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The Existence of a Diffuse Electric Double Layer at Cellulose Fibril Surfaces and its Role in the Swelling Mechanism of Parenchyma Plant Cell Walls

Ilan Shomer,[a]* Haim Frenkel[b] & Corina Polinger[a]

[a]Department of Food Science, Institute for Technology and Storage of Agricultural Products, [b]Department of Soil Physical Chemistry, Institute of Soils and Water, Agricultural Research Organization, The Volcani Center, PO Box 6, Bet Dagan 50250, Israel

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ABSTRACT

The swelling behavior of parenchymatous cell wall was studied in aqueous and non-aqueous solutions. The swelling was significantly linearly correlated with the static dielectric constant of the liquid. Increasing the dielectric constant above that of water (e.g. formamide) does not increase the swelling accordingly. In addition, the swelling is dependent on the type of the adsorbed ions, tending to decrease with the increased valency. The swelling in NaCl solution was higher than in $CaCl_2$ *solution at the same concentration. The swelling mechanism of the plant cell wall system is attributed to the attraction and repulsion forces acting in the diffuse double layer of the fibrillar surfaces, considering the limitation due to the fibrillar branching which forms interfibrillar connections.*

INTRODUCTION

The swelling of plant cell walls is of great importance for some biological and biotechnological activities, such as hydraulic conductivity, nutrients uptake, transportation of assimilates, texture, etc. (Colvin, 1981). The cell wall is the skeleton which determines the shape of the cell, and it consists of subunits which are connected by means of covalent and hydrogen bonds, in a defined network (Vian, 1982). This network, which is composed of polysaccharides and proteins, controls the cell volume

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*To whom correspondence should be addressed.

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(Carpita, 1985). However, the non-proteic cell wall components are largely responsible for the mechanical properties of the wall (Nakajima *et al.*, 1981).

The cellulosic matrix is arranged in bundles of macro- and microfibrils which are bundles of elementary fibrils or micelles (Essau, 1965; Fahn, 1974; Burgess, 1979; Carpita, 1985). Within a micelle the molecules are joined in long chains by primary valencies and in the lateral direction they are bound together by hydrogen bonds (Rogers & Perkins, 1968; Hall *et al.*, 1974).

Shomer *et al.* (1984) and Shomer & Levy (1988) showed that under conditions prevailing in a macerate of parenchyma tissue, the insoluble solids of the cell wall, and especially those of the cellulose, swell and hold large quantities of water in aqueous media. In these studies, the ultrastructural and swelling changes in the cell wall and its composition showed that the microfibrillar lattice of the cellulose is the factor which is responsible for the swelling rather than other factors such as pectin, protein or hemicellulose. Furthermore, enzymatic removal of pectin enabled greater swelling of the cell wall due to the increased volume of the cellulose lattice (Shomer *et al.*, 1984). It has been suggested that the main volume of the precipitate results from water penetration into the cell wall intermicrofibrillar space which, according to Carpita *et al.* (1979), has a diameter range between 3·5 and 5·2 nm, depending on the cell source.

Plant cell walls behave as a polyanionic matrix (Crasnier *et al.*, 1985), and in some cases they are considered as ion exchangers (Amory & Dufey, 1984). Therefore, the electrical properties may be of great practical importance in the regulation of cell wall swelling.

There are numerous studies of the structure of the cell wall and of the laying down of the cellulose microfibrils and their orientation (Preston, 1974, 1979, 1988; Vian, 1982).

Plant cell walls are hydrated naturally, and this makes it difficult to evaluate the swelling capacity which may be better studied in comparison with its non-swelled state. Hence an understanding of the swelling mechanism of plant cell walls *per se*, which is of basic interest in plant cell biology, has to be studied in dehydrated matter and in the absence of other factors such as plasma membranes and active enzymes. In this context, while the structure of the cell wall has been studied comprehensively, the mechanism involved in determining the causes of the porosity formation inside the cross-linked cell wall deserves basic study.

The present study dealt with the swelling mechanism of dried cell walls as examined by controlled hydration rates, obtained by water, a range of organic solvents and in mixtures of water with organic solvents.

Representative mixtures of these liquids were tested with and without electrolytes such as NaCl and $CaCl_2$.

RESULTS AND DISCUSSION

The swelling of dried cell wall material (CWM) in water–methanol mixtures is shown in Fig. 1. The swelling increases along with the percentage of water in the mixture. The lowest degree of swelling in this system was obtained in pure methanol. The amount of water adsorbed onto the cell walls was 50 g/g dried cell walls, whereas only 12 g was adsorbed in a solution of 20% ethanol. It is difficult to distinguish between capillary adsorption and purely surface-adsorbed water. However, the effect of methanol is quite pronounced.

If we hypothesize that the cell wall behaves in the same manner as an inert ion exchanger (Amory & Dufey, 1984), then the swelling of cell walls in water is due mainly to the hydration tendency of the fixed ionic groups and the counter ions, the osmotic activity of the counter ions, and the electrostatic repulsion between neighboring fixed ionic groups. As

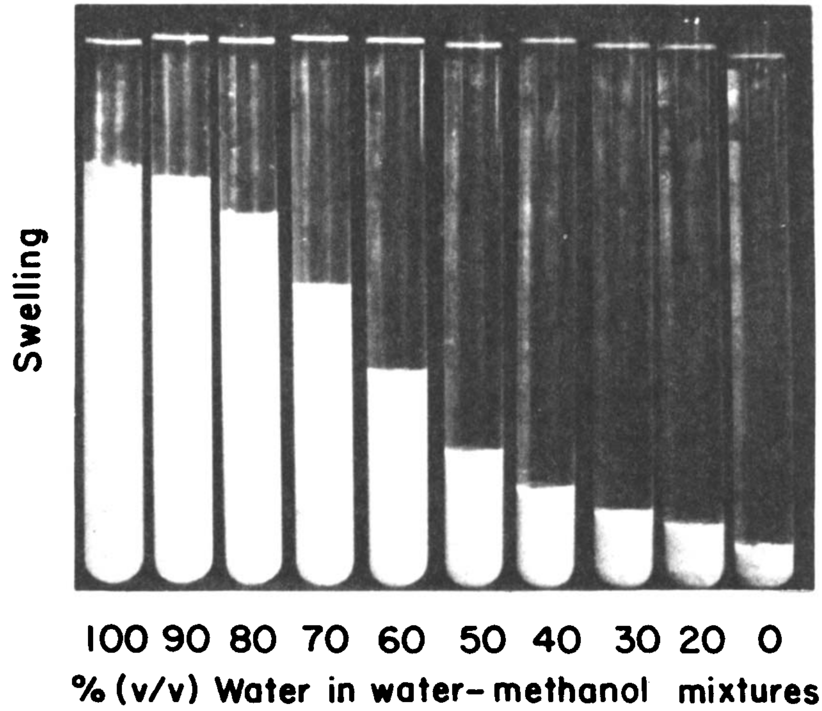

Fig. 1. Swelling of cell wall macerate in water–methanol mixtures.

swelling of the cell walls proceeds, the opening up of the matrix exposes additional available sites for adsorption of water molecules. Swelling equilibrium is a balance of opposing forces: the osmotic and the electrostatic forces which are balanced by the tendency of the expanded microfibrillar matrix to contract. Swelling in polar non-aqueous solvents is quite similar. The solvation tendency of the ions, the osmotic pressure difference and the electrostatic repulsion between fixed charges are the most important factors. The less polar the solvent, the weaker the solvation tendency of the ions. The lower the dielectric constant of the solvent, the stronger are the electrostatic interactions between ions of opposite charges (Helfferich, 1962). This can be seen in Fig. 2, which depicts the swelling of CWM in a mixture of water and organic solvents. In this figure swelling is presented as a relative swelling, which is the ratio between the volume of swollen CWM in a specific solvent mixture and its volume in distilled water. From the data presented in Fig. 2 it is evident that for mixtures of acetone, methanol and ethanol the swelling increases as the mole fraction of the organic solvents decreases. The distinct rates of reduction in swelling, in those mixtures, occurred between 0% and 30% mole fraction of the organic solvents. Thereafter, a continuous slow decrease in swelling occurs in all of those systems, as the mole fraction of the organic solvents continues to increase. A maximum reduction in swelling was reached in the pure solvents.

The swelling of CWM in formamide mixtures is quite different: the dielectric constant of formamide is greater than that of water. If the dielectric constant plays the major role in the swelling behavior, one

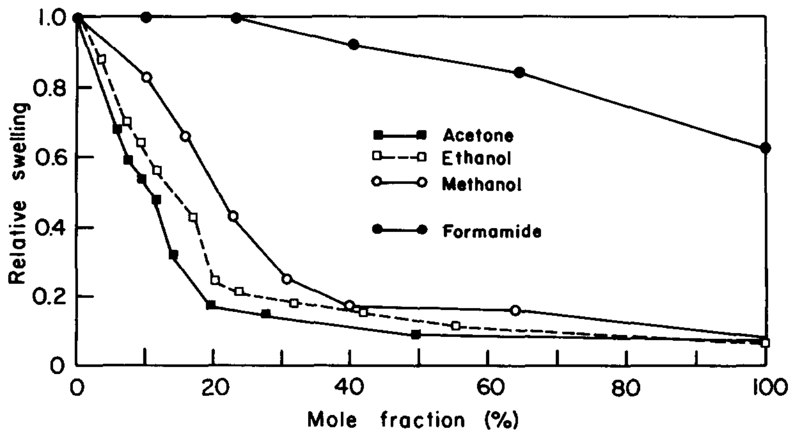

Fig. 2. Relative swelling of cell wall macerate in various organic solvents as a function of their mole fraction.

would expect that even more swelling would occur in formamide than in water. However, the cell wall swells to only a limited extent, because the packed chain of the matrix can unfold and make room for solvent molecules, but the cellulose chains cannot separate completely because they are cross-linked. Therefore, the addition of formamide to water up to ~25% mole fraction, does not lead to increased swelling. A further addition of formamide reduces the swelling to only a limited degree. This could be explained by the hydrogen bonds formed between the cellulose chains and the solvent, and by the possible tendency of the formamide molecules to be 'complexed' with water. Above ~25% mole fraction relatively strong hydrogen bonds between formamide molecules and cellulose chains reduce the swelling.

The dielectric constant of the dispersing medium has a strong effect on the degree of swelling, as can be seen from the results presented in Fig. 3. The sugar composition of the insoluble cell walls was not changed as a result of the solvent treatments. The neutral sugars (including rhamnose, arabinose, xylose, mannose, galactose, glucose), galacturonic acid (its degree of esterification) and proteins were not significantly different from each other in their amounts and ratios (Table 1). As the dielectric constants of the solvent (Table 2) decrease, swelling also decreases. Furthermore, a significant linear correlation exists between the observed swelling and the bulk static dielectric constant of the solvents, although the scatter of experimental points prevents the confident assignment of a specific functional form to the results. No sign-

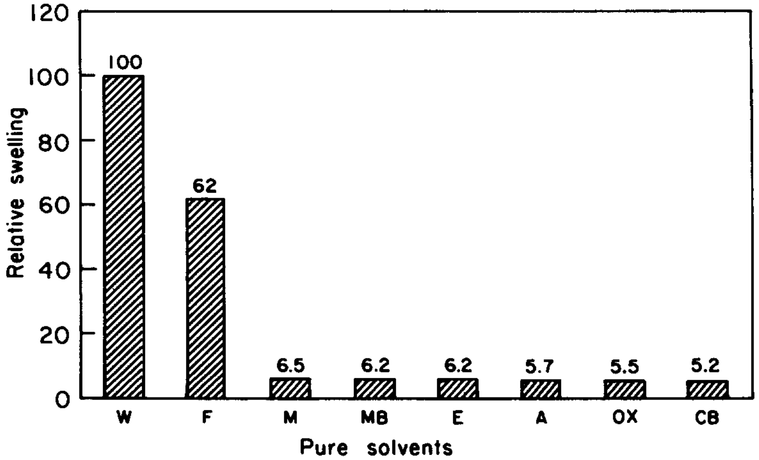

Fig. 3. Relative swelling of cell wall macerate in various mixtures of organic solvents in water as a function of the static dielectric constant of the mixtures.

TABLE 1

Sugar Composition, Pectin Content (% of dry matter), Degree of Esterification, and Protein Content (N × 6·25, Determined by Kjeldhal) of the Dry Matter (± S.D.)[a]

Sugar content[b]						*Galac. acid*[b]	*D.E.*	*Protein*[b] *(N × 6·25)*
Rh	*Ar*	*Xy*	*Ma*	*Ga*	*Gl*			
0·44 ± 0·06	2·08 ± 0·18	4·18 ± 0·39	4·10 ± 0·90	3·53 ± 0·59	27·98 ± 1·66	22·74 ± 0·70	63·59 ± 7·70	17·50 ± 2·50

[a] The results were obtained from treatments of the various solvents. The results are the average of cell wall samples treated with water, methanol, ethanol acetone and formamide. Each treatment of the solvent-treated cell walls was done in two repetitions.

[b] Rh: rhamnose; Ar: arabinose; Xy: xylose; Ma: mannose; Ga: galactose; Gl: glucose; Galac.: galacturonic; D.E.: degree of esterification.

TABLE 2
Static Dielectric Constants, Dipole Moment, Viscosity and Density of Solvents used in the Study

Solvent	*Chemical formula*	*Dielectric constant at 20°C*	*Dipole moment*	*Viscosity CPS at 20°C*	*Density (kg/m³)*
o-xylene	C_8H_{10}	2·57	0·62	—	0·880
Chlorobenzene	C_6H_5Cl	5·71	1·69	0·799	0·878
Methyl benzoate	$C_8H_8O_2$	6·59	—	—	—
Acetone	C_3H_6O	21·00	—	0·316[a]	0·790
Ethanol	C_2H_6O	25·00	1·69	1·200	0·789
Methanol	CH_4O	33·62	1·70	0·597	0·791
Water	H_2O	80·37	1·85	0·890	0·997
Formamide	$CHNH_2O$	109·00	3·68	3·300	1·127

[a] At 25°C.

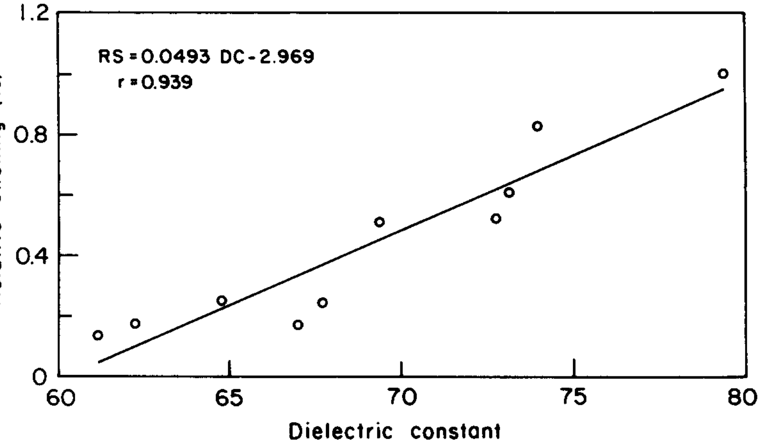

Fig. 4. Relative swelling of cell wall macerate in the following pure solvents: water (W), formamide (F), methanol (M), methyl benzoate (MB), ethanol (E), acetone (A), *o*-xylene (OX) and chlorobenzene (CB).

ificant correlations were found to exist between the swelling of the cell wall and the dipole moment of the liquid. These results suggest that the bulk dielectric constant of the mixtures is a major determinant of the total swelling of the cell wall, despite a wide range of solvent molecular properties. However, from the scattering of the results (Fig. 4) it is evident that the dielectric constant is not the sole determinant of swelling in this system. Some other factors may affect the swelling such as hydration tendency of the ions, structure role of water and the activity of the organic solvents in the mixture (Berendson, 1975; Franks, 1975).

The diffuse double layer at the cell wall surfaces consists of the cellulose microfibrillar charge and the compensating counter ions which reside in the liquid immediately adjacent to the surfaces. The counter ions are subjected to two opposing tendencies: (i) the electrostatic attraction of cations to negatively charged surfaces and (ii) the diffusion of cations from the surface of the microfibrils, where their concentration is high, into the surrounding solution where their concentration is lower. These two opposing tendencies result in a decreasing counter ion concentration from the surface to the bulk solution. Divalent ions are attracted to the surface with a force twice as great as that in the case of monovalent ions (Van Olphen, 1977). Thus the diffuse double layer in a divalent ion system is more compressed toward the surface. The greater the compression of the ionic atmosphere toward the surface, the less the overlap of the diffuse counter ion atmospheres and the smaller the swelling pressure, between the two positively charged ionic atmospheres. This can be seen in Fig. 5, where swelling of the cell wall in water, $CaCl_2$

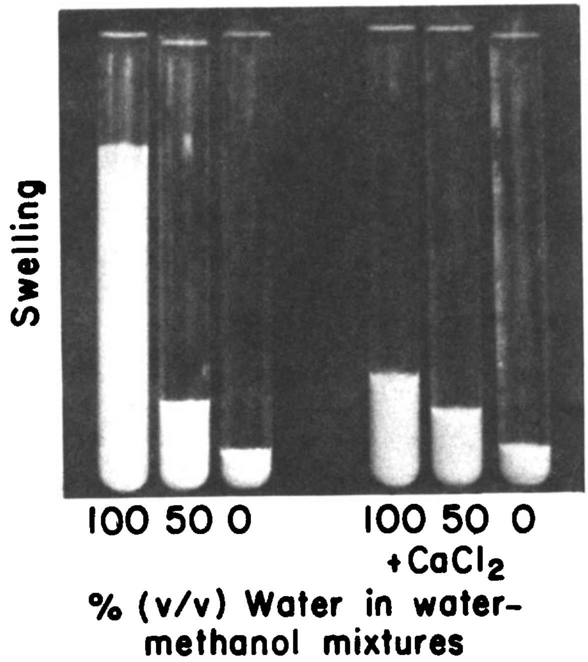

Fig. 5. Swelling of cell wall macerate in water, NaCl–$CaCl_2$ mixtures (SAR-16) and $CaCl_2$ solutions at electrolyte concentration of 0·3 M.

(0·3 M) and NaCl/$CaCl_2$ at a sodium adsorption ratio (SAR) of 16 is presented. The SAR is defined as follows in eqn (1).

$$\mathrm{SAR} = (\mathrm{Na}^+)/(\mathrm{Ca}^{2+})^{1/2} \qquad (1)$$

where all concentrations are given in mmol/litre.

While the maximum swelling of the cell wall occurs in distilled water, the addition of electrolytes, which suppressed the diffuse double layer, decreased swelling drastically (Fig. 5). The effect of $CaCl_2$ was more pronounced than that of the NaCl mixture at the same concentration. The Gouy theory (Van Olphen, 1977) predicts that the thickness of the diffuse double layer will also decrease when the dielectric constant of the medium is reduced. Therefore, the theory predicts that polar solvents will restrict the swelling of the cell wall already immersed in electrolyte solution. This is shown in Fig. 6, where the relative swelling of the cell walls in water, NaCl + $CaCl_2$ (SAR-16), and $CaCl_2$ is presented. It is evident that the addition of ethanol to NaCl–$CaCl_2$, and to $CaCl_2$ mixtures enhanced the restriction of swelling, especially in the lower mole fraction range. At a mole fraction of 25% ethanol, no difference in swelling was observed in the different systems.

The lattice of cellulose fibrils, and its (at times) bound matter such as pectin, neutral sugars, proteins, etc., is the skeleton which determines the swelling ability of the cell wall. The attraction and repulsion forces among the fibrils are considered by us to be dependent on the electrical properties of their surfaces. Although the charging rate of the cell wall may be derived from other fibrillar bound components such as pectin

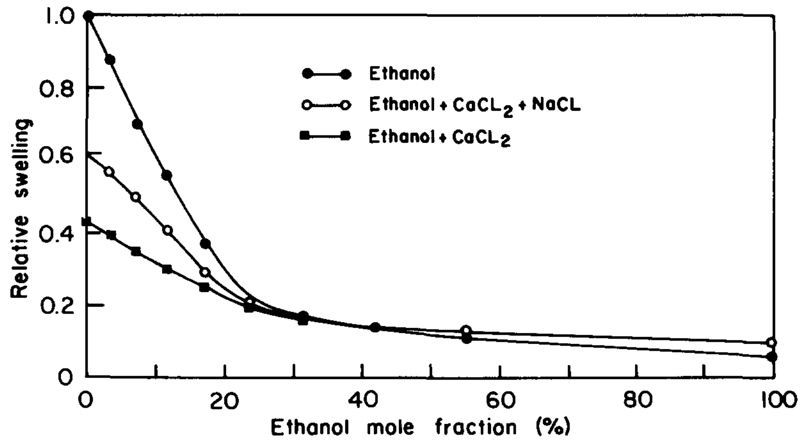

Fig. 6. Relative swelling of cell wall macerate in ethanol–water, ethanol–NaCl/$CaCl_2$ (SAR-16), and ethanol $CaCl_2$ solutions as a function of the ethanol mole fraction.

and protein, the final charge is the factor that determines the electrical properties of the fibrillar lattice.

CONCLUSIONS

Swelling of a given cell wall is affected by both the composition of the adsorbed exchangeable cation (residing on the microfibril surfaces) and the concentration of the electrolytes in the bulk solution. The diffuse double layer repulsion forces, also called the swelling pressure, decrease with the increase in the valence of the adsorbed ions. Because adsorbed Na^+ ions form a diffuse layer, high swelling pressures develop between the microfibrillar surfaces. Conversely, low swelling pressure develops between the microfibrils when Ca^{2+} ions are adsorbed. This was because of the attraction of the Ca^{2+} to two negative charges, which resulted in greater attraction forces between the counter ions and the negative charged surfaces. The presence of an organic liquid exhibits a similar effect. Decrease in the dielectric constant of the solution causes a decrease in the thickness of the diffuse double layer, thereby causing the microfibrils to come closer together, which results in less swelling. A significant linear relationship exists between swelling of the cell wall and the dielectric constant of the bulk solution.

EXPERIMENTAL PROCEDURE

The CWM was separated from a macerate of tomato (*Solanum lycopersicum* L.) fruit pericarp tissue. The pericarp tissue, free from epidermal layers and seeds, was mashed and then immediately heat-inactivated, for 10 min at 90°C. The obtained macerate included mainly whole cell walls, which were treated according to Ahmed & Labavitch (1977). The macerate was washed several times in distilled water, chloroform–methanol 1:1 (v/v) and acetone, respectively, then air dried and lyophilysed.

The composition of insoluble polysaccharides and pectin was determined on freeze-dried insoluble matter. Neutral sugars were analysed according to Sloneker (1972), on the alditol acetates of the hydrolysed polysaccharides by gas liquid chromatography (Hewlett Packard 5790A series Gas Chromatograph) with a flame ionization detector. The analysed solution was injected into a column of 3% SP-2340 on Supelco Port 100/120 mesh, under the following conditions: the column temperature was increased from 120°C to 220°C at the rate of 30 ml/min;

injection was at 230°C; detector was at 250°C; nitrogen was used as the carrier gas at a rate of 30 ml/min; oxygen and hydrogen were used for combustion at rates of 250 and 25 ml/min, respectively. Pectin was determined according to Blumenkrantz & Asboe-Hansen (1973) and the degree of esterification according to Wood & Siddiqui (1971). For these analyses, the CWM was washed several times with analytical acetone and dried.

Swelling behavior of the cell wall was studied in aqueous and non-aqueous solutions of acetone, ethanol, formamide and methanol, and the non-aqueous solvents *o*-xylene, chlorobenzene, methyl benzoate, acetone, ethanol, methanol and formamide. Some of the physical and chemical properties of the pure solvents are given in Table 2. The effect of Ca^{2+} and Na^{+} cations on swelling was also investigated in a mixture of 0·3 M NaCl–$CaCl_2$ salts in aqueous solutions of ethanol. The swelling experiments (in three replicates each) were carried out as follows: 300 mg of dried CWM was placed in a 14-mm diameter tube, 20 cm in length, and the desired liquid was added to the volumetric capacity and shaken gently overnight, to enable free swelling. Then the tube with the obtained suspension was placed vertically and left to enable free precipitation of insoluble cell walls, and the height of the CWM precipitate in the tubes was measured daily for 10 days. The values of swelling among the three replicates always agreed within 5%.

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