

Determination of Effective Partition Coefficients of Alkali Chlorides by Measurements of Concentration Potential across the *Nitella flexilis* (L.) Ag. Isolated Cell Wall

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ABSTRACT

We report a study in which we estimate by means of non-equilibrium processes the value of an effective partition coefficient, χ , a parameter that we use to take into account the non-ideal behaviour of the electrolyte of the external medium in the cell wall space. This parameter is defined by the ratio of the salt partition coefficient to the activity of the non-diffusing charges. To that purpose, concentration potential in NaCl, LiCl and CuCl₂ solutions was measured across an isolated cell wall of *Nitella flexilis* at different pH. No significant difference appeared between the potential values measured in the presence of NaCl or LiCl (pH 5.5, 4.0 and 3.0). At pH 3.0, in CuCl₂ solutions, the *Nitella* wall behaved as an uncharged membrane.

Two methods—high concentrations and least-squares approximation—were used to calculate from these results the value of the effective partition coefficient. Both gave similar values of χ . At a given pH, the values of χ are independent of the concentration of the external medium over a large range of concentration ratios. The activity of the exchange sites was estimated by the experimentally determined fraction of adsorbed monovalent cations. The ionic concentrations in the wall space were calculated at pH 5.5 and 3.0 by introducing the values of χ in the relations which correlate them to the ionic concentrations in the wall for that given value of the activity of the carboxylate sites. From their values, it appears that the negative fixed charges of the wall were functionally shaded by the condensation of a part of the adsorbed monovalent cations. The activity of the former was, therefore, reduced to a value considerably lower than that estimated by the cationic exchange capacity during equilibrium processes.

Key words: *Nitella flexilis* (L.) Ag., cell wall, concentration potential, partition coefficient, ion condensation.

INTRODUCTION

The role that the primary plant cell wall plays during the ionic exchanges between the external medium and the cytoplasmic phase depends on its ion exchange characteristics, mainly on its cationic exchange capacity (CEC) but also on its adsorption selectivity with regard to the different ions. These properties are conditioned by the activity of the negative fixed charges arising from the ionization at the physiological pH of the constitutive polyuronic acids. These charges interact with the diffusing ions and impose on them an apparent mobility and a concentration within this compartment different from the one these ions have in the external solution. The study of the ions-wall interaction always comes up against the

same difficulty: to what extent may we compare the cell wall solution with a real aqueous phase in which only electrostatic interactions take place? Any answer to this question would condition the choice of the reference standard state for the ions within this compartment and would permit an estimation of the respective activity coefficients of both diffusing ions and fixed charges. In the absence of unquestionable information, this compartment is usually considered as an aqueous medium subject to an homogeneous electrical field and the non-ideal behaviour of the ions is expressed through a correction coefficient inferred from theory and accessible through experimentation.

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Applying Schlögl's theoretical analysis (1954) of the electrical potential which appears across a membrane charged with known CEC separating electrolytic solutions, Demisch and Pusch (1979) proposed as correction parameter the value of the electrolyte partition coefficient between the membrane and the surrounding medium. In this paper, we apply this formalism to the cell wall of the freshwater alga *Nitella flexilis* (L.) Ag., in order to reconsider the correction coefficient as an adjustable parameter called the effective partition coefficient. It is defined by the ratio of the electrolyte partition coefficient—which expresses the non-ideal behaviour of the electrolytes in the wall space—to the activity of the fixed charges of the exchanger. This activity is not a constant and its value can vary at the same time as that of the partition coefficient. Once the effective coefficient has been experimentally determined, its variations are discussed according to the polyelectrolyte theory (Manning, 1969a, b) with a view to proposing an estimation of the fixed charges activity of uronic acids of the *Nitella* wall.

The choice of electrolytes tested, NaCl and LiCl, is a consequence of an experiment carried out under strict equilibrium conditions. We have noted that, in the absence of divalent cations in the *Nitella* wall, they induce important structural modifications in the wall and their respective effects of the CEC loss are very different (Gillet, Van Cutsem, and Voué, 1989).

THEORY

The free energy variation associated with the exchange of one equivalent of ions between an external 1:1-valent electrolyte and a plant cell wall considered as an exchanger is governed by the following equilibrium equation,

$$\Delta G = \Delta G_0 + RT \ln \left(\frac{\bar{a}_+ \bar{a}_-}{a_+ a_-} \right) \quad (\text{Eq. 1})$$

with \bar{a} and a the activities in the exchanger and the external solutions. Introducing the mean activity coefficient γ_{\pm} and replacing ΔG_0 by its value, we obtain,

$$\frac{\bar{c}_+ \bar{c}_-}{c_+ c_-} = \left(\frac{\gamma_{\pm}}{\gamma_{\pm}^0} \right)^2 \exp \left[-\frac{2(\mu_{\pm}^0 - \mu_{\pm}^{\circ})}{RT} \right] \quad (\text{Eq. 2})$$

with μ_{\pm}^0 the mean standard chemical potential of the phase.

The square root of the left-hand side of the preceding equation is equal, by definition, to K_s , the salt partition coefficient (Guggenheim, 1959; Tsimboukis and Petropoulos, 1979). The following equation gives us, therefore, the thermodynamic meaning of this coefficient:

$$K_s = \left(\frac{\gamma_{\pm}}{\gamma_{\pm}^0} \right) \exp \left[-\frac{\Delta \mu_{\pm}^0}{RT} \right] \quad (\text{Eq. 3})$$

As the right hand side of Eq. (2) reduces to unity in a

medium where the activity coefficients are unitary and the reference chemical potential identical to that of the surrounding medium, the partition coefficient K_s is, therefore, clearly a correction factor which permits the plant cell wall to be assimilated with an ideal Donnan medium.

The concentration potential appearing across the plant wall considered as an exchanger is given by (Demisch and Pusch, 1979)

$$\Delta \psi = -\frac{RT}{F} \ln \left[\frac{c_{\text{in}} (1 + 4c_{\text{out}}^2 \chi^2)^{1/2} + 1}{c_{\text{out}} (1 + 4c_{\text{in}}^2 \chi^2)^{1/2} + 1} \right] + \frac{RT}{F} \frac{\bar{u}_+ - \bar{u}_-}{\bar{u}_+ + \bar{u}_-} \ln \left[\frac{(1 + 4c_{\text{out}}^2 \chi^2)^{1/2} + \frac{\bar{u}_+ - \bar{u}_-}{\bar{u}_+ + \bar{u}_-}}{(1 + 4c_{\text{in}}^2 \chi^2)^{1/2} + \frac{\bar{u}_+ - \bar{u}_-}{\bar{u}_+ + \bar{u}_-}} \right] \quad (\text{Eq. 4})$$

with \bar{u} the ionic mobility in the exchanger. In this relation, we introduced the effective partition coefficient, χ defined by the K_s/A^- ratio, with A^- the activity of the ionized fixed charges of the wall. Either term of that relationship, derived from the T.M.S. theory (Teorell, 1953), describes the contribution to the concentration potential of the Donnan or diffusion potentials, respectively. The streaming potential generated by a possible osmotic pressure difference between the 'in' and 'out' solutions is considered to be negligible.

MATERIALS AND METHODS

(1) Biological material

Experiments were carried out on the freshwater alga *Nitella flexilis* (L.) Ag. The walls of unparasited mature internodal cells (third to fifth internodes) were isolated as described by Van Cutsem and Gillet (1983). Selected walls were cut into pieces of 15 mm long. Three pieces were put in 20 cm³ polyethylene vials filled with treatment solutions, before being stocked in a thermostated room (25 °C). All the samples were successively equilibrated in 10 mN CuCl₂ and 10 mN NaCl or LiCl. Copper chloride solutions were used during pretreatment because the specific interactions of the cupric ions with the exchange sites of the wall (Van Cutsem and Gillet, 1982) prevent the weight and the CEC losses more than do the Mn²⁺ ions (Gillet *et al.*, 1989). The solutions were renewed seven times over 8 h.

(2) Concentration and liquid junction potential

Three different electrolytes were used to measure the concentration potentials: NaCl, LiCl and CuCl₂. A wall treated in the same electrolyte as the one which was going to be used for the potential measurement was mounted between two plexiglass chambers. The compartment facing the inner side of the wall was filled with a 10 mN solution whereas the concentration of the same electrolyte solutions contained in the other chamber was varied from 0.5 to 300 mN. Each compartment was stirred at 700 rev. min⁻¹ by means of magnetic stirrers to reduce the thickness of the unstirred layers. The concentration potential which appeared across the wall was measured by means of reversible Ag/AgCl electrodes. Both Ag/AgCl electrodes were calibrated: for each solution used, the electrical potential existing between

the Ag/AgCl electrode and a calomel reference electrode (Schott Geräte—W. Germany) dipped in the same solution was measured and recorded for further use. The compartment facing the inner side of the wall was considered as the reference phase. A Keithley 604 differential electrometer was used as impedance adapter and 4096 measurements were recorded by a Nicolet Explorer III 2090 digital oscilloscope at the speed of 5 ms per point. The potential retained value was chosen as the mean value of the recorded signal corrected by subtracting from it the difference of the corresponding electrode potentials previously recorded. When the acidified solutions were used, both compartments were simultaneously acidified by adding 1 N HCl until the pH became equal to 4.0 or 3.0. A 7 min time lapse corresponding to the maximum equilibrium time of the system was, in this case, necessary to obtain a stable potential value before its recording.

The osmotic pressure of the different electrolytic solutions was not corrected by adding the required amount of mannitol, the streaming potential effects being negligible. Preliminary experiments have shown that, for the largest used concentration ratio (i.e. $c_{out}/c_{in} = 30$), the differences between the potential measured with solutions containing or not added mannitol were only equal to 0.9 mV for NaCl and 1.4 mV for LiCl, whereas the standard variations of the concentration potentials ($n = 5$)—caused by the intrinsic variations of the biological material—were 3.4 mV and 3.5 mV with the NaCl and LiCl solutions, respectively.

The liquid junction potential, which appeared whenever two CuCl_2 solutions with different concentration were brought into contact, was measured according to the same process in the absence of a wall between the two compartments.

(3) Ionic exchange kinetics

The experimental technique was used to determine the variations of the copper, sodium or lithium wall content as a function of the immersion time of the latter in a NaCl or LiCl solution at pH 4.0 or pH 3.0. Three wall pieces 15 mm long isolated from the same internodal cell were used for each determination of the protons amount fixed by the wall after immersion times varying from 30 s to 7 min.

The first two pieces were useful for determining the initial ionic content of the walls and their maximum CEC, the latter being measured after a new equilibrium sequence in 10 mM CuCl_2 . The quantity of protons fixed by the wall was calculated by subtracting the CEC measured after immersion from the maximum CEC. This experimental process was used to minimize the individual fluctuations of the plant cell walls.

(4) Determination of the wall ionic content

After treatment, the walls were quickly dried between two Whatman No. 3 filter papers and weighed by means of a Cahn RG model electrobalance before the extraction of the ionic content in 1 N HCl. The extracts analysis was carried out by atomic absorption spectroscopy (Van Cutsem and Gillet, 1983).

RESULTS

The concentration potential which appears across the *Nitella* wall, brought in contact with two solutions of the same electrolyte (NaCl, LiCl or CuCl_2) at different concentrations, becomes stable after 7 min stirring.

The potential measured in the presence of NaCl or LiCl shows similar variations with respect to the concentration ratio (Fig. 1). As the concentration ratio increases, it reaches a maximum when $\log(c_{out}/c_{in}) = 0.60$, afterwards it linearly decreases. At acidic pH (pH 4.0 and pH 3.0), a

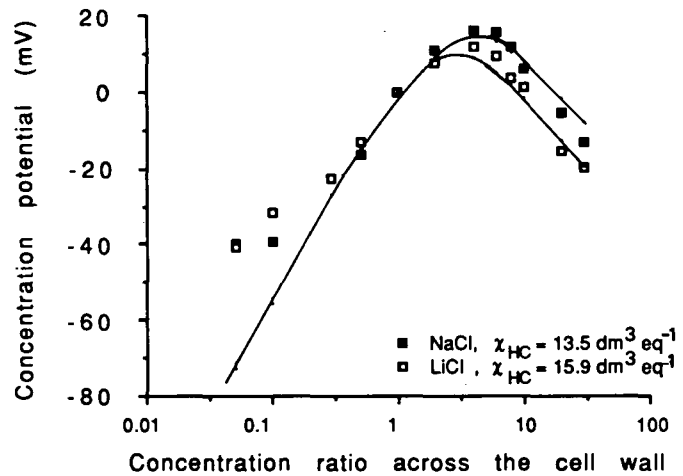


FIG. 1. Concentration potential measured at pH 5.5 across a *Nitella* isolated cell wall as a function of the concentration ratio c_{out}/c_{in} , with c_{in} being fixed at 10 mM. The potential calculated on the basis of Eq. 4 with the value of the effective partition coefficient χ_{HC} determined at high concentration (for NaCl, $\chi_{HC} = 13.5 \text{ dm}^3 \text{ eq}^{-1}$ and for LiCl, $\chi_{HC} = 15.9 \text{ dm}^3 \text{ eq}^{-1}$) are drawn in full lines.

similar variation of $\Delta\psi$ with respect to the concentration ratio is noted (Fig. 2a, b). Nevertheless, its absolute value remains lower than that measured at pH 5.5 and the linear decrease of the potential which is observed when $\log(c_{out}/c_{in}) > 0.60$ is less pronounced.

The potential appearing across a wall bathed in CuCl_2 solutions at pH 3.0 shows different behaviour from that observed in the presence of sodium and lithium chlorides (Fig. 3). The potential remains quasi-constant on a large concentration range and rapidly decreases as the concentration ratio becomes higher than 7.0. At this pH, the concentration potential values are similar to those measured for a liquid junction over the same range of concentration ratios.

The analysis of the cationic fraction of the walls immersed in the acidified NaCl or LiCl solutions shows that all the cupric ions are not exchanged and that at the beginning of the kinetics these occupy more than 50% of the exchange sites (Fig. 4a, b). Moreover, they are proportionally less displaced when the kinetic experiments are carried out at pH 4.0. Finally, at fixed pH, the wall seems to be 15% more selective for the Na^+ cations than for the Li^+ ones.

DISCUSSION

(1) Estimation of the effective partition coefficient

The general formulation of the concentration potential $\Delta\psi$ (Eq. 4), as proposed by Demisch and Pusch (1979), reduces to simplified forms when the c_{out}/c_{in} ratios become either very low or very high.

At the highest concentration ratio and at pH 3.0, the concentration potential is given by

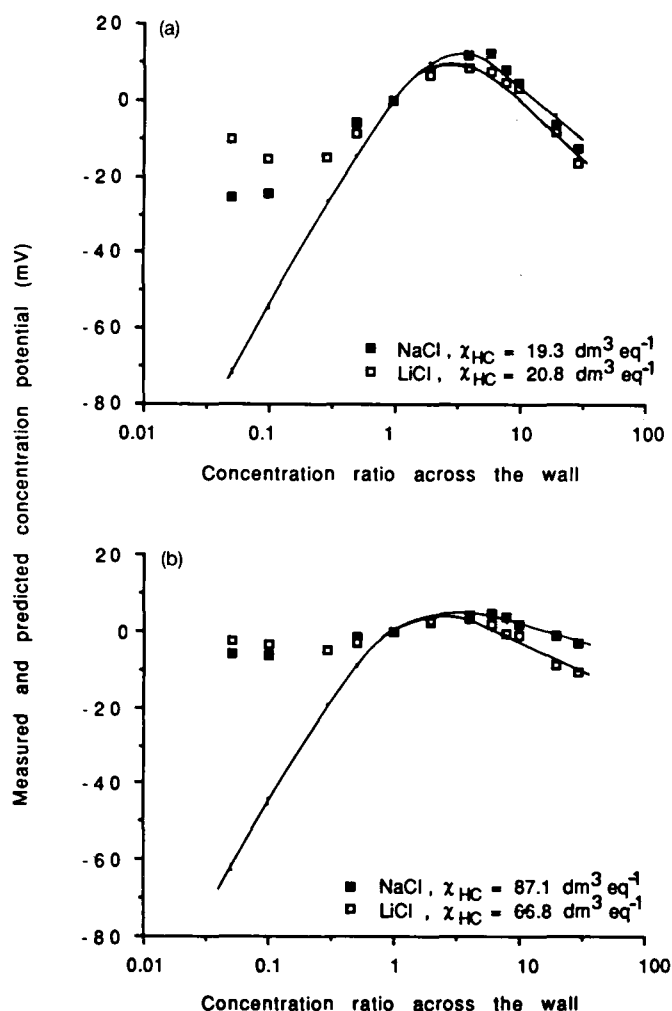


FIG. 2. Variations of the concentration potential with the pH of the surrounding medium (a: pH 4.0 and b: pH 3.0). Data are presented as a function of the concentration ratio c_{out}/c_{in} . The same symbols are used as in Fig. 1. The potential calculated on the basis of Eq. 4 with the high concentration values of χ , χ_{HC} , indicated in the low right-hand corners, is drawn in full lines.

$$\Delta\psi \approx \frac{\bar{u}_+ - \bar{u}_-}{\bar{u}_+ + \bar{u}_-} \frac{RT}{F} \ln \left(\frac{c_{out}}{c_{in}} \right) - \frac{RT}{F} \ln \left[\frac{c_{in} \sqrt{1 + 4\chi^2 c_{out}^2} + 1}{c_{out} \sqrt{1 + 4\chi^2 c_{in}^2} + 1} \right] \quad (\text{Eq. 5})$$

In fact, at this pH, the recombination of the carboxylate sites of the polyuronic acids with the protons is high-rated and the chlorides are no longer prevented from diffusing. Therefore, the concentration potential is the sum of a liquid junction potential given by the Henderson equation (Lakshminarayanaiah, 1972) and of two Donnan potentials which remain at the interfaces because of the existing competition between the cations at high concentration and the protons at the level of these exchange sites ($pK = 3.36$, Morvan, Demarty, and Thellier, 1979).

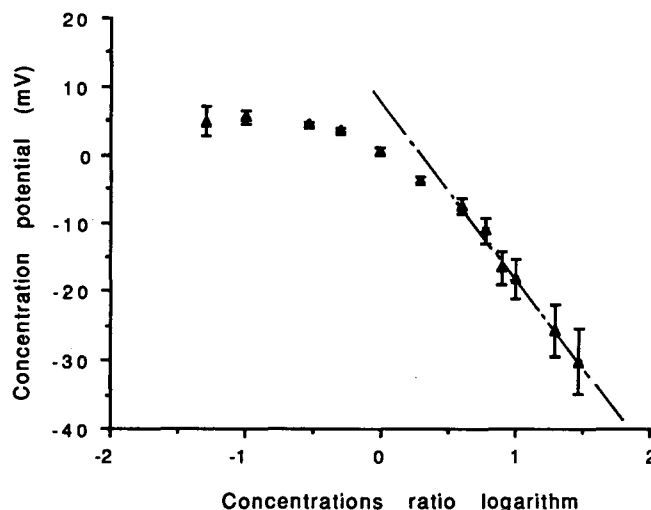


FIG. 3. Concentration potential measured across a *Nitella* wall separating CuCl_2 solutions at pH 3.0 and at different concentrations. Dotted line interpolates the measurements of the liquid junction potential carried out over the range of the high concentration ratios.

The second term of Eq. (5) is nearly independent of c_{out} for $c_{out} \geq 80$ mN. So that $\Delta\psi$ linearly varies with the logarithm of c_{out} since c_{in} is fixed at 10 mN. The slope of the straight line which gives us the $\Delta\psi$ variations at the high concentration ratios allows the ionic mobilities ratios in the wall $\bar{u}_{Na}/\bar{u}_{Cl}$ and $\bar{u}_{Li}/\bar{u}_{Cl}$ to be calculated (Table 1). These are lower than one and increase as the solutions are acidified. At pH 3.0, the \bar{u}_+/\bar{u}_{Cl} values for Na^+ and Li^+ are similar to those of the ratio of their limiting ionic equivalent conductances in aqueous solution. Their increase is attributed to the reduction in the number of interactions between the cations and the exchange sites of the wall in consequence of the progressive neutralization by the protons of the cell wall space (Fig. 4).

At these high concentration ratios, the \bar{u}_+/\bar{u}_{Cl} ratios are equal to the ratios of the corresponding transport numbers, the latter being, therefore, lower than one. Gillet and Lefebvre (1981) have shown that, in the *Nitella* isolated cell wall—but with no divalent cations present—the ratio of the transport number of the K^+ cations to the one of the Cl^- anions, \bar{t}_K/\bar{t}_{Cl} , is equal to 2.16 at pH 5.8. Nevertheless, both sets of results are not contradictory because the \bar{t}_K/\bar{t}_{Cl} value was calculated from concentration potentials measured at low concentration ratios. In such a concentration range, the ratio of the transport numbers is no more equal to the ratio of the corresponding ionic mobilities. It directly depends on the Donnan exclusion phenomenon and is related to the \bar{u}_+/\bar{u}_- ratio by

$$\frac{\bar{t}_+}{\bar{t}_-} = \frac{\bar{u}_+}{\bar{u}_-} \frac{c_+}{c_-} \quad (\text{Eq. 6})$$

with \bar{c}_+ and \bar{c}_- the cationic and anionic concentrations in the wall space (Demisch and Pusch, 1979).

Once the ionic mobilities ratio is determined from the

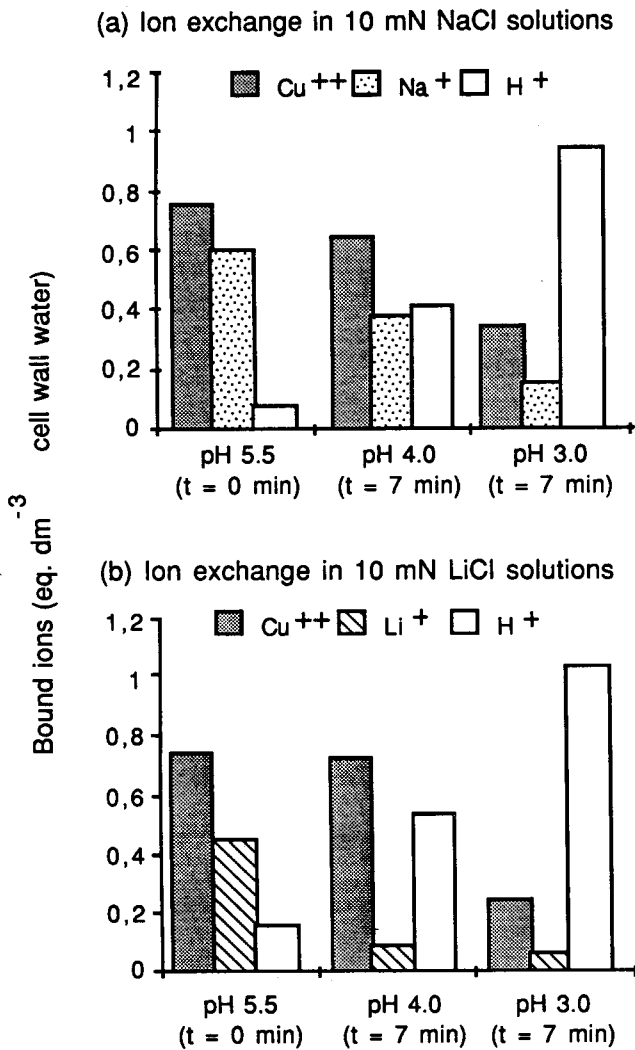


FIG. 4. Ionic composition of the *Nitella* cell wall. (a) Quantity of copper and sodium ions initially bound in a wall in equilibrium with a 10 nM NaCl solution (pH 5.5). Quantity of these ions remaining in the wall after 7 min of ion exchange in a 10 mM NaCl solution at pH 4.0 or pH 3.0 (b) Same as (a) with lithium chloride substituted for sodium during both cell wall treatment and ion exchange process.

slope of the curve of potential, the second term of Eq. (5) is calculated to get the value of χ for this special kind of ionic environment (Table 2). Then we introduce into Eq. (4) the $\overline{u_+}/\overline{u_{Cl^-}}$ and χ values obtained in the limiting case of the high concentrations at pH 3.0 and give c_{out} the

TABLE 1. Effect of the external pH on the ionic mobilities ratios in the *Nitella flexilis* (L.) *Ag* isolated cell wall

$\lambda_+^0/\lambda_{Cl^-}^0$ is the ratio of the limiting ionic equivalent conductance of the cation over that of the chloride ion.

	pH of the external medium			$\lambda_+^0/\lambda_{Cl^-}^0$
	5.5	4.0	3.0	
NaCl	0.17	0.24	0.68	0.66
LiCl	0.14	0.22	0.52	0.51

TABLE 2. Effective partition coefficients of sodium and lithium chlorides in the *Nitella* cell wall

pH_{out} is the pH of the external solution. χ_{HC} is the value of the effective partition coefficient calculated from the measurements of the concentration potential at high concentration and χ_{LS} is the 'best-fit' values of χ calculated on the basis of the Eq. 4 by a least-squares method. All the values of χ are expressed in dm^3 of cell wall water per equivalent.

pH_{out}	NaCl		LiCl	
	χ_{HC}	χ_{LS}	χ_{HC}	χ_{LS}
5.5	13.5	13.1	15.9	15.3
4.0	19.3	19.0	20.8	20.3
3.0	87.1	81.5	66.8	65.2

experimentally tested values. We note (Fig. 2b, full lines) that the concentration potential thus predicted agrees with our measurements until the concentration ratio decreases around 0.5. It can be concluded that the ionic mobilities ratio and the effective partition coefficient are independent of the Na^+ and Li^+ concentration for concentration ratios ranging from 0.5 to 30. The divergence observed at the lowest concentration ratios can be attributed to the proton diffusion whose concentration at either side of the wall may no longer be neglected with respect to that of the other cations, in consequence of the Donnan accumulation.

When the method is applied to the results we obtained with solutions at higher pH (pH 4.0 and 5.5), there is less agreement between the measured and the computed potential but this is acceptable because the exclusion of the chlorides is more pronounced at these pH (Figs 1 and 2a, full lines). From this, we can conclude that the ionic mobilities ratio and the effective partition coefficient are also at these pH independent of the concentration over the same concentration range.

As at a given pH, χ is shown to be constant over a wide concentration range, we try to determine its value in another way from the measured concentration potential without making any hypothesis concerning its nature. We introduce the $\overline{u_+}/\overline{u_{Cl^-}}$ values for Na^+ and Li^+ in Eq. (4) and determine by means of a non-linear least squares algorithm (Brown and Dennis Jr., 1972) the 'best-fit' value of χ for every experimentally tested pH. This 'best-fit' value is the χ value which permits at best the potential computed from Eq. (4) to be adjusted to that measured over all the concentration range tested. It is noted that the χ values so-determined are, at the different pH, similar to the previous ones (Table 2). As foreseen, they increase as the solution pH decreases since the fixed charge activity is lowered by the progressive protonation of the carboxylate sites of the wall.

(2) Estimation of the exchange sites activity

As χ is a parameter related to the activities—and not to the concentration—we are going to estimate from the χ

previously determined values the effective fraction of the fixed charge of the wall involved in the ionic diffusion phenomena. The effective fraction concept was first introduced by Toyoshima, Kobatake, and Fujita (1967) to explain the results of their concentration potential measurements across artificial membranes. The relations between χ , A^- and the ionic concentrations in the wall have been proposed by Heyde and Anderson (1975):

$$\bar{c}_+ = \left(\frac{A^-}{2}\right) (\sqrt{1 + (2\chi c_s)^2} + 1) \quad (\text{Eq. 7a})$$

$$\bar{c}_- = \left(\frac{A^-}{2}\right) (\sqrt{1 + (2\chi c_s)^2} - 1) \quad (\text{Eq. 7b})$$

with \bar{c}_+ and \bar{c}_- the cationic and anionic concentrations in the wall and c_s the electrolyte concentration in the external medium. By introducing into these equations the different estimations of A^- as given by the CEC measurements, it is possible to obtain an estimation of the cationic and anionic concentrations in the cell wall medium.

A rough estimation of the fixed charge activity is inferred on the basis of the results obtained at pH 3.0 with the CuCl_2 solutions. The concentration potential values show that a copper saturated wall ($X_{\text{Cu}} = 0.80$, $X_{\text{H}} = 0.20$, with X the partial molar fraction in the wall) behaves like a neutral membrane at high concentration ratios (Fig. 3). Therefore, it seems reasonable to believe that the cupric ions—specifically adsorbed on the non-diffusing charges (Van Cutsem and Gillet, 1982)—do not contribute to the mobility phenomena in the wall. The fixed charge activity should, therefore, be given by the fraction of the adsorbed monovalent ions (Fig. 4). Substituting in Eqs (7a) and (7b) the monovalent fraction for A^- and its 'best-fit' value for χ , we find at pH 5.5 as well as at pH 3.0 that the estimation of A^- leads at high concentrations to an accumulation of ions in the wall greater than the one theoretically expected in an ideal Donnan space ($K_s = 1$) (Fig. 5). Although the abnormal accumulation of the chlorides can be partially explained by the appearance of monovalent copper complexes (CuCl)⁺ (Sposito, Holtzclaw, Johnston, and LeVesque-Modore, 1981), it is probably the consequence of an overestimated fixed charge activity rather than that of the activity coefficients of the diffusing ions. Indeed, the latter are implicitly included in the estimation of the χ effective partition coefficient (Eq. 3).

Since the obvious disproportion between the CEC and the carboxylate sites activity can not entirely be explained by the specific adsorption of the cupric ions, it is reasonable to suppose that the decrease of A^- is a consequence of a strong electrostatic screening of the polyuronic acids negative charges by the alkali cations. Owing to coordination effects, a part of the monovalent ions should be specifically adsorbed on the exchange sites (review in Poonia and Bajaj, 1979) where it would constitute the

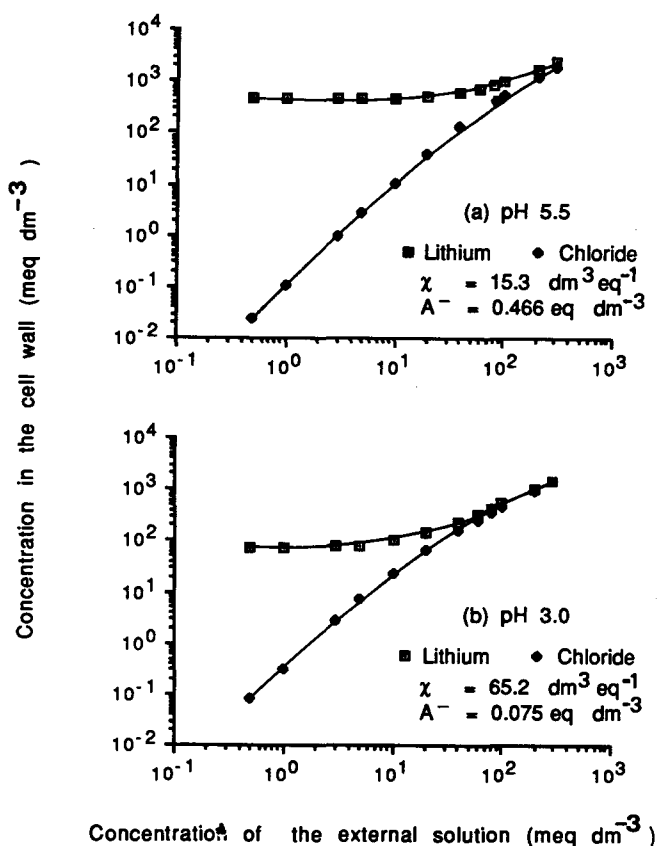


FIG. 5. Lithium and chloride concentration in the wall as a function of the concentration of the external solution. The data are calculated on the basis of Eqs 7a and 7b. The χ_{HC} and A^- values are given in the lower right-hand corners.

immobile bound ions fraction already supposed by Tyree (1968) in his conductivity experiments. If we compare the wall with a solution of polyelectrolyte (Demarty, Ripoll, and Thellier, 1980; Kloareg, Demarty, and Mabeau, 1987; Richter and Dainty, 1989a, b), we can connect the fixed charge activity decrease to the condensation of a part of the alkali cations on the non-diffusing negative charges (Manning, 1969a, b). In fact, counterions of valence z condense when the value of the linear charge density parameter ξ defined by

$$\xi = \frac{e^2 \alpha \bar{N}}{\epsilon k T b} = 0.713 \frac{\alpha \bar{N}}{b} \quad (\text{at } 25^\circ\text{C}) \quad (\text{Eq. 8})$$

is greater than $|z|^{-1}$. The fraction of the polyion charge non-neutralized by the condensation is equal to ξ^{-1} times the effective CEC (Manning, 1969a). In Eq. (8), e is the electronic charge, ϵ the dielectric constant, k the Boltzmann constant, T the absolute temperature, \bar{N} the mean number of fixed ionizable groups per monomeric unit, α the polyion dissociation degree and b the monomeric unit mean length (nm). On the other side, the maximum activity of the non-neutralized non-diffusing charges equals by definition χ^{-1} (Eq. 3). Therefore, we get

TABLE 3. Linear charge density parameter (ξ) and mean length of the monomeric unit (b) associated to some alkaline forms of the *Nitella* isolated wall

ξ is a dimensionless parameter and b is expressed in nm.

pH	NaCl		LiCl	
	ξ	b (nm)	ξ	b (nm)
5.5	8.1	0.09	7.1	0.10
4.0	7.5	0.10	2.1	0.35
3.0	13.5	0.05	4.9	0.15

$\chi^{-1} = \xi^{-1} Y$, with Y being the concentration of the non-diffusing charges, which do not undergo either protonation or specific adsorption of divalent cations. The equation permits us to calculate the ξ value from the experimentally determined effective fraction of the CEC (Fig. 4). If we compare the ξ values thus obtained with the one calculated from equation (7) for the α -D-polygalacturonic acid, we note that the ξ values obtained are not consistent with the value $\xi = 1.64$ usually accepted (Table 3). The mean lengths b of the monomeric units associated with these ξ values, for the most part almost equal to 0.1 nm (Table 3), allows us to assume that, in the presence of cupric ions adsorbed into the wall, the adsorption of the Na^+ and Li^+ alkali cations is also the result of the exchange sites chelation, therefore, reducing the related mean length of the monomeric unit.

In conclusion, it emerges that both electrolytes tested show a similar behaviour in the presence of specifically adsorbed copper and that the carboxylate sites activity of the *Nitella* wall during non-equilibrium processes is considerably lower than their concentration estimated during equilibrium processes (CEC). It also follows from this that owing to the exchange sites electrostatic screening, only a small fraction of the wall counterions actively contribute to the ionic diffusion phenomena.

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