Proton and Copper Adsorption to Maize and Soybean Root Cell Walls

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ABSTRACT

A surface complexation model which has been used to describe inner-sphere complexation on metal oxide surfaces was applied to the adsorption of Cu by isolated cell walls of 4-day and 28-day-old maize (Zea mays L. cv WF9 × Mo17) and 21-day-old soybean (Glycine max [L.] Merr. cv Dare) roots. Concentration dependence of the titration data prevented the determination of unique pK and capacitance values for the 4-day maize cell walls, though mean values obtained for the intrinsic pK of the titratable carboxyl groups were 3.0 (4-day maize), 3.6 (28-day maize), and 3.0 (21-day soybean) as determined by potentiometric titration with either NaOH or HCl in 20 millimolar NaCl. The constant capacitance model was applied to Cu sorption data from rapid batch equilibrium experiments in an ionic medium of 20 millimolar NaClO₄. Speciation calculations indicated that the formation of a bidentate surface complex was sufficient to describe the experimental data for all three types of plant material, with only one value for the pK and capacitance density. The intrinsic constants of Cu complexation by a neutral site are: log K = $-0.3 \pm 0.1$, $-0.2 \pm 0.3$, and $0.9 \pm 0.1$ for 4-day and 28-day maize, and 21-day soybean, respectively. The integral capacitance density parameter, which describes the relationship between surface charge density and electrical potential, is several times larger than for crystalline mineral surfaces. This result indicates that the surface electrical potential remains low even when the surface charge density is high. Such behavior is characteristic of gels and porous oxides.

Cell walls behave as ion exchangers because of their pectic polysaccharide and glycoprotein constituents. The cell walls and water-filled intercellular spaces of the root cortex are accessible to solutes from an external solution. Although not considered as a major factor in ion absorption by plants, the exchange characteristics of cell walls may exert some influence on uptake behavior. The presence of exchange sites at the root surface was first noted in 1904 and their effect on ion uptake has since been a topic of controversy. Recent reviews (6–8) highlight aspects of this subject.

It is well established that adsorption in the root apoplast is controlled by cell wall exchange properties (8, 28). Whether this affects membrane uptake is still unresolved. Cell wall exchange properties may influence ion availability for uptake, diffusion rates in the apoplast, the chemical and electrical environment of the membrane and its transporters, growth of the cell wall, and function of cell wall enzymes (1).

To test the possibility that root exchange properties can alter nutrient uptake by plants, an uptake stimulation model which includes cell wall ion exchange is needed. Such a model requires equilibrium constants for cell wall ion exchange to predict the root cell wall ionic composition. Copper was selected as a test case in this investigation because it is known to be strongly and selectively bound by organic matter (24). Electron spin resonance (ESR) spectroscopy of Cu bound to cell walls of unripe apple fruit cortex (10) indicated that Cu probably loses part of its hydration shell to form inner-sphere complexes, or is held in a rigid 'eggbox' conformation because of stereochemical factors. At low solution Cu concentrations, wheat, ryegrass, and red clover can retain Cu bound to root cell walls even when the shoots become Cu-deficient (11).

Only a few investigators have attempted to develop predictive models of cell wall ion exchange behavior. Early applications of the Donnan theory of membrane equilibrium were qualitatively correct, but not capable of quantitative predictions (4). Bush and McColl (4) and Van Cutsem and Gillet (26) determined cell wall thermodynamic exchange constants for K, Mg, Ca, and H exchange on chard (Brassica oleracea var acephala) leaves and for Ca, Cu, and Zn exchange on Nitella flexilis, a freshwater alga. These investigators noted the selectivity of divalent ions, especially Ca (4) and Cu (26). The predictive, mechanistic models which have been applied are of two types: the first assumes the cell wall behaves like a polyelectrolyte or linear, charged macromolecule with ions 'condensing' on its surface until a stable value for the ratio of ionized sites to charge density is reached (6). The second assumes a Stern-like layer for selectively adsorbed species (H and Ca, for example) and a diffuse double layer for non-specifically adsorbed ions (2, 19). Because of the strong affinity observed for Cu adsorption to cell walls, a third molecular model was selected for this study to describe the cell wall-electrolyte interface. The Constant Capacitance model, developed by Stumm, Schindler and coworkers (29), can be regarded as the limiting case of the basic Stern model described above for conditions (high ionic strength, high potential) which lead to high diffuse layer capacitance. This model has not previously been used to describe adsorption by plant cell walls.

THEORY

Molecular models of surface complexation are applied for predictive purposes to estimate conditional equilibrium con-

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stants which apply over a range of pH, surface charge, and solution composition. In addition, their application may suggest mechanisms of molecular behavior which can then be tested independently by experimentation. The constant capacitance model, reviewed elsewhere (21, 29), is one of several which have been applied successfully to describe the interface between aqueous solutions and metal oxide and hydroxide surfaces. While titration and metal adsorption data can be fitted equally well to various molecular models (29), the models differ in their assumptions about the chemical and electrical components of the surface complexation reaction. The constant capacitance model was chosen to describe the adsorption of Cu ions to cell wall surfaces for three reasons. First, pH dependent and specific adsorption of Cu and other metals has been documented for cell walls and other organic materials (4, 19, 24, 27). Second, this model assumes that the primary potential-determining ion, the proton, occupies the same interfacial layer as the chemically adsorbed Cu ion, which is the simplest interpretation. Finally, the constant capacitance model, as the high ionic strength limiting case of the basic Stern model, may be more suitable for describing the soil solution-root interface.

Like oxide surfaces, charged sites of the cell wall develop their charge at the solution interface by proton transfer. We consider an average surface site, SOH, which in this case is most likely a carboxyl group of a galacturonic acid chain. The model is based upon three assumptions: (a) only inner-sphere complexes form between the surface sites, protons, and the specifically adsorbed ions; (b) no complexes are formed with ions in the background electrolyte; and (c) the surface charge density (σ) is related to the surface potential (Ψ) by a proportionality constant, C, the integral capacitance density (in units of Farads/m²), which acts as an adjustable parameter of the model (i.e. σ = CV(Ψ)) (21).

If we consider the proton dissociation reaction for the carboxyl groups of the cell wall:

\[
\text{SOH} = \text{SO}^- + H^+ \tag{1}
\]

the thermodynamic exchange constant for protons is then:

\[
K_{ex} = \frac{[\text{SO}^-][H^+]}{[\text{SOH}]} \tag{2}
\]

\[
= \frac{f_{\text{SO}}-[\text{SO}^-][\gamma H^+]}{f_{\text{SO}}[\text{SOH}]}
\]

where [ ] represents activity and [ ] represents concentration. The rational and solution activity coefficients (\(f_{\text{SO}}\), \(f_{\text{SOH}}\), and \(\gamma H^+\)) represent the deviations from ideal behavior of the complexes on the cell wall surface and the ions in solution, respectively. The solution activity coefficient for H⁺ is ignored in the calculation of the constant capacitance model because the chosen reference state is the constant ionic medium reference state (21). The ratio of the rational activity coefficients (\(f_{\text{SO}}/f_{\text{SOH}}\)) is represented in the constant capacitance model by \(\exp(-F\Psi/RT)\), where R is the molar gas constant, T is the absolute temperature, and F is the Faraday constant. This expression, a special case of the van der Waals statistical mechanical model, represents the deviation from ideality of the cell wall exchanger caused by long range electrostatic interactions among the charged surface species (SO⁺) (21). Since we cannot measure \(\Psi\) experimentally but can measure \(\sigma\), we use the relationship between \(\Psi\) and \(\sigma\) in assumption (3) above and substitute the expression \(\exp(-F\sigma/\text{CRT})\) for the ratio of the activity coefficients.

These assumptions give the model expression for the intrinsic equilibrium constant for proton dissociation:

\[
K_{\text{OH(int)}} = \frac{[\text{SO}^-][H^+]}{[\text{SOH}]} \exp(-F\sigma/\text{CRT}). \tag{3}
\]

The intrinsic constant is not equal to the thermodynamic exchange constant (Eq. 2), but is proportional to it. The inequality arises from a difference in the standard states of the surface species involved in the two expressions (21). The conditional equilibrium constant for this reaction is written:

\[
\epsilon K_{\text{OH}} = \frac{[\text{SO}^-][H^+]}{[\text{SOH}]} \tag{4}
\]

and can be determined experimentally for each point of a titration curve. By inspection, one can see that \(K_{\text{OH(int)}} = \epsilon K_{\text{OH}}\) when \(\sigma\) is equal to zero, since the exponential term is then equal to 1.0. Thus, the intrinsic equilibrium constant can be determined by extrapolation of the conditional equilibrium constant to zero net surface charge (21).

The constraint equations which permit calculations with the constant capacitance model include equations for mass and charge balance and for the surface complex stability constants involved in the reactions being described. In this case, the reactions to be considered are:

\[
\text{SOH} + H^+(aq) \rightleftharpoons \text{SOH}_2^+(aq) \tag{5}
\]

\[
\text{SOH} \rightleftharpoons \text{SO}^-(aq) + H^+(aq) \tag{6}
\]

\[
\text{SOH} + \text{Cu}^{2+}(aq) \rightleftharpoons \text{SOCuOH}(aq) + H^+(aq) \tag{7}
\]

\[
2 \text{SOH} + \text{Cu}^{2+}(aq) \rightleftharpoons (\text{SO})_2\text{Cu}(s) + 2H^+(aq) \tag{8}
\]

\[
\text{SOH} + \text{CuOH}^+(aq) \rightleftharpoons \text{SOCuOH}(s) + H^+(aq). \tag{9}
\]

Titration data suggest that no protonation of the carboxyl groups occurs in the experimental pH range for Cu adsorption, so reaction 5 will be ignored. The equation for the equilibrium constant for reaction 6 is given in Equation 3 and those for reactions 7 through 9 are:

\[
K_{\text{Cu}_1(int)} = \frac{[\text{SOCu}^+][H^+]}{[\text{SOH}][\text{Cu}^{2+}]} \exp(F\sigma/\text{CRT}) \tag{10}
\]

\[
K_{\text{Cu}_2(int)} = \frac{[\text{SO}][\text{Cu}][H^+]^2}{[\text{SOH}][\text{Cu}^{2+}]} \tag{11}
\]

\[
K_{\text{Cu}_3(int)} = \frac{[\text{SOCuOH}][H^+]}{[\text{SOH}][\text{Cu}^{2+}]} \tag{12}
\]

Since there are no charged surface complexes in Equations 11 and 12, the intrinsic constant is equal to the conditional equilibrium constant. The additional constraints of mass and charge balance are required for solution of the equilibrium problem.
\[
[\text{SOH}]_r = [\text{SOH}] + [\text{SO}^-] + [\text{SOCu}^+] 
\]
\[
+ 2([\text{SO}_2\text{Cu}] + [\text{SOCuOH}]) \tag{14}
\]
\[
\sigma = [\text{SOCu}^+] - [\text{SO}^-] \tag{15}
\]

where \([\text{SOH}]_r\) represents the total carboxyl acidity and is equivalent to \(\sigma_{\text{max}}\) (maximum surface charge density).

**MATERIALS AND METHODS**

**Cell Wall Isolation Procedure**

Maize seeds (Zea mays L. cv WF9 × Mo17) were germinated for 4 d in darkness at 28°C on blotter paper moistened with 0.1 mM CaCl₂ solution. Cortical sleeves of the primary root were separated from the remainder of the seedling (12), frozen and fragmented in liquid N₂ and suspended in a 100 mM potassium phosphate buffer (pH 7.0). The cell wall mixture was placed in a cell disruption bomb (Parr 4636) and equilibrated at 10.35 MPa (1500 p.s.i.) N₂ gas for 20 min. Discharged cells were collected, washed, and filtered 8 to 10 times with double distilled water and twice with 20 mM NaCl. Two samples for dry weight determination and about 5 g (wet weight) for lipid P determination were saved from each preparation. After drying at 60°C for 24 h, the preparations were about 2 to 3% of the filtered wet weight. Since phospholipids are contained in the plasma membrane but not in the cell wall, a chloroform-methanol extraction and lipid phosphorus determination (3) was used to indicate the amount of the membrane and cytoplasmic contents removed. For all cortical cell wall preparations, lipid P was decreased to 12 ± 8% of that present in the intact root on a dry weight basis.

To compare exchange properties of older roots, seedlings started in low light for 5 d were transferred to aeroponic tanks for further growth. After 21 to 24 d, the roots were excised, rinsed with cold distilled water, and excess water removed. The cell wall disruption and isolation procedure described above was repeated.

For soybeans (Glycine max [L.] Merr. cv Dare) the seeds were germinated in autoclaved coarse vermiculite for 7 d, and then transferred to the aeroponic tanks. After 14 d, the roots were excised and cell walls were isolated as described above.

**Determination of Total Acidity**

In the pH range of the proton titration and Cu adsorption experiments (pH 3–8), carboxyl groups appeared to be the primary source of charge on the cell wall, based on the resemblance of the titration curves to those for weak carboxylic acids. To quantify \([\text{SOH}]_r\), a modified version (9) of the calcium acetate method for determination of COOH acidity was used. Samples were prepared as described in the cell wall isolation procedure. After rinsing with distilled water and filtration, 40 to 50 g of sample (wet weight) were suspended in approximately 250 mL distilled water and brought to pH 3 with HCl. The suspension was drained on a nylon filter and 5 to 10 g subsamples (100–300 mg dry weight) were placed in 125-mL screw-cap Erlenmeyer flasks. The filtrate was collected for use as a titration blank. The volume of solution in the cell walls was estimated, and CO₂-free water was added to bring the total volume of solution to 40 mL. Ten mL of 0.5 mM Ca(OAc)₂ stock solution was also added. The flasks were closed tightly and shaken at moderate speed for approximately 12 h at room temperature, then removed and retained for steam distillation. For each sample, 400 mL of distillate was collected in a calibrated flask and retained for titration. Each sample and blank solution was titrated with 0.01 N NaOH to pH 8.50 using the expanded scale of the pH meter. The COOH content (mol COOH/kg cell wall) of the sample was calculated according to the equation:

\[
\frac{[\text{mL NaOH (sample)} - \text{mL NaOH (blank)}]}{\text{NaOH concn (mol/L)}} \times \text{dry weight of sample (g)} \tag{16}
\]

**Proton Titration Experiments**

The indifferent background electrolyte chosen for the proton titrations was NaCl, since these ions are thought to have only electrostatic interactions with organic matter (24) and cell walls (28). The ionic strength of 20 mm was selected as a value commonly found in soil solutions (15). Approximately 10 to 20 g of the salt-washed, filtered cell walls were placed in the jacketed titration vessel of a Metrohm 614 Impulsomat automatic titration system with a calculated volume of 20 mL NaCl to bring the final volume (including the solution volume of the cell walls) to 250 mL. The suspension temperature was maintained at 25.0 ± 0.1°C. Water saturated N₂ gas was bubbled into the mixture to minimize the presence of CO₂. The mixture was continually stirred with a magnetic stirrer mounted in a glass cage to prevent grinding against the walls of the vessel. A Ross combination electrode (Orion) was used to measure pH with a Metrohm 632 pH meter. Additions of standardized 0.02 N NaOH and HCl were made through an automatic dispenser. For forward titrations, HCl was added to bring the initial pH to 3.0, and for back titrations the initial pH was adjusted to pH 8.5 or 10.0. Aliquots of acid or base were added to raise or lower the pH by approximately 0.1 pH unit. Amounts added and pH were recorded two minutes after each addition.

**Copper Adsorption Experiments**

For the Cu experiments, NaClO₄ was used instead of NaCl to prevent the formation of the interfering complex, CuCl⁺ (23). After washing 10 times with double distilled water, 40 g (wet weight) of the cell wall preparations were brought to 100 mL with distilled water. Aliquots (5 g) were dispensed from this continuously stirred suspension into 50 mL polylamellar centrifuge tubes using an automatic pipette with an enlarged tip opening. Appropriate volumes of 0.1 mol/kg NaClO₄ (4 g), 0.1 or 1.0 mmol/kg (CuClO₄)₂ (2 g), and deionized water were added to bring the total solution concentration to approximately 0.02 mol/kg NaClO₄ and 0.01 or 0.1 mmol/kg CuClO₄. Appropriate aliquots of 5, 10, or 50 mM HClO₄ or NaOH were added to each pair of duplicate samples to adjust the initial pH range from 4 to 8 for maize, or 2 to 7 for soybean, in 0.5 pH unit increments. The necessary amounts of acid and base were determined by titrating two samples in a -Cu solution, one with HClO₄ and the other with NaOH.
For all Cu adsorption experiments, the final cell wall concentration was between 1 and 2 g/kg, and final suspension weight was 20 g.

After making all the required additions, the tubes were shaken for 30 s and centrifuged for 90 min at 10,000 rpm. A study of the effect of length of equilibration times indicated that there was no significant change in the amount of Cu sorbed after 0 to 10 h of shaking. After centrifugation, the supernatant solutions were decanted and filtered through 25 mm 0.4 μm Nuclepore polycarbonate membranes held in Swin-Lok filter holders attached to 30 mL plastic syringes. The samples were collected under vacuum and pH was measured immediately with a microcombination electrode (Fisher) and a Beckman 171 pH meter. Samples were stored in polycarbonate bottles and refrigerated, with the Cu concentration determined using flame atomic absorption spectroscopy (Perkin Elmer 5000) within 24 h.

Data Analysis for Proton Titration Experiments

Acidic properties of the cell wall surface are most clearly represented by calculation of the formation function for each point on the titration curve (22). This function calculates δnOH, which is the number of moles of H* neutralized per g cell wall during a titration.

The first step of the calculation is to determine the net OH consumed for each point of the cell wall titration curve according to the equation:

\[ n_{OH}(\text{sample}) = (OH_a - OH) - (H_a - H) \]  
where \( OH_a \) = concentration (mol/L) of total OH added (total volume of NaOH solution added × base concentration)/total volume of solution, \( H_a \) = concentration of H added (calculated in the same fashion), and OH and H are the concentrations remaining in solution, and are calculated using the following equations:

\[ OH = 10 \exp[(pH - pK_a)/\gamma_{OH}] \]  
\[ H = 10 \exp[(-pH)/\gamma_H] \]  
both in mol/L, where \( K_a \) is the activity product for H₂O and \( \gamma \) is the single ion activity coefficient, which is calculated from the Davies equation (20) and has the value 0.870 for an ionic strength of 20 mM.

The same calculation is made for each point of the blank titration, using Equations 16 to 18. The blank titration function is fit with a polynomial regression equation of the form:

\[ n_{OH}(\text{blank}) = \beta_0 + \beta_1(pH) + \beta_2(pH)^2 + \ldots + \beta_m(pH)^m \]  
This function is applied to calculate \( n_{OH}(\text{blank}) \) for each data point of the cell wall titration. Finally, the formation function is calculated from the following equation:

\[ \delta n_{OH}(\text{mol/kg cell wall}) = \frac{n_{OH}(\text{sample}) - n_{OH}(\text{blank})}{CW} \]  
where \( CW \) is the concentration of cell walls in kg (dry weight)/L.

To normalize the formation function, \( \delta n_{OH} \) is transformed to the fraction of the total sites which are dissociated (\( a_{OH} \)). The inflection points were determined by graphical methods applied to the formation function plots and were considered equivalent to complete dissociation of the carboxyl sites. The \( \delta n_{OH} \) value for that point is adjusted to be equivalent to SOH⁻; then all other formation function values of lower pH are recalculated accordingly and divided by the total acidity to give the fraction of sites which are dissociated:

\[ a_{OH}(i) = \frac{\delta n_{OH}(i) + [SOHT - \delta n_{OH}(\text{endpoint})]}{[SOHT]} \]  
where SOHT = total carboxyl acidity determined by the calcium acetate method, and \( \delta n_{OH}(\text{endpoint}) \) is the value of the formation function at the apparent COOH titration endpoint.

Data Analysis for Adsorption Experiments

The amount of Cu sorbed was determined by difference between the amount added and the amount remaining in solution. The supernatant concentrations were converted to mol Cu in solution by multiplying by the solution weight for each sample. The mol of Cu added initially were similarly determined by multiplying the weight of added Cu(ClO₄)₂ by the stock solution concentration. The following equation was used to determine Cu adsorption:

\[ n_{Cu}(\text{mol/kg}) = \frac{\text{mol Cu(initial)} - \text{mol Cu(final)}}{\text{kg cell wall}} \]  

RESULTS AND DISCUSSION

Proton Titrations

In Figure 1, formation functions for forward and back titrations are plotted for three concentrations of root cortical cell walls of 4-d maize seedlings. Though the curves have similar shape and magnitude, their \( \delta n_{OH} \) values vary as a function of the titration starting point, which can differ for each curve. To avoid the possibility of irreversible conformational changes or dissolution of cell wall constituents, the minimum pH in all titrations was 3. The consumption of OH⁻ in the titration of carboxyl groups from pH 3 to 6 is evident; around pH 6 the curve flattens and other groups apparently begin to be titrated. The shape of the formation functions for 28-d maize and 21-d soybean root cell walls (Fig. 2) is similar to the 4-d maize, although the magnitude for soybean is much larger. Dicots generally have higher uronic acid contents and root cation exchange capacities than monocots (8). As determined by the calcium acetate method, total carboxylic acidity of the soybean root cell walls was 670 ± 43 mmol/kg cell wall, compared to 174 ± 14 mmol/kg for 4-d maize cortex and 165 ± 21 mmol/kg for 28-d maize roots.

The titration curves of maize and soybeans in these experiments are similar to those obtained by others for algal and higher plant cell walls (7, 16). Dufey et al. (7) suggested that the groups titrated above pH 7 may be -NH₂, -SH, and phenolic -OH groups. In the experiments reported here, titration above pH 8 caused a yellowish or greenish coloration
of the cell wall suspension. Wacquant (28) observed a yellow coloration of roots treated with base and attributed this to the ionization of phenolic OH groups. He also found that the exchange capacity of roots increased irreversibly after treatment with base and suggested this was due to alkaline hydrolysis of the esterified uronic acids. Since the esterified groups make up 15 to 40% of the total exchange sites (28), these demethylated carboxyl groups are probably responsible for the majority of OH consumed at the upper end of the titration curve. Only at pH values greater than 8.5, where plant growth rarely occurs, do these additional groups form a significant component of the total sites. Since there are few titratable groups between pH 7 and 8.5, and the formation of Cu hydroxide and carbonate complexes is significant in that range, Cu sorption in this investigation was restricted to pH values below the COOH endpoint (i.e. below pH 7.5).

The normalized formation function, calculated according to Eq. 21, is shown in Figure 3 for the formation functions plotted in Figure 1. Above pH 4, the titrations appeared reversible and displayed no cell wall concentration effect. However, at pH 3, the \( \alpha_{OH} \) values range from 0 to nearly 0.5 with lower cell wall concentrations having the lower values. Possible explanations for the observed concentration effects cannot be confirmed with the experimental data presented here. One hypothesis is that aggregation or interparticle interaction at higher cell wall concentrations enhances the electrostatic repulsions between sites, which inhibits proton dissociation. Unstirred cell wall preparations were observed to aggregate over time, but it seems unlikely that significant particle-particle interaction could occur in the continually stirred and bubbled solution. Another related possibility is that the interaction of cell wall fragments may cause carboxyl sites to be less accessible during the short equilibration time. The total acidity determinations, which were used to normalize the formation functions, equilibrated for 12 h. While short equilibration times have been recommended for proton titrations of organic matter (24), longer times produce different titration results (16). Finally, the differences could be due to effects of cell wall-associated divalent ions, particularly Ca, on lateral electrostatic interactions of the dissociated sites. Ionic composition of the cell walls was not determined, but it is unlikely that Ca concentrations would be higher in the more dilute cell wall suspensions.

For each point of the titration, once \( \alpha_{OH} \) is calculated, it is possible to calculate the conditional equilibrium constant (\( K_{OH} \) in Eq. 4). The equation can be written in terms of mole fractions (\( N \)) or as a function of \( \alpha_{OH} \), the fraction of dissociated sites:

\[
\frac{\alpha_{OH}}{N_{SOH}} = \frac{a_{OH}[H^+]}{1 - \alpha_{OH}}.
\]

(23)

Taking the negative logarithm of both sides:

\[
\log(K_{OH}) = \text{pK} + \log([1 - \alpha_{OH}]/\alpha_{OH})
\]

(24)

which is the familiar Henderson-Hasselbalch equation. This is a standard approach to determine the pK of variable charge mineral surfaces, organic matter and proteins (24, 25). As
Calculated from root cortical cell walls, from (pKOH[\text{int}]) the Figure 3. The smooth curve represents the function calculated by the speciation model, MICROQL, for an intrinsic proton dissociation constant (pKaoH[int]) of 3.0, determined by averaging intercept values from Figure 4A, and a capacitance density (C) of 5.3 F m⁻², determined by averaging slope values from Figure 4A and applying Equation 25.

mentioned earlier, pH is considered equal to the negative log of the H⁺ concentration (i.e. γ⁺ = 1) since we are assuming the Constant Ionic Medium Reference State and the concentration of H⁺ is generally much less than the ionic strength of the medium (20).

In Figure 4, pKOH is plotted against αOH, or fraction of dissociated sites. Only the values of αOH from 0.2 to 0.8 are plotted, since other functional groups may interfere at lower or higher values. Although the slopes vary, all three types of cell wall preparation show some increase in pKOH with increasing αOH. This is the expected result of electrostatic interactions between sites: the enhanced repulsion between sites makes it more difficult for protons to ionize (decreasing K) with an increasing degree of dissociation (24). This pattern is most prominent for the soybean (Fig. 4C) and higher concentrations of the 4-d maize (Fig. 4A) and for soybean 28-d maize (Fig. 4B) and lower concentrations of the 4-d maize. While the slope of this curve may be affected by several factors (25, 30), the greater COOH density of the soybean cell walls may have resulted in greater interaction between sites; hence, a steeper slope (30). Contamination by membrane or cytoplasmic constituents may also have caused enhanced electrostatic interaction in the older, harder to purify, soybean root cell walls. However, the 28-d maize root cell walls were also more difficult to purify than the 4-d maize cortical cell walls, but did not demonstrate the enhanced repulsion between sites. Neither did the 28-d maize titration data reflect the inhibition of proton dissociation observed for higher concentrations of the 4-d maize cortical cell walls, although the concentrations were higher (1.9 g cell wall/L compared to 1.5 g cell wall/L). Other possible reasons for the concentration dependence observed in Figure 4A are discussed above.

**Figure 4.** Negative common logarithm of the conditional equilibrium constant (Eq. 24) for the proton dissociation reaction plotted against fraction of dissociated sites for root cell walls of: A, 4-d maize cortex; B, 28-d maize; and C, 21-d soybean. Values plotted for all formation functions in Figures 1 and 2, but only for 0.2 < αOH < 0.8 to avoid interference of other functional groups. Straight line represents the mean values of intercept (pKaoH[int]) and slope from linear equations calculated for each titration.

**Constant Capacitance Model**

For the application of the Constant Capacitance model, Figure 4 serves two purposes. Extrapolation of the curves to the point of zero charge (αOH = 0) gives the value for the
intrinsic equilibrium constant \( pK_{\text{OH}}(\text{int}) \) defined in Equation 3. The slope of the curve is an indication of the importance of lateral electrostatic interactions and is related to the capacitance parameter, \( C \), by the equation:

\[
p'K_{\text{OH}} = pK_{\text{OH}}(\text{int}) + \frac{F\sigma_{\text{max}}}{2.3 \, CRT} \alpha_{\text{OH}}
\]  

Because of the concentration effects described above, it is not possible to determine unique values of intrinsic \( pK \) and \( C \) for the 4-d maize. Linear regression equations were calculated for the data from each of the five titrations and the values for intercept and slope were averaged (Table I). The same procedure was applied for the 28-d maize and 21-d soybean. Mean values for the intrinsic \( pK \) were 3.0 \( \pm \) 0.5 (4-d maize), 3.6 \( \pm \) 0.1 (28-d maize), and 2.9 \( \pm \) 0.1 (21-d soybean). These values are close to 3.23, the \( pK \) of galacturonic acid (14), and to other values of cell wall \( pK \) (2, 30).

The values for \( C \), calculated with Equation 25, were 5.3, 8.8, and 3.6 \( F/m^2 \) for 4-d maize, 28-d maize, and 21-d soybean, respectively. These values for the capacitance are 4 to 8 times those generally obtained for crystalline oxide surfaces (29). Higher values of \( C \) are also found for gels and porous oxides (13, 17). Lyklema (13) and Perram et al. (17) suggest that the high surface charges and low electrokinetic potentials of these materials are explained by accommodation of charge by counterions behind the particle surface. Differences in charge density between soybean and maize roots may explain some of the variation of \( C \), although the initial ionic composition of the cell walls may also have influenced this parameter. Bush and McColl (4) observed a steeper slope for \( p'K_{\text{OH}} \) versus \( \alpha_{\text{OH}} \) measured on chard leaf cell walls for different electrolytes (K), than for selectively bound Ca and Mg.

Table I. Intrinsic Equilibrium Constant \( (pK_{\text{OH}}(\text{int})) \) and Capacitance \( (C) \) Determined from Intercept and Slope of Curves in Figure 4A for Titrations of 4-d Maize Seedlings

<table>
<thead>
<tr>
<th>Cell Wall Suspension Concentration</th>
<th>Type of Titration</th>
<th>( pK_{\text{OH}}(\text{int}) )</th>
<th>( C )</th>
</tr>
</thead>
<tbody>
<tr>
<td>g/L</td>
<td></td>
<td>F/m²</td>
<td></td>
</tr>
<tr>
<td>0.8</td>
<td>Back</td>
<td>3.40</td>
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</tr>
</tbody>
</table>

The concentration dependence of the titration data yields a range of \( C \) values for 4-d maize cortical cell walls (Table I) from 2.7 to 13.7. Clearly, the causes of this concentration effect need to be investigated by further experimentation. Methodology may be improved so that unique values of intrinsic \( pK \) and \( C \) apply to a range of suspension concentrations. Otherwise, it will be necessary to select concentrations for adsorption experiments which most closely approximate the behavior of intact root cell walls.

To test the constant capacitance model and the values for \( pK_{\text{OH}} \) (int) and \( C \) determined above we used MICROQL-UCR (5), a chemical equilibrium program, to solve Equation 3 for the proton dissociation reaction. The program calculates equilibrium speciation in electrolyte solutions alone or in contact with adsorbing surfaces. Calculations were made for the speciation of the cell wall surface at different pH values. The parameters required by the model and values used are given in Table II. Total acidity values were used for maximum adsorption density (SOH₂ or \( \sigma_{\text{max}} \)). The values for specific surface area (SSA) were calculated on the basis of a mean distance of 0.86 nm between neighboring COOH groups on a galacturonic acid chain (18):

\[
\text{SSA} (m^2/g) = (0.43 \, \text{nm})^2 N_A(\sigma_{\text{max}}) 10^{-18}
\]  

(26)

where \( N_A \) = Avogadro constant. The values for concentration of the solid were determined experimentally. In Figures 3 and 5, the normalized formation functions are plotted for the three types of cell wall material. The smooth curves represent the values for \( \alpha_{\text{OH}} \), or fraction of dissociated sites, calculated by MICROQL. The model calculations based on the titration derived mean values for \( pK_{\text{OH}} \) (int) and \( C \) correspond closely to the data only at pH > 4. To apply the model so that it describes titration behavior observed at lower pH, values for \( pK \) (int) and \( C \) must be determined for each suspension concentration, which significantly limits its usefulness.

Copper Adsorption

With the intrinsic proton dissociation constant and parameters of the model established, and tested for goodness of fit against the proton titration data, MICROQL was used to solve Eqs. 3 and 10–14. The final pH and total \([\text{Cu}^{2+}]\) were entered for each data point of the copper adsorption isotherms (Tables III–V). For each of the possible Cu sorption reactions

| Table II. Parameters Used to Fit Proton Titration and Adsorption Data with MICROQL |
|---------------------------------|-----------------|-----------------|-------|
|                                 | 4-d Maize Root  | 28-d Maize Root | 21-d Soybean Root |
|                                 | Cortical Cell Walls | Root Cell Walls | Root Cell Walls |
| Adsorption density (mol/g)      | 1.74 × 10⁻⁴     | 1.65 × 10⁻⁴     | 6.70 × 10⁻⁴     |
| Specific surface area (m²/g)    | 60.8            | 57.6            | 234.0           |
| Concentration of solid (g/L)    |                 |                 |                  |
| For proton                       | 1.2             | 1.9             | 1.2             |
| For Cu                           | 1.57            | 2.07            | 1.60            |
| Capacitance (F/m²)               |                 |                 |                  |
| For proton                       | 5.3             | 8.8             | 4.0             |
| For Cu                           | 4.0             | 4.0             | 4.0             |
Figure 5. Fraction of dissociated sites \((n_{\text{cut}})\) as a function of pH. Calculated from formation functions plotted in Figure 2 for one replicate each of 28-d maize and 21-d soybean root cell walls, with inflection points considered equivalent to 100% dissociation of carboxyl sites; all other values determined from Equation 21. The smooth curves represent functions calculated by the speciation model, MICROOL, for intrinsic proton dissociation constants and capacitance densities determined from intercept and slope values, respectively, of regression lines in Figure 4, B and C. For 28-d maize, \(pK_{\text{Cu}(\text{int})} = 3.6, C = 8.8 \text{ F/m}^2, 1.9 \text{ g cell walls/L}\); for 21-d soybean, \(pK_{\text{Cu}(\text{int})} = 2.9, C = 3.6 \text{ F/m}^2, 1.2 \text{ g cell walls/L}\).

### Table III. Solution pH and Cu Concentration, and Cu Sorbed by Root Cortical Cell Walls of 4-d Maize Seedlings (\([\text{NaClO}_4]\) = 20 mmol/kg, \([\text{Cu}]_T = 10.52 \pm 0.05 \mumol/kg, 1.57 \text{ g Cell Walls/kg}\))

<table>
<thead>
<tr>
<th>pH</th>
<th>([\text{Cu}]_T) ((\mu\text{mol/kg}))</th>
<th>([\text{Cu}]_{\text{cut}}) ((\mu\text{mol/kg cell walls}))</th>
</tr>
</thead>
<tbody>
<tr>
<td>3.17</td>
<td>10.17 ± 0.05</td>
<td>0.21 ± 0.05</td>
</tr>
<tr>
<td>3.57</td>
<td>9.26 ± 0.05</td>
<td>0.81 ± 0.01</td>
</tr>
<tr>
<td>4.03</td>
<td>7.43 ± 0.05</td>
<td>1.97 ± 0.03</td>
</tr>
<tr>
<td>4.57</td>
<td>5.26 ± 0.20</td>
<td>3.49 ± 0.20</td>
</tr>
<tr>
<td>5.11</td>
<td>2.75 ± 0.10</td>
<td>4.91 ± 0.11</td>
</tr>
<tr>
<td>5.64</td>
<td>1.71 ± 0.00</td>
<td>5.61 ± 0.05</td>
</tr>
<tr>
<td>6.22</td>
<td>1.53 ± 0.05</td>
<td>5.65 ± 0.16</td>
</tr>
<tr>
<td>6.63</td>
<td>1.57 ± 0.00</td>
<td>5.60 ± 0.07</td>
</tr>
<tr>
<td>6.84</td>
<td>1.82 ± 0.05</td>
<td>5.57 ± 0.11</td>
</tr>
</tbody>
</table>

*Mean of 2 replicates ± 1 SD.

(Eqs. 7–9), a value for \(K_{\text{Cu}}\) was determined by fitting to the known values of Cu sorbed and Cu remaining in solution. The values for \(K_{\text{Cu}}\) were averaged to determine the \(K_{\text{Cu}}\) (int) for the reaction.

For all three types of cell wall material, the best simulation of the adsorption data was achieved when the surface complex was assumed to be bidentate (SO\(_2\)Cu). However, a better fit to the 4-d maize sorption data was achieved when the value for capacitance was changed to 4 \(\text{F/m}^2\) from 5.3 \(\text{F/m}^2\) (determined from the proton titration data; see Fig. 6A). In Figure 6, the sorption data and the simulated data are shown for all three types of cell wall materials, assuming a bidentate Cu surface complex, a pK of 3, and \(C = 4 \text{ F/m}^2\). The values for the equilibrium constant which were obtained were log \(K\) (int) = −0.3 ± 0.1 (4-d maize), −0.2 ± 0.3 (28-d maize), and 0.9 ± 0.1 (21-d soybean). For 28-d maize (Fig. 6B), the Cu sorption data can be simulated either by using the values of pK (3) and C (4 \(\text{F/m}^2\)) given above, or determined from the proton titration data. Both sets of model parameters overestimated the sorption of Cu above pH 5. For both the 28-d maize and 21-d soybean, the experimentally obtained decrease in sorption at pH > 6 was not predicted by the model.

The relative sensitivity of the model simulations to selected values for pK\(_{\text{Cu}}\) (int) and C depends on the pH range represented by the adsorption data. Where adsorption occurs at pH values primarily above (4-d maize) or below (21-d soybean) the intrinsic pK, pKa and C are more sensitive than when adsorption occurs at pH values close to the pK (28-d maize). This is consistent with the fact that the cell walls are highly buffered in that pH range.

Simulated adsorption curves for the three possible surface complexes (SO\(_2\)Cu, SO\(_2\)Cu\(^+\), and SOCuOH) are presented in
Figure 6. pH dependence of the adsorption of Cu ions to root cell wall surfaces of: A, 4-d maize cortex; B, 28-d maize; and C, 21-d soybean. Percent adsorption (relative to maximum adsorbed in each experiment) measured in rapid batch equilibrium experiments described in "Materials and Methods." All three types of cell wall material were suspended in 20 mmol/kg NaClO₃. Suspension concentrations of cell walls and total Cu were: A, 1.57 g cell wall/kg and 10.5 μmol Cu/kg; B, 2.07 g cell wall/kg and 12.3 μmol Cu/kg; and C, 1.60 g cell wall/kg and 8.68 and 87.2 μmol Cu/kg. Using values of $K_{OB}$ (int) and $C$ determined from Equation 25, MICROQL was used to determine a value for $K_{OB}$ for each data point of the copper adsorption isotherms (Tables II-IV); these were subsequently averaged to determine $K_{OB}$ (int). Solid lines represent the calculated results when $pK_{OH} =$ 3.

Figure 7. Comparison of model predictions for pH dependence of Cu adsorption to 4-d maize cortical cell walls. Data obtained as described in Figure 6. Lines represent the results calculated by MICROQL when $pK_{OH}$ (int) = 3 and $C = 4$ for each of the three possible surface complexes.

Figure 7, assuming $pK_{OH}$ (int) = 3 and $C = 4$. The model curves can be compared to Cu adsorption data for the 4-d maize cortical cell walls. Although differences are not striking, the bidentate complex yields the best approximation of the data. This is consistent with ESR studies (10) that propose an "eggbox" model for divalent ion binding by the galacturonic acid chains. In such a model, the Cu is chelated by two COOH groups on adjacent polymer chains, and may lose a portion of its hydration shell upon binding (10). Spectroscopic studies are required to confirm which surface complex forms.

CONCLUSIONS

The results suggest that the Constant Capacitance model may be useful for predictions of proton and Cu adsorption to root cell walls. The application of the model is hindered by the inability to determine constant values of the intrinsic parameters and the relative insensitivity of these parameters when adsorption occurs in the pH range near the pK. Further investigations are necessary to establish whether concentration-independent parameters can be established and to test the model against adsorption data collected over a range of cell wall and metal concentrations and solution ionic strengths. If concentration dependence of the proton titrations and, possibly, metal adsorption studies cannot be overcome, it will be necessary to determine which behavior is most similar to conditions experienced by intact root cell walls bathed in soil solution.

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We thank Dr. Garrison Sposito for helpful discussions, and anonymous reviewers for their suggestions.

$C = 4$, and the surface complex which forms is assumed to be bidentate (SO₂Cu). Hatched lines represent the calculated results when $pK_{OH}$ and $C$ have the values determined from the proton titrations (Fig. 4).
LITERATURE CITED


