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ARTICLE

Comparison between Methods using Copper, Lanthanum, and Colorimetry for the Determination of the Cation Exchange Capacity of Plant Cell Walls

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The determination of the cation exchange capacity (CEC) of plant cell walls is important for many physiological studies. We describe the determination of cell wall CEC by cation binding, using either copper (Cu) or lanthanum (La) ions, and by colorimetry. Both cations are strongly bound by cell walls, permitting fast and reproducible determinations of the CEC of small samples. However, the dye binding methods using two cationic dyes, Methylene Blue and Toluidine Blue, overestimated the CEC several-fold. Column and centrifugation methods are proposed for CEC determination by Cu or La binding; both provide similar results. The column method involves packing plant material (2−10 mg dry mass) in a chromatography column (10 mL) and percolating with 20 bed volumes of 1 mM La or Cu solution, followed by washing with deionized water. The centrifugation method uses a suspension of plant material (1−2 mL) that is centrifuged, and the pellet is mixed three times with 10 pellet volumes of 1 mM La or Cu solution followed by centrifugation and final washing with deionized water. In both methods the amount of La or Cu bound to the material was determined by spectroscopic methods.

KEYWORDS: Cell wall; dye; ion exchange; pectin

INTRODUCTION

The cation exchange capacity (CEC) of plant cell walls is imparted mostly by the presence of negative charges on cell wall carbohydrate polymers. The predominant source of negative charge is the galacturonic acid residues in pectin (found in dicots and some monocots) and glucuronic acid in glucuronoarabinoxylan (found in grasses) (*1*−*3*). It has been estimated that 70−90% of the total charge of cell walls is contributed by pectin or glucuronoarabinoxylan, with the reminder contributed by cell wall proteins and lignin (*4*). The CEC is pH dependent, due to dissociation of the weakly acidic carboxyl groups, with a pK_a of ca. 4.4 (*5*). However, the physiological pH of cell walls is around pH 5−6 (*2*, *6*), at which between 80% and 97% of carboxyl groups are dissociated and contribute to the CEC, whereas other functional groups play only a minor role at neutral pH. Dicots have a higher CEC than grasses (*7*). The CEC is important for nutrient acquisition by plants and for the functioning of plasma membrane transport processes (*4*, *8*). Also, the CEC may affect the susceptibility of plants toward aluminum toxicity (*9*−*11*). The CEC also affects the swelling behavior, texture, and drying characteristics of plant material, which are important factors in the food processing industry (*12*).

Several methods have been proposed to determine the CEC of plant material: (a) binding of rare earth elements (e.g., lanthanum) (*13*), (b) binding of copper (*14*), (c) titration with alkali (*9*, *14*, *15*), (d) colorimetric quantification of sugar acids, (e) binding of a cationic dye (*16*), and (f) X-ray microanalytical techniques (*17*). Some of these techniques are time-consuming, require specialized equipment, or have not been validated and compared to one another.

The use of rare earth elements has the benefit of low background concentrations in plant material accompanied by the high valency of the rare earth elements (e.g., Nd^{3+}, Pd^{3+}, La^{3+}) that results in strong binding of these cations to the cell wall, which minimizes losses of the bound cations during washing steps. The addition of polyvalent cations to the cell wall shifts the equilibrium toward complete dissociation of carboxyl groups, due to their high affinity for carboxyl groups. This gives results that are virtually independent of solution pH and ionic strength.

The use of Cu binding has similar benefits, but there is a higher background concentration of Cu in roots and it binds slightly more weakly to cell wall material than do trivalent cations (*5*).

Titration relies on the conversion of cell wall material to the H form (i.e., all bound cations need to be displaced by protons). The free acid groups are then back-titrated to neutrality in a solution containing Ca, Mg, or Na ions (*14*, *17*−*19*). Either the bound cations can be quantified or the titer can be used to estimate the CEC (*17*). Similar approaches have been described to measure the CEC of soil colloids (*20*). While the titration method has been widely used, pectin losses may occur during conversion of the cell wall material to the acid form, resulting in the underestimation of CEC (*2*, *21*). Furthermore, not all bound cations are displaced by protons, leading to a further underestimation of the CEC.

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The colorimetric assay employs 3-phenylphenol, which reacts specifically with galacturonic and glucuronic acid present in pectin and hemicellulose, respectively (*22*). While these uronic acids are the predominant source of charge, the contribution of proteins and lignin to the CEC is ignored by this method. Furthermore, the presence of an excess of neutral sugars (i.e., glucose in cellulose) may interfere with uronic acid determination, as does the methyl esterification of the uronic acids (*23*).

Dye binding uses the ability of cationic dyes to bind to anionic charges. Metachromatic dyes, such as Methylene Blue and Toluidine Blue O, are positively charged thiazine dyes which undergo color changes (i.e., shifts in wavelength) on binding to negatively charged surfaces (*16*, *24*−*26*). The color shift indicates aggregation between the dye molecules and charge transfer when bound to the substrate (*27*). Methods based on dye binding have been developed to determine the charge density (i.e., the cation exchange capacity) of clay suspensions (*24*, *26*, *28*) and of some organic materials (e.g., carrageenan, humic substances) (*29*, *30*).

Given the importance of the CEC of cell walls in the accumulation and transport of many essential nutrients and toxic elements, the aim of this study was to compare several methods for the routine determination of the CEC of plant cell wall material on milligram quantities of plant material and to determine the factors influencing the sensitivity and reliability of the assays.

MATERIALS AND METHODS

Plant Material and General Methods. Seeds of bean (*Phaseolus vulgaris* L. cv. Sinatra), wheat (*Triticum aestivum* L. cvv. Kennedy and Sunbrook), sunflower (*Helianthus annuus* L. cv. Hysun 38), maize (*Zea mays* L. cv. Hycorn 424), plantain (*Plantago lanceolatum* L. cv. Tonic), triticale (x *Triticosecale* Wittm. ex A. Camus cv. Kiewiet), chicory (*Chicorum intybus* L. cv Grouse), lotus (*Lotus corniculatus* L. cv. Goldie), and cowpea (*Vigna unguiculata* L. Walp. cv. Red Caloona) were germinated in rolled paper towels soaked in 1 mM $CaCl_2$. The seeds were germinated on a laboratory bench at ambient temperature (25−26 °C) and room light.

Roots or shoots of 5−10 day old seedlings were pooled, cut into small sections, and collected on ice and either immediately homogenized or frozen for 2−8 weeks before homogenization with a Ten Broek homogenizer at 4 °C. The slurry was washed on stainless steel screens (64 μM aperture) with 1−2 L of ice-cold deionized water and centrifuged (900*g* RCF for 5 min) and the pellet collected. Light microscopic investigation of the cell wall material did not reveal any recognizable intracellular components in the cell wall preparations, and the slurries consisted predominantly of fragmented cell walls. To determine the effect of environmental conditions on the CEC, roots from seedlings sown and harvested at two different dates (1 month apart) were collected and analyzed separately. Additionally, plant material was oven-dried and mill-ground, the results of this procedure being compared to those for homogenized material. The homogenized slurry was stored for no more than 1−2 days at 4 °C; otherwise, it was frozen for later use. An aliquot of the slurry was either packed in 10 mL disposable columns (0.8 × 4 cm) with an integrated reservoir (e.g., BioRad PolyPrep or EconoColumn) for the column method or placed in centrifuge tubes for the centrifugation method.

Column Method with Cu or La. Homogenized frozen and thawed slurry was packed by gravity flow into BioRad PolyPrep columns (any other type of column can also be used) to give a bed volume of 0.2−1 mL that was washed 10 times with the bed volume (i.e., 10 × 0.2−1 mL) of deionized water or HCl (0.02−0.1 M). The cell wall material was converted to the metal form with 20−50 bed volumes (i.e., 20−50 × 0.2−1 mL) of 1 mM $CuCl_2$ or $LaCl_3$ at room temperature, before washing with 20 bed volumes (20 × 0.2−1 mL) of deionized water. The cell wall material was dried, weighed, and digested with 5 mL of nitric acid/perchloric acid (5/1 v/v) and 1 mL of hydrogen peroxide (30%) and the cation concentration determined by inductively coupled plasma optical emission spectroscopy (ICP-OES).

Centrifugation Method with Cu or La. Aliquots (2−5 mL) of homogenized frozen and thawed slurry were centrifuged, and the pellet (ca. 0.5 mL) was washed twice with acid (10 pellet volumes of 0.01 M HCl) or water. This was followed by conversion to the metal form, in which the pellet was added to 5 mL of 5 mM $CuCl_2$ or $LaCl_3$ solution and the mixture was stirred for 5 min and allowed to stand for 0.5 h on ice. After centrifugation (2000*g* RCF for 10 min), the pellet was resuspended in a fresh aliquot of metal chloride solution and the mixture was stirred and reacted for 0.5 h on ice, followed by centrifugation. The pellet was washed three times with deionized water (5 mL) by centrifugation and dried at 65 °C, the dry weight was determined prior to being digested in 5 mL of acid as above, and the cation concentration was determined by ICP-OES.

Table 1. Effects of Washing Steps on the Measured Cation Exchange Capacity (CEC) using the Column Method[a]

prewash	CEC of sunflower	volume of final wash	CEC of cowpea
water	541 ± 93 a	1	696 a
0.02 M HCl	543 ± 6 a	5	693 a
0.1 M HCl	524 ± 55 a	10	704 a
		20	694 a
		50	687 a

[a] Cell wall material from sunflower was washed with 10 volumes of water or acid prior to La sorption and the CEC determined. Cowpea material in the La form was rinsed with increasing volumes of water and the CEC determined. Where applicable, the results are the mean ± standard deviation of three determinations, expressed as $\mu mol_+ g^{-1}$. Significant differences (Tukey's *t* test, $P < 5\%$) between treatments and within a plant species are denoted by different letters. The roots were collected from 5−10 day old seedlings germinated in 1 mM $CaCl_2$ solution.

Colorimetric Method. Methylene Blue (3,7-bis(dimethylamino)-phenothiazin-5-ium) and Toluidine Blue O (3-amino-7-(dimethylamino)-2-methylphenothiazin-5-ium) were obtained from Merck and used without further purification. Aqueous dye solutions (0.02−0.0002% Methylene Blue; 0.05−0.0005% Toluidine Blue) were used to stain citrus pectin solutions (Sigma Chemical Co.) whose degree of esterification was varied by alkaline de-esterification (*31*). Slurries (1 mL; equivalent to 1−2 mg dry weight) of homogenized cell wall materials of maize and sunflower were mixed with 1 mL of the dye solutions. After mixing for 25 min, the adsorption curves were determined on the supernatant after centrifugation. Wavelength spectra were recorded on a GBC Model 916 UV−vis spectrophotometer between 450 and 700 nm using a 1 cm path length cuvette. The molar extinction coefficients of 95 000 M^{-1} cm^{-1} for Methylene Blue (*32*) and 38 250 M^{-1} cm^{-1} for Toluidine Blue (*27*) were used to quantify the dye.

Statistical Analyses. In all instances, CEC was expressed as micromoles of cationic charge bound per unit of dry weight of cell wall material ($\mu mole_+ g^{-1}$), calculated from the concentration of the cation bound multiplied by the charge of the cation. Measured CEC values were analyzed for significant differences by the general linear models procedure in SAS version 8. Significant differences (Tukey's *t* test) at $P < 5\%$ between treatments were established when no interactions between main effects were determined. Experiments were replicated between two and six times, as outlined in Results.

RESULTS

Adsorption Methods. *Column Method with Cu or La.* Washing cell material with dilute acid prior to metal adsorption resulted in a large reduction in bed volume but a weight loss of only 4 or 6% with 0.02 or 0.1 M HCl, respectively. Also, the CEC was not significantly reduced ($P = 0.968$) due to acid washing (**Table 1**).

No weight losses were observed upon adding the metal solutions or during the subsequent washing steps (data not shown). Flow rates of solutions through the columns ranged from 0.1 to 2 mL min^{-1}. Column wash volumes of 1 to 50 bed volumes gave the same results for the CEC ($P = 0.240$, **Table 1**).

There was no significant difference in measured CEC when using Cu or La as test ion for maize ($P = 0.339$) and lotus ($P = 0.862$) (**Table 2**). With chicory, CEC values measured with La were significantly ($P = 0.027$) higher than with Cu (**Table 2**).

Centrifugation Method with Cu or La. Mixing cell wall material with Cu or La solutions in a centrifuge tube and replacing the supernatant solution also allowed for determination

of the CEC. Replacement of the metal solution with fresh solution permitted removal of desorbed ions. No significant differences in CEC were observed between two or three solution exchanges (data not shown). There were no significant differences between 1 or 5 mM metal chloride saturating solution for CEC determination (**Table 3**) when 10 pellet volumes of metal solutions was used. The minimum contact time of 0.25 h was chosen (**Table 4**), though ancillary experiments showed that the exchange is complete within 3 min (data not shown).

Table 2. Influence of the Test Ion (Cu or La) on the Measured Cation Exchange Capacity (CEC) of Maize, Chicory, or Lotus Cell Wall Material Using the Column Method[a]

	CEC		
test ion	maize (Hycorn)	chicory	lotus
Cu	77 ± 7 a	457 ± 46 b	428 ± 31 a
La	67 ± 7 a	615 ± 43 a	435 ± 29 a

[a] Results are the mean ± standard deviation of four determinations, expressed as $\mu mol_+ g^{-1}$. Significant differences (Tukey's *t* test, $P < 5\%$) between test ions and within a plant species are denoted by different letters. The roots were collected from 5−10 day old seedlings germinated in 1 mM $CaCl_2$ solution.

Table 3. Effect of the Concentration of the Test Ion on the Measured Cation Exchange Capacity (CEC) of Bean Root Cell Wall Material Using the Centrifugation Method[a]

test ion	concn	CEC
Cu	1	437 ± 9
	5	441 ± 25
La	1	449 ± 32
	5	459 ± 39

[a] Results are the means ± standard deviation of four determinations, expressed as $\mu mol_+ g^{-1}$. Concentrations are given in mM. There were no significant differences (Tukey's *t* test, $P < 5\%$) between measured CEC values. The roots were collected from 5−10 day old seedlings germinated in 1 mM $CaCl_2$ solution.

Table 4. Influence of the Contact Time on the Cation Exchange Capacity (CEC) of Bean Root Cell Wall Material As Measured with Cu or La Test Ions Using the Centrifugation Method[a]

	CEC	
contact time	Cu test ion	La test ion
0.25	422 ± 16	529 ± 80
3.5	458 ± 33	452 ± 32
17	457 ± 16	438 ± 38

[a] Values are the mean of two determinations ± standard deviation, expressed as $\mu mol_+ g^{-1}$. The contact time is given in h. There were no significant difference (Tukey's *t* test, $P < 5\%$) between measured CEC values. The roots were collected from 5−10 day old seedlings germinated in 1 mM $CaCl_2$ solution.

Colorimetric Method. Mixing cell wall material with positively charged Methylene Blue or Toluidine Blue resulted in the adsorption of the dye on the cell wall material with the consequent depletion of dye from the solution.

The absorbance measurements were made at 660 nm (for Methylene Blue) or 600 nm (for Toluidine Blue), showing that the amount of dye bound increased curvilinearly with the amount of dye added (**Figure 1**). The CEC determined by La sorption on the material (dashed horizontal line in **Figure 1**) is substantially less than the amount of dye bound (Qmax).

Application of the Methods. The column and centrifugation methods were compared, with both methods giving comparable results (**Table 5**). The repeatability of the La method was high, as shown by the low SD (**Table 5**), but there were significant differences between sample batches (**Table 6**).

Differences between the CEC values of source materials were clearly evident when comparing root and shoot material of the same species (**Table 7**). The shoot material of sunflower (mainly epicotyl and first leaf pair in sunflower) and wheat (the coleoptile and first leaf in wheat) had approximately twice the CEC of the root material. The difference between root and shoot material from Tonic plantain was less distinct.

Storage of the plant material prior to processing had some effect on the measured CEC (**Table 8**). Material initially oven-dried or immediately frozen prior to homogenization gave lower CEC values than freshly homogenized material.

Table 5. Comparison between the Centrifugation and Column Methods for Determining CEC Using La Cations[a]

	CEC	
material	centrifugation	column
sunflower root	521 ± 23	514 ± 19
maize root	73 ± 11	66 ± 3

[a] Values are means ± standard deviation of six replicates, expressed as $\mu mol_+ g^{-1}$. There were no significant differences (Tukey's *t* test, $P < 5\%$) between methods for either plant material. The roots were collected from 5−10 day old seedlings germinated in 1 mM $CaCl_2$ solution.

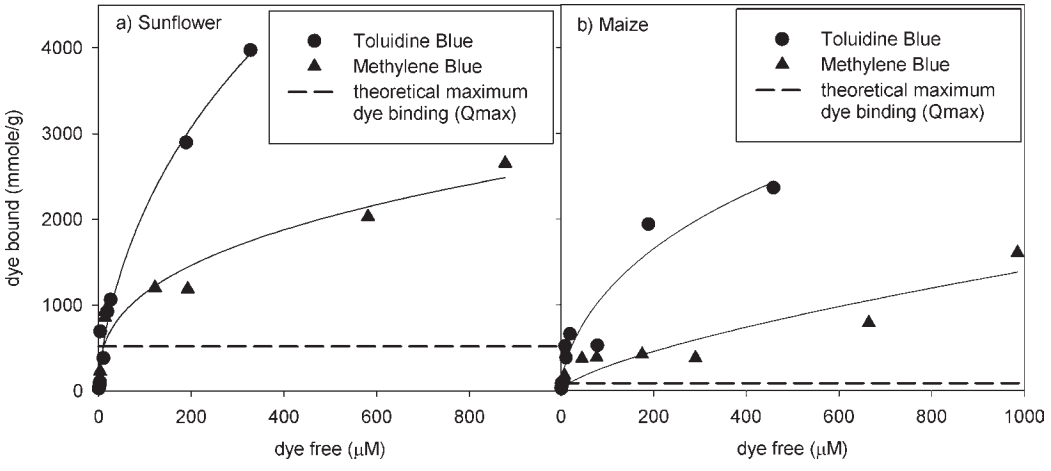

Figure 1. Adsorption curves of Toluidine Blue (●) and Methylene Blue (▲) on (a) sunflower and (b) maize cell wall material. The solid lines represent the Langmuir−Freundlich adsorption isotherms fitted to the data (symbols). The dashed horizontal line represents the CEC of the material (in $\mu mol_+ g^{-1}$) determined by La sorption.

Table 6. Effect of Harvest Date of x*Triticale* "Kiewiet" Root Material on the CEC[a]

harvest date	CEC
June 2	128 ± 3 a
July 1	105 ± 3 b

[a] Root material from seedlings sown and harvested 4 weeks apart was collected, stored frozen, and analyzed at the same time with the column method using La. Values are expressed in $\mu mol_+ g^{-1}$ ± standard deviation of four samples, and the experiment was repeated three times. Significant differences (Tukey's *t* test, $P < 5\%$) between harvest dates are denoted by different letters. The roots were collected from seedlings grown in 1 mM $CaCl_2$.

Table 7. CEC Values of Root and Shoot Material from Various Plant Species[a]

	CEC	
source	root	shoot
sunflower	325 ± 8 b	599 ± 20 a
maize	35 ± 1 b	96 ± 6 a
tonic plantain	245 ± 3 b	287 ± 8 a

[a] The CEC was measured by the column method and is expressed as $\mu mol_+ g^{-1}$ ± standard deviation of five to six determinations. Significant differences (Tukey's *t* test, $P < 5\%$) between root and shoot CEC within a plant species are denoted by a different letter. The roots were collected from 5−10 day old seedlings germinated in 1 mM $CaCl_2$ solution.

Table 8. Influence of Sample Preparation and Storage on the CEC of Maize and Wheat Cell Wall Material. [a]

source	treatment	CEC
Hycorn maize	homogenizer	159 ± 5 a
	mill ground	143 ± 3 b
sunbrook wheat	dried	91 ± 10 b
	fresh	120 ± 11 a
	frozen	85 ± 9 b

[a] Values are expressed as $\mu mol_+ g^{-1}$ ± standard deviation of four determinations. Significant differences (Tukey's *t* test, $P < 5\%$) between treatments for each plant species are denoted by different letters. The roots were collected from 5−10 day old seedlings germinated in 1 mM $CaCl_2$ solution.

DISCUSSION

Adsorption Methods. *Column Method with Cu or La.* The 4−6% weight loss during acid washing may be attributed to losses of acid soluble cell wall material such as proteins and pectin, although the CEC was not significantly reduced due to acid washing (**Table 1**). Greater losses of pectin and CEC were claimed for algal cell walls from *Nitella* (*21*), and this may be attributed to the different cell wall composition in algae. A light microscopic investigation showed that the cell walls appeared crumpled in the acidic solution (data not shown). This may be due to protonation of carboxyl groups, leading to decreased repulsion of cell wall polymers. The acid washing appears to have physical effects more pronounced than chemical effects. Although we consider the acid-washing step optional, the effect of acid washing should be checked for the plant material of interest.

Cell wall material contained in small chromatography columns was readily converted to the Cu or La form with 25 bed volumes, sufficient to completely exchange the originally bound cations. No weight losses were observed upon adding the metal solutions or during the subsequent washing steps (data not shown), suggesting that the conversion of the cell wall material does not change the pectin composition of the cell wall.

The Cu or La ions are strongly bound to the cell wall material (*5*) and do not incur significant losses with extensive washing, since wash volumes of 1−50 bed volumes gave the same results for the CEC (**Table 1**). We chose to digest the Cu- or La-loaded cell wall material with acid (dry ashing was not tested but could be used as an alternative digestion method) prior to determination of Cu or La ions, avoiding the time-consuming step of eluting the Cu or La ions with acid and analyzing the eluate.

Both Cu and La ions gave comparable results for the CEC of maize and lotus cell wall material (**Table 2**). With chicory, CEC values measured with La were higher than with Cu (**Table 2**) and we attribute this to sample variability rather than to true differences in CEC. The agreement between the CEC values determined with Cu and La ions was generally very good (compare **Tables 2**−**4**). Native cell wall material contains less La than Cu, but the concentrations in cell wall material will likely depend on the type of source material and the growing conditions (*33*). Therefore, blank controls (prior to metal addition) need to be included to correct for background levels.

On the basis of the results, the suggested column method is as follows: place cell wall slurry in small chromatography columns to give a packed bed volume of 0.2−1 mL (equivalent to a dry weight of 7−35 mg). If desired, wash the bed with 20 volumes of 0.02 M HCl. Add 20 volumes of 1 mM La or Cu chloride, followed by rinsing with 10 volumes of deionized water. The retained material can then be analyzed for bound cations.

Centrifugation Method with Cu or La. The CEC could also be determined by mixing cell wall material with Cu or La solutions in a centrifuge tube and exchanging the supernatant solution two or three times. Since both cations bind strongly to cell wall material, competition with desorbed cations for binding sites is low. Either 1 or 5 mM Cu or La chloride solutions could be used for the exchange without any significant influence on the CEC (**Table 3**) when 10 pellet volumes of metal solutions was used. Although a minimum contact time of 0.25 h was chosen (**Table 4**), ancillary experiments (data not shown) revealed that the exchange is complete within 3 min. Washing of the cell wall material two to three times with deionized water removed excess metal ions. An advantage of the centrifugation method is that the contact time between the metal solution and the cell wall material can be controlled better than with the column method, ensuring that the results obtained with the centrifugation method are less variable.

The suggested protocol for CEC determination by the centrifugation method is as follows: place the slurry of cell wall material in a graduated centrifuge tube, centrifuge briefly (2000*g* for 5−10 min), and discard the supernatant. Record the volume of pellet and mix with 10 pellet volumes of 1 mM Cu or La solution. Mix repeatedly for 0.25 h, centrifuge, and replace the supernatant with a fresh volume of metal solution. Repeat this step for a third time. After centrifugation, mix the pellet with 10 pellet volumes of deionized water, mix briefly, centrifuge, and replace the supernatant with fresh deionized water. Repeat for a third time. The pellet can then be analyzed for bound cations.

Colorimetric Method. Methylene Blue or Toluidine Blue dyes are cationic dyes and adsorb onto the negatively charged cell wall material with the consequent depletion of dye from the solution. Therefore, the change in adsorption of the supernatant can be measured and used to calculate the amount of dye bound to plant material. Absorbance measurements showed that the amount of dye bound increased curvilinearly with the amount of dye added (**Figure 1**) and showed no plateau corresponding to the CEC. Indeed, the CEC determined by La sorption on the material (dashed horizontal line in **Figure 1**) was substantially less than the amount of dye bound (Q_{max}). This discrepancy between La sorption and dye binding is possibly due to aggregation of the dye on the cell wall material and is manifested in the curvilinear adsorption curves. The amount of dye bound by sunflower cell

wall material was higher than that bound by maize, reflecting the higher CEC of dicotyledonous plants such as sunflower as compared to that of the Poaceae (*7*). Also, the CEC maize cell wall measured with the La method is similar to values reported in the literature for maize and wheat, suggesting that the CEC measured with La is correct, whereas dye binding overestimated the CEC. Similarly, the CEC values determined on commercial citrus pectin with varying degrees of esterification agreed well among the La adsorption method, alkaline titration, and galacturonic acid assay, but dye binding overestimated the CEC of the pectin samples (data not shown).

Aggregation of dye molecules is a characteristic of metachromatic dyes such as Methylene Blue and Toluidine Blue, resulting in a color change (*25*). Due to the possible aggregation of the dye on negative charges of the cell wall material, there is no stoichiometric relationship between dye molecules bound and the number of charges. Therefore, under the current experimental conditions, dye binding could not be used to quantify the CEC of plant cell wall material.

Application of the Methods. The La adsorption method was chosen as the routine method due to low background levels and strong binding of La to cell wall material. Further, unlike Al for example, La undergoes no precipitation or polymerization reactions in the range of pH 1−7 (according to PhreeqcI modeling of a 1 mM $LaCl_3$ solution).

Initially, the column and centrifugation methods were compared, with both methods giving comparable results (**Table 5**). The repeatability of the La method was high, as shown by the low standard error (**Table 5**), but there were significant differences between sample batches (**Table 6**) which may be attributed to differences in the age of the plant material at collection and to differences in growing conditions (*7*, *34*).

Root and shoot material of the same species differed in the CEC (**Table 7**). The shoot material of sunflower (mainly epicotyl and first leaf pair in sunflower) and wheat (the coleoptile and first leaf in wheat) had approximately twice the CEC of the root material, and this may be related to the greater ability to take up nutrients from the soil. In contrast, the difference in CEC between root and shoot material from Tonic plantain was less distinct.

The effect of sample preparation and storage on the plant cell CEC was also determined (**Table 8**). Material homogenized with a Ten Broek homogenizer had a slightly higher CEC than mill-ground material. This can be attributed to the finer particle size and higher number of exposed binding sites with the homogenized material, whereas mill-ground material was dried first which may have "coagulated" or irreversibly aggregated the binding sites (*35*, *36*) and resulted in lower CEC measurements.

The CEC was slightly affected by the storage of the plant material prior to processing (**Table 8**) with oven-dried or frozen material having lower CEC values than freshly homogenized material. This may again be caused by irreversible aggregation of cell wall polymers during freezing or drying, resulting in fewer available binding sites. We have not tested the effect of freeze-drying on the CEC.

In conclusion, the Cu and La adsorption methods are both reliable, have a high sensitivity, and can be used on small quantities of samples (< 10 mg dry weight) with intra-assay and interassay coefficients of variation of between 0.5 and 5%. Both cations are suitable for use in either column or centrifugation methods. No precipitate of Cu or La occurs on detemination of the CEC up to pH 7. Since the La background concentration in plants is very low, the La ion is preferred over Cu. Both Cu and La bind strongly to plant material, thereby minimizing desorption losses during the washing step, and the strong binding of La makes adsorption virtually independent from pH in the range pH 4−7. The proposed adsorption methods have been successfully used on a variety of plant species and for extended periods.

ABBREVIATIONS

CEC, cation-exchange capacity; ICP-OES, inductively coupled plasma optical emission spectroscopy.

SAFETY

Metal salts of Cu and La are toxic, and skin or eye exposure or ingestion should be avoided by using personal protective equipment. Digestion of samples with concentrated acid may result in vigorous reactions, in addition to risks due to the corrosive nature of the acid. The digestions should only be performed in a fume hood with full face shield, acid-resistant gloves, and lab coat.

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