will increase to $C_{\text{A}^-} = C_B V/(V_0 + V)$ mmol mL^{-1} . At the equivalence point, $C_A V_0 = C_B V_e$, where V_e is the volume of KOH added when the equivalence point is reached. Therefore, $C_{\text{HA}} = (C_A V_0 - C_B V)/(V_0 + V) = C_B(V_e - V)/(V_0 + V)$

The dissociation equilibrium of HA is: $\text{HA} \leftrightarrow \text{H}^+ + \text{A}^-$

$$\frac{\{\text{H}^+\}[\text{A}^-]}{[\text{HA}]} = K_a,$$

where K_a is dissociation constant, $\{\}$ represents equilibrium activity and $[\]$ represents equilibrium concentration.

At equilibrium, suppose x mmol of HA has dissociated. To a good approximation,

$$[\text{A}^-] = C_{\text{A}^-}^- + x \approx C_{\text{A}^-}^- \quad (\text{A1.0a})$$

$$[\text{HA}] = C_{\text{HA}} - x \approx C_{\text{HA}}, \quad (\text{A1.0b})$$

the proton activity is thus:

$$\{\text{H}^+\} = K_a \frac{[\text{HA}]}{[\text{A}^-]} \approx K_a \frac{C_{\text{HA}}}{C_{\text{A}^-}^-} \approx K_a \frac{V_e - V}{V}$$

According to the definition of pH, $\{\text{H}^+\} = 10^{-\text{pH}} = K_a \frac{V_e - V}{V}$. By rearranging and generalizing this equation, we obtain a linear relationship between V and $V \times 10^{k_1 - \text{pH}}$:

$$V \times 10^{k_1 - \text{pH}} = k_2(V_e - V) \quad (\text{A1.1})$$

where k_1 is an arbitrary value assigned so that the antilogarithms will fall in a suitable range such as 0 to 100 or 0 to 1,000, k_2 is a constant including the equilibrium constant; here

$$k_2 = 10^{k_1 * K_a}$$

When we plot $(V \times 10^{k_1 - \text{pH}})$ against V (volume of KOH added), we obtain a straight line that has a slope of k_2 and can be extrapolated to $y=0$ to get V_e .

We define the expression $V \times 10^{k_1 - \text{pH}}$ as Gran's function 1 (Gran 1).

A1.2 Dibasic Weak Acid and Strong Base

For a dibasic acid, H_2A , after the first equivalence point V_{e1} has been reached, the second mass action equation is:

$$\{H^+\} = K_{a2} \frac{[HA^-]}{[A^{2-}]}$$

$[HA^-] \approx C_{HA^-} = (2C_A V_0 - C_B V) / (V_0 + V) = C_B (V_{e2} - V) / (V_0 + V)$, where V_{e2} is the second equivalence point, and $[A^{2-}] \approx C_{A^{2-}} = C_B (V - V_{e1}) / (V_0 + V)$,

These last three equations are combined to give:

$$\{H^+\} = K_{a2} \frac{V_{e2} - V}{V - V_{e1}} = 10^{-\text{pH}} \tag{A1.2}$$

Rearrange Eq. (A1.2) into:

$$10^{\text{pH}} (V_{e2} - V) = 1/K_{a2} (V - V_{e1})$$

Transform it into a more general form:

$$10^{\text{pH} - k_3} (V_{e2} - V) = k_4 (V - V_{e1}) \tag{A1.3}$$

Equation (A1.3) is used to determine the first equivalence point V_{e1} .

The required value of V_{e2} in this equation can be estimated with sufficient accuracy from the first-derivative curve of the titration curve.

We define the expression $10^{\text{pH} - k_3} (V_{e2} - V)$ as Gran's function 2 (Gran 2).

To determine the second equivalence point, rearrange Eq. (A1.2) and generalize it into:

$$10^{k_5 - \text{pH}} (V - V_{e1}) = k_6 (V_{e2} - V) \tag{A1.4}$$

The value of V_{e1} in this equation is usually determined before V_{e2} using Eq. (A1.1) and Eq. (A1.3).

We define the expression $10^{k_5 - \text{pH}} (V - V_{e1})$ as Gran's function 3 (Gran 3).

A1.3 After the Final Equivalence Point has been Passed

After all the protons on the weak acid HA or H2A have been neutralized by KOH, $C_{OH^-} = C_B (V - V_e) / (V_0 + V)$, $C_{H^+} = k_w / C_{OH^-}$, $\{H^+\} = \gamma C_{H^+} = 10^{-\text{pH}}$, $\gamma = \text{activity coefficient}$

Combine these last three equations:

$$10^{\text{pH}} \gamma k_w (V_0 + V) = C_B (V - V_e)$$

Knowing that C_B and γ keep constant during a titration, we generalize the above equation into:

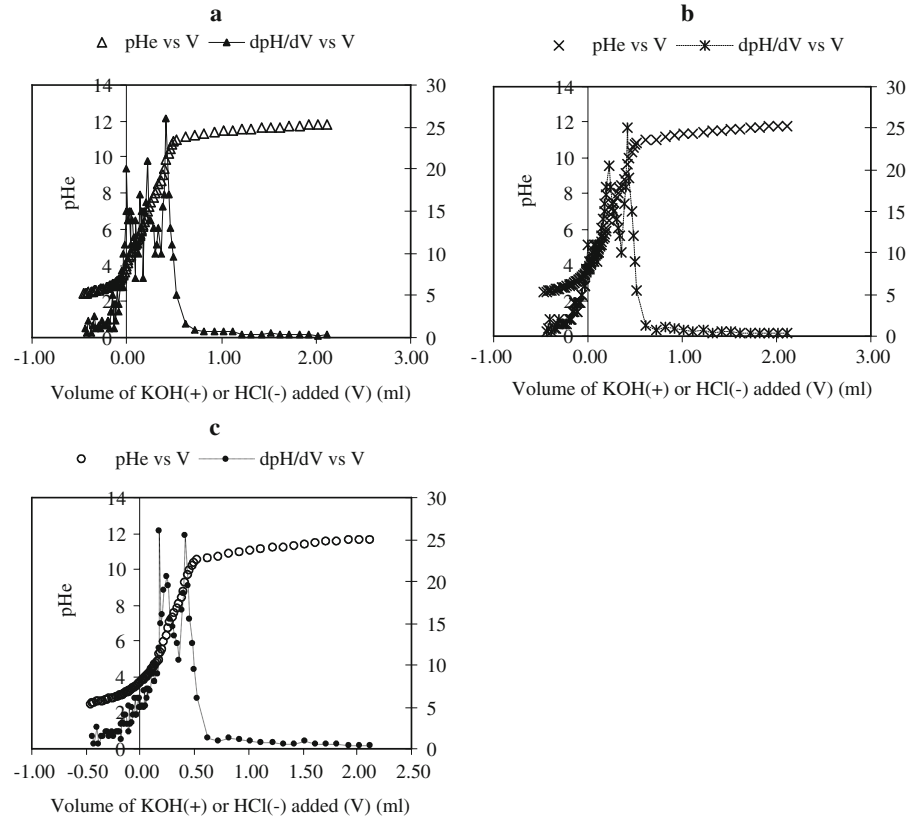
$$10^{\text{pH} - k_7} (V_0 + V) = k_8 (V - V_e) \tag{A1.5}$$

Equation (A1.5) is used for plotting titration data of both monobasic and dibasic acids when the final equivalence point has been passed.

We define the expression $10^{\text{pH} - k_7} (V_0 + V)$ as Gran's function 4 (Gran 4).

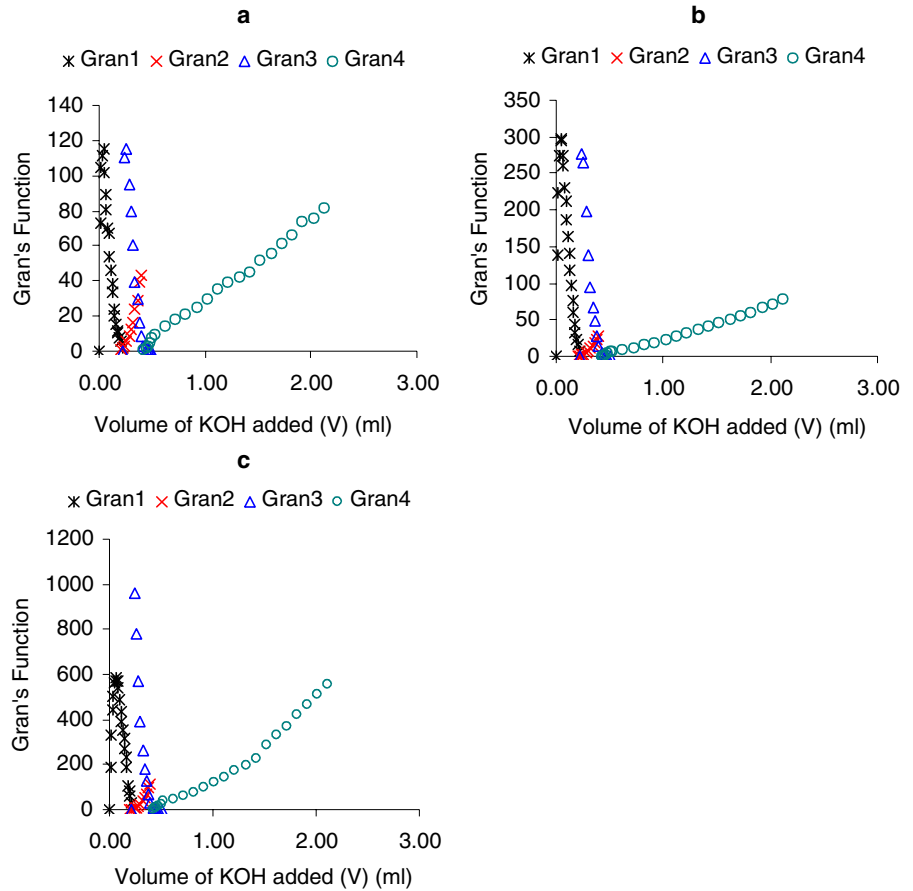
A2. Estimates of Total Number of Cation-binding Sites by KOH Titration

Fig. 3 Determination of the equivalence points from first-derivative curves



A3. Determination of the Equivalence Points Using Gran's Methods (Experimental Data)

Fig. 4 Gran's Functions (Gran 1 to Gran 4) are plotted against V (volume of KOH added, in mL). The x -intercepts of the approximately *straight lines* represent the two equivalence points V_{e1} and V_{e2} . **a** Ionic strength (I)=1 mM; **b** I =10 mM; **c** I =100 mM. Explanations of the Gran's functions are found in A1. V_0 =10 mL. **a** Gran 1= $V \times 10^{8-pH}$, Gran 2= $10^{pH-7}(V_{e2}-V)$, Gran 3= $10^{9-pH}(V-V_{e1})$, Gran 4= $10^{pH-11}(V_0+V)$; **b** Gran 1= $V \times 10^{8-pH}$, Gran 2= $10^{pH-6}(V_{e2}-V)$, Gran 3= $10^{9-pH}(V-V_{e1})$, Gran 4= $10^{pH-11}(V_0+V)$; **c** Gran 1= $V \times 10^{8-pH}$, Gran 2= $10^{pH-5}(V_{e2}-V)$, Gran 3= $10^{9-pH}(V-V_{e1})$, Gran 4= $10^{pH-10}(V_0+V)$

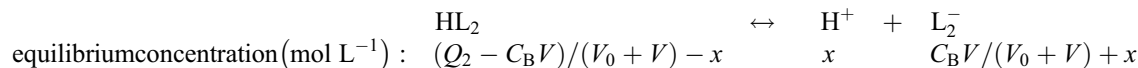


A4. Dissociation Constants (K_a) of the Weak Acids

As was discussed in Section 4, the titration curves had two full inflexion points on the KOH titration side, which suggested the titration of weak to very weak acidic groups (designated as L_2 and L_3 in Figure A3) with OH^- . As for the HCl titration side, it represents the titration of a stronger (though still weak) acidic group (designated as L_1 in Figure A3) with H^+ .

A4.1 Titration with KOH

After determining the first equivalence point V_{e1} , we calculated the total number of cation exchange sites



$x = [H^+] = \{H^+\}/\gamma_H$, where γ_H is the activity coefficient calculated by the Davies equation.

The mass action relationship is:

$$\begin{aligned} \{H^+\} &= K_{a2} \frac{[HL_2]}{[L_2^-]} \Rightarrow -\log \{H^+\} \\ &= -\log K_{a2} - \log \frac{[HL_2]}{[L_2^-]} \Rightarrow \text{pH} \\ &= \text{p}K_{a2} + \log \frac{[L_2^-]}{[HL_2]} \end{aligned}$$

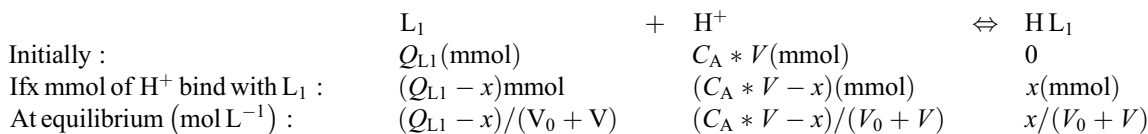
Therefore, plotting the equilibrium pH against $\log([L_2^-]/[HL_2])$ at each titration point, we obtained

(in mmol) of type 2 (Q_2) as: $Q_2 = C_B * V_{e1}$. Now we have Q_2 mmol of weak acid (designated as HL_2) present in V_0 mL of KCl solution with an ionic strength of 1, 10, or 100 mmol L^{-1} . The initial concentration of HL_2 is thus $Q_2/V_0 \text{ mol L}^{-1}$. After adding V mL of KOH into the solution, the system consists of $C_{HL_2} = (Q_2 - C_B V)/(V_0 + V) \text{ mol L}^{-1}$ of HL_2 and $C_{L_2} = C_B V/(V_0 + V) \text{ mol L}^{-1}$ of $K^+L_2^-$. After this, if $x \text{ mol L}^{-1}$ of HL_2 has dissociated, the equilibrium concentrations of the species are:

a straight line intersecting the y -axis at $\text{p}K_{a2}$. Similarly, we can estimate K_{a3} , the dissociation constant of the third type of ionogenic sites (L_3^-) (Figure A3).

A4.2 Titration with HCl

Relatively strong acidic groups (designated as L_1) are considered to exist as zwitterions or neutral form under the initial pH of 4. When we add HCl (with a standardized concentration of $C_A \text{ mol L}^{-1}$) into a solution that contains $Q_{L1}/V_0 \text{ mol L}^{-1}$ of L_1 , the following equilibrium reaction occurs:

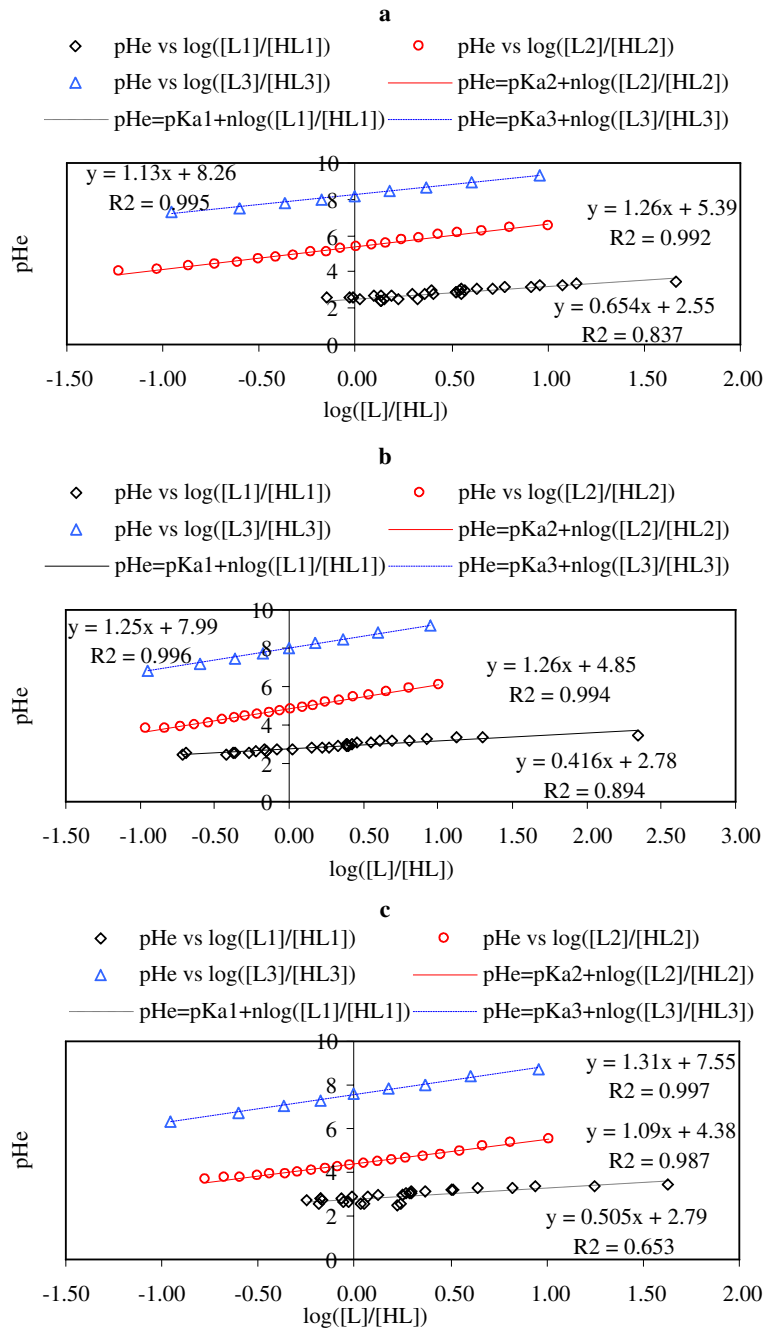


$[H^+] = (C_A * V - x)/(V_0 + V) = \{H^+\}/\gamma_{H^+} = 10^{-\text{pH}_e} / \gamma_{H^+}$, from this relationship, we can solve for x corresponding to each V . Equilibrium concentration of L_1 ($[L_1]$) and HL_1 ($[HL_1]$) can be calculated subsequently for each V .

By the same reasoning, using the modified Henderson–Hasselbach equation,

$$\text{pH}_e = \text{p}K_{a1} + n \log ([L_1]/[HL_1])$$

Fig. 5 Estimated dissociation constants of the acidic ligands. Equilibrium pH (pHe) was plotted against $\log([L]/[HL])$ giving a straight line intersecting the y-axis at pKa. Scatter dots represent data; smooth lines represent linear regression with equations 4 and R2 values stated beside each line. (a) $I = 1 \text{ mM}$; (b) $I = 10 \text{ mM}$; (c) $I = 100 \text{ mM}$



Plotting pH_c against $\log([L_1]/[HL_1])$, we should obtain a straight line with y -intercept of $\text{p}K_{a1}$ (Figure A3).

Because we could not obtain a Q_{L1} value from the titration curve, we instead wrote a linear regression program in Microsoft® Excel to find Q_{L1} and n by maximizing the coefficient of determination (R^2). From here, we determined the total amount of cation exchange sites of the first, relatively strong, type of acidic group (Q_{L1}) to be $190 \mu\text{mol}_c \text{ g}^{-1} \text{ DW}$.

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