



Review

Solvation and expansion of neutral and charged chains of a carbohydrate polyelectrolyte: Galacturonan in water. A critical revisiting

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ABSTRACT

Experimental and theoretical data have been revisited to shed light onto the aspects of hydration and chain expansion of pectic acid (galacturonan) upon charging. The prediction of the variation of the number of solvation water molecules between the two limit ionization states from theoretical calculations was confirmed to a very high accuracy by the corresponding number evaluated from dilatometric measurements. The relevance of hydration to the mechanism of bonding of calcium ions by sodium pectate is discussed. Characterization of polymer expansion has been obtained by calculating the values of the characteristic ratio and/or the persistence length on the respective populations and comparing the theoretical predictions with experimental data. The results show that a charged chain in typical conditions of ionic strength is more expanded than its neutral counterpart, whereas the ideal limit (3_1 and 2_1) helical conformations in the uncharged and totally charged conditions, respectively, share the same value of the linear advance of the helical repeat, when the ionic strength tends to infinite. Total divergence between theoretical predictions and experimental evidence rules out the possibility that carboxylate charge reduction by protonation and by methyl esterification are equivalent in determining the solution behavior of galacturonan.

1. Introduction

Environmental consciousness and increased attention for healthy food and nutrition has brought even more the importance of natural polymers from renewable sources to the attention of the general public, but also to that of technologists and scientists [1]. Plant polysaccharides, both from terrestrial and marine sources, have undergone a steady increase of interest: “*Plant cell-wall carbohydrates, which together form the most abundant natural compounds on earth, are our most important renewable natural resource.*” [2], Pectin is ranking in an ideal “top ten” of the food carbohydrate polymers; interestingly, new, unexpected applications out of the food field seem at hand, like as “*an extraordinary natural kinetic hydrate inhibitor*” [3]. Besides being used as a bio-sorbent to remove toxic metals from waste water, its application in the food industry is mainly as a gelling agent. Both properties are deeply rooted in the complex chemical structure, including its polyelectrolyte nature as a polyanion, and in the delicate balance of polymer-polymer and polymer-solvent-(co)solute interactions. Water is the solvent of choice for pectin, both in plant and in food. Among co-solutes, cations play a major role

(recognized as early as in 1931) [4], specifically interacting with the carboxylate group of constituent galacturonic acid [5]. Divalent cations, like Ca^{2+} , form sequences in the so-called “egg-box” junctions [6] (duly revisited in the “shifted egg-box” model [7]) of gels at neutral pH; on the other side, protonation of COO^- groups by H^+ give rise to interchain junctions in the acid gel [8]. In all cases the solvation of the OH-rich polyuronide by water (hydration) is fundamental for the structural [9–12], phyto-physiological and functional [13,14] behavior of pectin. Pectin is a collective name comprising various types of linear or branched chains (whose main types are referred to as homogalacturonan, rhamnogalacturonan I, and rhamnogalacturonan II) [2]. In more restricted definition, most of the scientific literature refer to the linear polymer of α -(1 → 4)-linked D-galacturonic acid (GalA), occasionally interrupted by α -(1 → 2)-linked α -L-rhamnopyranose residues. A variable proportion of the galacturonic acid residues is methyl-esterified. [2] Pectins are operationally grouped into low-methoxyl-pectins (LMP) and high-methoxyl-pectins (HMP), the degree of methylation marking the difference ranging from 40% to 50% depending upon the authors. Often, and depending upon the context, the pectin and pectate words refer to

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the salt form of HMP and very low LMP, respectively, and pectinic acid and pectic acid to the corresponding acid forms, respectively. Similarly, the term poly(galacturonic acid) (galacturonan, PGA; [Scheme 1](#)) refers not only to the “perfect” homopolymer, but also to linear GalA-polymers, interspersed with neutral sugars and/or esterified GalA residues, altogether for not more than about 10%.

The hydration of pectin has been the topic of some studies, both experimental and theoretical, starting from the first one dating back to 1938 [15]. The relevance of hydration in relation to the response of pectic material to in vivo osmotic stress prompted a series of extensive investigations, essentially based of methods of classical physical chemistry of polymer solutions and gels [13,14,16–18]. Other studies, both experimental (including thermodynamic [19], spectroscopic [20], capillary viscometry and sedimentation [21] approaches) and theoretical [12,22], were more focused on the interaction with water of LMP and poly(galacturonic acid) chains in dilute aqueous solutions.

The main goals of the present paper are to achieve a better understanding of both hydration and expansion of galacturonan, by critically revisiting previously published data - both theoretical and, particularly so, experimental - also as the polymer response to typical factors affecting polyelectrolyte conformation like the degree of charging (by protonation of the uronate group) and the ionic strength of the medium. Galacturonan is able to produce ionotropic gels by addition of ions, either the H^+ ion (at low pH), or the Ca^{2+} one, at neutral pH, that justifies our attention being put onto the limiting cases of uncharged poly(galacturonic acid), on one side, and to fully charged poly(galacturonate) on the other. In that respect it should be noted that most available data pertain to samples in which the variations of polymer charging have been achieved by modulating the level of esterification of the carboxylic group. Albeit equivalent from the electrostatic point of view, the two processes are chemically very different, making the comparison of the two charge neutralization modes very useful, recalling the warning “that validation of computer predictions requires a close interplay between experimentalists and theoreticians.” [23].

Reference will be made to previous work of this group, both experimental [19] and theoretical [12], as well as to relevant literature results, in order to critically revisiting those two important aspects of pectic acid behavior. A redrawing of the most relevant among the quoted data from this laboratory has been collected in the Supplementary Material to ease data accessibility.

2. Hydration

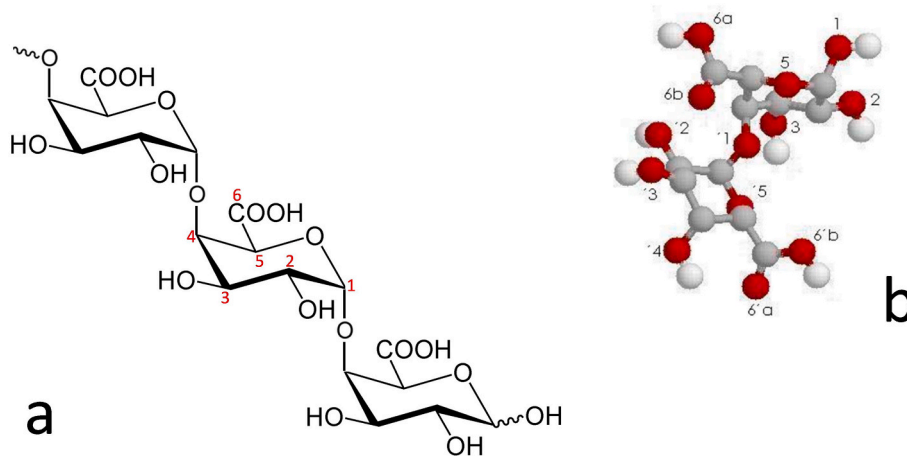
As stated in the opening, the first goal of this paper is to get more

detailed information of the hydration of pectic acid and sodium pectate. In water, hydrophilic solutes and - even more so - ionic ones bind large amounts of solvent molecules in the process called electrostriction. Electrostriction has received since long a high attention both from the fundamental and the applied viewpoints. Marcus has given some very comprehensive theoretical surveys; [24,25] a thorough reference to many different experimental techniques (albeit missing dilatometry) to study hydration is given in the Introduction of the recent paper by Penkov [20].

The only reference to galacturonan moiety in the sequence of master papers by Engelsen and co-workers on the hydration of carbohydrates [e.g. references [26, 27]] is to primary cell wall potato pectin [28]. However, the galacturonic acid component in the investigated samples accounted for a bare 8.3 ± 2.1 (mol %) in the native sample, to reach a relative abundance as low as 25.6 ± 3.6 (mol %) in the debranched sample. Moreover, some of the COOH groups were esterified (“the degree of acetylation of GalA was $46 (\pm 1)\%$ in all samples.”), although “it can be concluded that the degree of methoxylation is low and the methoxylated GalA's are primarily located in the immobile regions”. “Overall, it is observed that the arabinan side chains are readily hydrated, whereas at least some of the galactan side chains remain sufficiently immobilized for CP, even at the highest level of hydration.....The Rha-GalA backbone was reluctant to hydrate. This is surprising because the backbone carries negative charges but was supported by both 2H and ^{13}C NMR experiments.” [28]. Altogether, the results are only qualitative, pointing to a reduced mobility and hydration of the GalA units, that are buried in the rhamnogalacturonan core of the primary cell wall potato pectin.

Likewise, in the extensive modeling work by Pérez and co-workers on galacturonan/pectin, only the paper by Perić et al. explicitly tackled the problem of hydration, with explicit-solvent simulations (10 ns), mostly aimed at describing hydration around calcium counterion, defined as “water molecules with their oxygen atoms closer than 0.6 nm from the counter-ion in the proximity of a poly(galacturonate) chain” [29].

Probably the most recent work on hydration involving poly(galacturonate) is that by Huynh et al. [22], which was focused on the “interactions between polygalacturonate (polyGal) and four divalent cations ($M^{2+} = Ba^{2+}, Ca^{2+}, Mg^{2+}, Zn^{2+}$) that differ in size and affinity for water.”. The molecular dynamics (MD) simulations involved “umbrella sampling simulations to investigate the interactions of the divalent cations with either water or with the carboxylate group of Gal.”. In essence, the focus of the work was put on the hydration (and correlated change upon binding) of the different counterions rather than on that of the polyelectrolyte; no information was provided on the hydration of the galacturonan chain per se, in the absence of divalent ions.



Scheme 1. a. Schematic representation of the ideal poly(galacturonic acid) polymer. Numbering refers to the pyranosidic carbon atoms. b. Snapshot of a galacturonic acid dimer. Numbering refers to oxygen atoms linked to pyranosidic carbon atoms, prime-marked numbers referring to the non-reducing galacturonic acid unit oxygen atoms.

With more general reference to the biopolymer field, it should be recalled that the structural features of galacturonan - as well as of alginates, hyaluronan or xanthan, to name just some analogs - make the class of polyuronates a very peculiar one. Structurally, they are linear weak (homo)polyacids; among biopolymers, only nucleic acids (e.g. B-helix or single filament-DNA) are linear and (homo)polyacids, like polyuronates, but the “hard” nature [30] of the phosphate (strong acid) group makes them behaving very different as to both metal binding and protonation. Only the behavior of poly(L-glutamic acid) (or its aspartic analog), in the protein/polypeptide world, is very close to that of polyuronates, because the otherwise often globular conformation, the presence of significant hydrophobic interactions and, above all, the zwitter-ionic nature all contribute to pull the protein domain very far from that of the carbohydrate polyanions of interest. Only popular synthetic polycarboxylates, like poly(acrylic acid) or poly(methacrylic acid), share the definition of linear weak (homo)polyacids with the carbohydrate analogs; however, the much smaller size of the (helical) repeating unit and the much larger flexibility of the olefin backbone is at the root of the known big difference in the conformational behavior between those synthetic and the natural polycarboxylates.

2.1. Modeling hydration of galacturonan

The results of modeling from reference [12] have been reported here in Table 1. They provide a quantitative estimate of the amount of water molecules intimately bound to poly(galacturonic acid). Not unexpectedly, they show that charging the polyuronide brings about an increase of such number (per repeating unit) from about 11 to about 19 (+ 71%), in parallel with an almost similar increase in elongation (as persistence length) from 79.5 Å to 135 Å (+ 70%) (see further discussion on chain expansion).

The presence of two intramolecular hydrogen bonds (one direct, one with water, see Fig. 1) for the charged form agrees with the recent identification of two extra sites of rather strong hydrogen bonding manifested by higher oscillation frequency and contribution to dielectric response compared to that detected in water from terahertz time-domain spectroscopy [20].

The work by Noto et al. [9] preceded by a few years that of Guidugli [12] in using molecular dynamics with explicit solvent to study structure and dynamics of both the charged and uncharged form of PGA. Their results and those by Guidugli et al. roughly converge in identifying, in principle, three energy minima. However, one minimum is prevailing for the uncharged form, and two minima (plus a negligible third one) are significant for the charged form (see Figs. S1 and S2 in the Supplementary Information). As to hydration, they found that: “The charged dimer (in the most populated B' conformation) forms 50% more hydrogen bonds with surrounding water molecules” [9]. Were the bonds reported in their Table 3 directed all to different water molecules, then the hydration water molecules for the fully charged form are 15.3 and 13.96 for conformational minima A' and B', respectively, and for the protonated form 9.29 hydration water molecules, for both minima. Those values are lower than those reported by Guidugli et al. (see Table 1), by about 16% for the acid form, and by about 19% and 26% for minima A' and B' of the

Table 1

Mean values, per galacturonic monomer, of the number of direct and solvent-mediated intramolecular hydrogen bonds, number of solvent molecules linked by hydrogen bonds, and calculated persistence length and characteristic ratio for the neutral and ionized galacturonic acid.

charge	direct H-bonds	intramolecular H-bonds, water mediated	No. of H-bonded water molecules	$L_p \pm \sigma$ (Å)	$C_{\infty=900}$
neutral	0.04	0.09	11.1	79.5 ± 1.1	35
charged	0.96	0.88	18.9	135.0 ± 1.9	58

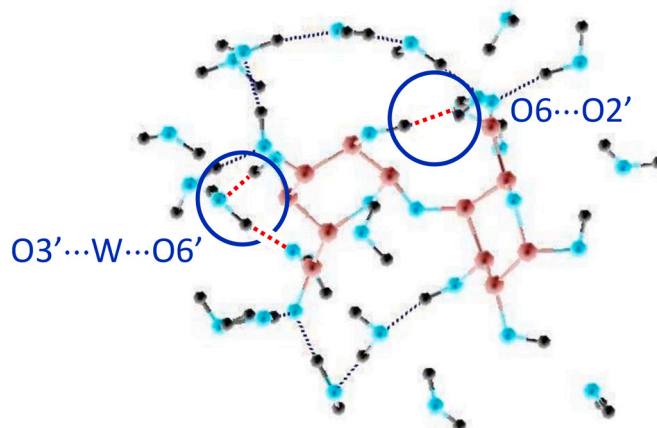


Fig. 1. A typical snapshot taken from the MD simulation of a galacturonic acid dimer in explicit water solvent showing the relevant direct and water-mediated intramolecular H-bonds. Solvent molecules H-bonded to the dimer are also shown.

fully charged form, respectively. The discrepancy is not large, in our opinion; at any event, the two contributions converge in finding that:

- the galacturonan chain is very hydrated in water and in all conditions of charging;
- the charged form is surrounded by a much larger number of water molecules than the uncharged one: from 50% (Noto et al.) to 69% (Guidugli et al.).

From the historical standpoint, the first and - to the best of our knowledge - only one experimental attempt at evaluating the hydration of the different forms of pectin chains is that of Stuewer: “While the degree of hydration of pectin is usually considered to be one of the factors involved in its jelling, no previous attempts to measure it directly have been made, although qualitative results have been reported [4]. The hydration of pectin and of a number of its derivatives has been measured by the method of a reference substance in water and in aqueous alcohol, in agreement with results by ultrafiltration. It is found that the hydrate water amounts to 0.25, 0.21, 0.38, and 0.35 g. of water to 1 g. of pectin, pectic acid, sodium pectate, and calcium pectate, respectively. That of pectinic acid resembles pectin; the three sodium and the three magnesium derivatives are somewhat more hydrated” [15]. On using the different values of the mass of the repeating unit for the quoted pectins, one finds the they correspond to 2.3 and 4.7 water molecules bound per repeating unit of pectic acid and sodium pectate, respectively. Those values are definitely lower than both those in Table 1 and those by Noto et al. [9], likely due to the semi-empirical method of determination which does not guarantee, a priori, full exchange of solvation molecules. However, it is interesting to point out that the ratio of the calculated numbers of bound water of sodium pectate on those of pectic acid is 2.0 in Stuewer's results, 1.7 in those of Tables 1 and 1.6 and 1.5 for the two minima in Noto's work, that is: they are always significantly larger than 1 and thus in agreement with the picture of a higher electrostriction upon chain charging.

2.2. Dilatometry and hydration

Dilatometric measurements, by their intrinsic thermodynamic nature, can directly provide information on variation of (molar) volume pertaining to the studied reaction. Albeit somewhat less popular in the recent years, this technique proved to be very informative on variations of hydration in synthetic and semi-synthetic (natural) polyelectrolytes [31–43] as well as in the study of biopolymers [44–51,54]. The recent illustrative paper by Kahn is a useful source of information about this approach, recalling his conclusions: “Volume is an extensive

thermodynamic property whose importance in understanding proteins has been emphasized by Kauzmann [50]. Capillary dilatometry is a way to measure changes in it directly, but technical difficulty has limited its use. The method described here brings it within the capacity of any biochemistry or biophysics laboratory. Insight into the role of hydrating water in reactions involving proteins and nucleic acids thus becomes more readily available.” [51].

The dilatometric results of the protonation reaction of both sodium pectate and monomeric sodium D-galacturonate are reported in Fig. S4 of the Supplementary Material, as a function of the degree of acid dissociation, α . Inspection of the results reported in Fig. S4 leads to the following comments:

- i. the integral changes of (molar) volume for the protonation of both sodium pectate and its monomeric analog are positive, much like all known poly(carboxylate) cases (and low MW acids as well);
- ii. the integral volume change plots are linear for both polymer and monomer, indicating that each sample is characterized by a constant, α -independent, value of the differential (molar) volume change of protonation, $\Delta\bar{V}_P$ (being $\Delta\bar{V}_P = d\Delta V_P/d\alpha$);
- iii. the values of $\Delta\bar{V}_P$ for sodium pectate ($+13.998 \pm 0.005$ mL/mol H^+) and sodium galacturonate ($+13.7 \pm 0.2$ mL/mol H^+) are so close as not allowing to claim a significant difference upon enchainment of the galacturonic acid ring as to volume change of proton binding ($\Delta\bar{V}_P$ for overall fitting is 13.88 ± 0.10 mL/mol H^+).

2.3. Chain hydration upon charge and conformation changes

Point ii. above brings to the structural conclusion that there is no indication of cooperative enhancement of hydration of neighboring carboxylate groups upon proton dissociation, in full agreement with all known results obtained on polyuronates, both native [52] and of semi-synthesis [53], and on poly-L-glutamic acid in the pH range of coil conformation [54]. However, this is at variance with what is observed for sodium poly(acrylate), PAA [31]. This is not surprising in view of the much smaller average distance between COO^- group projections on chain axis, b , in PAA ($b = 2.52$ Å) with respect to PGA ($b = 4.33$ Å), and hence a much higher linear charge density ξ ($\xi = l_b / b$, being l_b - the Bjerrum length- equal to 7.135 Å in water at 298.15 K). This parameter seems to be at the root of such observed hydration cooperativity in PAA: “The latter result was previously observed by Ikegami [by the method of refractivity measurement] [55], who ascribed it to a combination of a cooperative enhancement of hydration of neighboring carboxylate groups and of an overall charge effect on the thickness of a secondary hydration region.” [31].

A further structural conclusion at the polymeric level is that the change of hydration (as volume change of transition, $\Delta\bar{V}_T$) associated with the demonstrated pH-induced transition of conformation (“stiff” 3_1 helix \rightarrow “loose” 2_1 helix) of pectate is zero, at variance with the very large change in enthalpy [8,19]. To the best of our knowledge, the only reported volume change of (intramolecular) conformational transition of a polysaccharide is that of the “loose helix” \rightarrow “tight (single) helix” of κ -carrageenan, for which a “very small and negative, likely between -0.5 and -1 mL \cdot mol $^{-1}$ ” value has been reported [56]. Moreover, “ ΔV for the coil-helix transition [of poly-L-glutamic acid, PLGA] is in the order of 1 mL per amino acid residue” [54]; such an extremely small value of $\Delta\bar{V}_T$ is surprisingly comparable to that of galacturonan, in spite of the large structural difference of the two corresponding biopolymer families.

From the above, it is then possible to say that the so far reported experimental evidence suggests that intramolecular conformational transitions of ionic biopolymers are probably accompanied by very small, or even nil, values of $\Delta\bar{V}_T$.

2.4. Monomer vs. polymer hydration

The results commented in point iii. tell that the volume variations accompanying protonation are almost exclusively pertaining to the uronate moiety, with no contribution from enchainment, within error. This is further substantiated by the comparison with reported volume change of protonation of low MW carboxylic (and polycarboxylic) acids: $\Delta\bar{V}_P$ (all values in mL/mol H^+) of succinic acid is $+11.6$ (1st dissociation) and $+13.5$ (2nd dissociation), that of glutaric acid is $+12.2$ (1st dissociation) and 13.7 (2nd dissociation), and for 4-carboxypimelic acid is $+13.7$ (1st dissociation), $+13.7$ (2nd dissociation) and 15.6 (3rd dissociation), with an average value of $+13.4 \pm 1.3$ mL/mol H^+ [31].

A recent extensive paper based on terahertz time-domain spectroscopy of carbohydrates [20] has shown that: “The crucial effect of the axial OH(4) group on the increase of γ parameter is confirmed as well in the case of the comparison of galacturonic and polygalacturonic acids.” According to Reference [20] the hydration of Na^+ poly(galacturonate) appears closer to pure water than that of monomeric Na^+ galacturonate, pointing to a higher hydration of the latter molecule. The difference has been attributed to the presence of OH group on position C4 on Na^+ galacturonate vs. a bare glycosidic oxygen on the polymer (actually, this reasoning should be doubled having to additionally take into account the OH on C1). Clearly, the hydroxyl group adds to the H-bond acceptor capacity of the glycosidic oxygen also the H-bond donor capacity related with the presence of the H atom. This observation might erroneously induce to find a discrepancy with the present results from dilatometry which demonstrate an identical behavior upon dissociation, unless one notices that the quoted results based on spectroscopy pertain to the absolute hydration state of the various systems, whereas dilatometry can only measure differences among them. It is quite likely, then, that the absolute hydration of Na^+ galacturonate is larger than that of its polymeric analogue but that the difference of hydration between the charged ($\alpha = 1$) and the uncharged form ($\alpha = 0$) of both carbohydrates (i.e. $\Delta\bar{V}_P$) is the same, irrespective of enchainment.

2.5. Hydration and stereochemistry of glycuronans

The focus on the axial position of OH(4) in galacturonan leads to consider the $\Delta\bar{V}_P$ values of β -glucuronans (either bearing the glucuronic residue in the main chain or as a side-chain) which possess an equatorial OH(4) group. Such glucuronans are the C(6)-oxidized derivatives of glucose polymers (β -glucans). Interestingly, their $\Delta\bar{V}_P$ values are identical ($+14$ mL/mol H^+) [53] with that of poly(galacturonic acid), whose OH(4) is axial. Should one highlight a difference in the hydration changes upon protonation of (1 \rightarrow 4 linked) polyuronates, one would rather stress the role of the presence of an axial orientation of C—O linkages on either positions C(2) or C(3), or, in other words, the relative *cis* orientation of the C—O linkages on positions C2 and C3. In fact, the value of $\Delta\bar{V}_P$ of poly(guluronic acid) and of both algal and bacterial alginic acid (whose monomers are β -D-mannuronic acid and α -L-guluronic acid) is (for all compounds) equal to $+11.5$ mL/mol H^+ , with a significant relative decrease of -18% with respect to the value of the “all-equatorial” (*trans*) glycuronans. (For pure comparison purposes, it should be recalled that in the case of poly-L-glutamic acid, which is totally devoid of OH groups, “the [protonation] volume change indeed dropped from 11.4 mL in 0.01M NaCl to 11.1 mL in 0.2M NaCl.” [54], i.e. very close to that of alginates). The remarkable point is that the alginate samples differ - altogether - both as to monomer composition and as to (partial) acetylation; however, they are all characterized by the axial orientation of the C—O linkages either on position C(2) for M or C(3) for G, and consequently an always-*cis* orientation of vicinal diols. At variance, whereas the relative orientation of the C—O linkage on positions C(4) is different between galacturonan and the glucuronans (being axial

for the former case and equatorial for the latter ones), both groups share a common all-equatorial (*trans*) arrangement of the ring C—O bonds not involved in glycosidic linkages and an identical value of $\Delta\bar{V}_p$. The likely explanation is that the axial position of one OH group - either on C(2) in β -D-mannuronic acid or on C(3) in α -L-guluronic acid - is sufficient to hinder the C(6) carboxylate group achieving the maximum hydration, likely through effects over the pyranosidic ring. A schematic representation is given below in [Scheme 2](#).

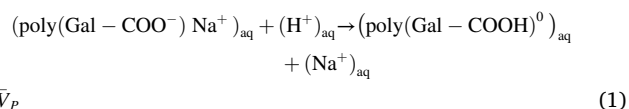
2.6. Hydration of uncharged glycans vs. charged glycuronans

Reference [20] also indicates that the characteristics of the hydration of salts of uronic compounds, either monomeric (namely, Na^+ galacturonate) or polymeric (Na^+ poly(galacturonate)), are more different from those of pure water than that of uncharged carbohydrates, that is confirmed by the present results, both theoretical and experimental. The Author points to an observed general behavior that “in the case of DNA we can speak of cooperative effects during the formation of hydration shells... [whereas] We do not observe such effects in aqueous polysaccharide solutions; on the contrary, we see the opposite tendency: polysaccharide induces less alterations in water structure than the composing monomers taken separately.” [20] Such behavior can be easily explained considering, on one side, the null ($\xi = 0$) linear charge density of the investigated “polysaccharides”, namely dextran and amylopectin (which should be more properly named “non-ionic polysaccharides”) or the comparatively small charge density of sodium pectate ($\xi = 1.65$) if compared, on the other, with the very large value of the charge density of B-DNA ($\xi = 4.2$). This point has already been mentioned here above in relation to the more charged polycarboxylate PAA [31].

2.7. Liberation of electrostricted water: comparison between theory and experiment

In his extensive survey on hydration, Marcus states that: “Several reports from X-ray and neutron scattering concluded that the water in the hydration shell of a protein is electrostricted, being 5-25% denser than bulk water” and “The density values reported for the surface layer of water of several proteins, such as chicken ovalbumin lysozyme (ranging from 1.08 to 1.22 $\text{g} \cdot \text{cm}^{-3}$) agreed with the values calculated from the surface charge densities and the corresponding electric fields (ranging from 2.34 to 5.69 $\text{GV} \cdot \text{m}^{-1}$) and permittivities” [24]. This adds to the evidence quoted by Chalikian, in his review [57] that “experimental observations ... indicate a ~ 10%–15% increase in the density of water of protein hydration relative to bulk water [58–60]. Fully in line with the above statements, an accepted value of the density of water electrostricted around polycarboxylates is

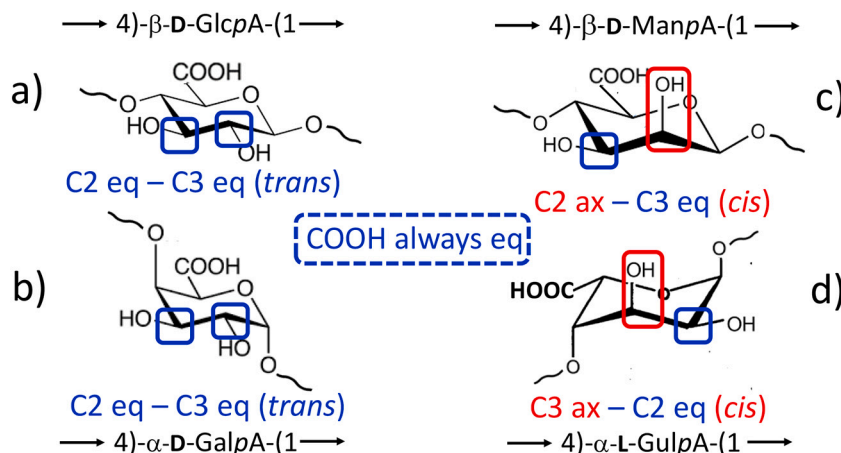
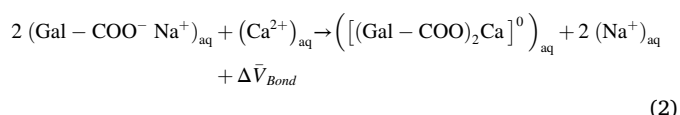
$1.1 \text{ g} \cdot \text{cm}^{-3}$ [31,55]. On this basis, one estimates (with $\Delta\bar{V}_p = +14 \text{ mL/mol H}^+$) the number of water molecules released from the hydration layer of sodium pectate upon protonation, n_w , for the reaction (Eq. (1)):



$n_w = \frac{\Delta\bar{V}_p}{\delta V_{\text{water}}^{\text{liberated}}} = 7.8$; $\delta V_{\text{water}}^{\text{liberated}} = \left(\frac{M_{\text{water}}}{\rho_{\text{water}}} \right) - \left(\frac{M_{\text{water}}}{\rho_{\text{water}}^{\text{electrostricted}}} \right) = 1.8 \text{ mL} \cdot (\text{mole released water})^{-1}$ [61]. This number perfectly coincides with the difference of the number of water molecules around one repeating unit, between Na^+ poly(galacturonate) and poly(galacturonic acid), reported in [Table 1](#) (namely: 18.9–11.1). The result is particularly encouraging, being the successful experimental corroboration of a theoretical prediction of hydration change of poly(galacturonic acid) / sodium poly(galacturonate).

2.8. Liberation of electrostricted water molecules in ionotropic gel formation

The found number of immobilized water molecules, given the average value of $\overline{\text{DP}}_n = 94$ of the investigated pectate sample [61], makes an (average) total of more than seven hundred electrostricted water molecules per chain upon passing from the fully protonated to the fully charged form! This large number of immobilized solvent molecules can be viewed as a large “entropy reservoir” for sodium pectate. This is confirmed by the large and negative values of the entropy change of the elementary step of dissociation, ΔS_{diss} , both before ($\alpha = 0.2$) and beyond ($\alpha = 0.9$) the pH-induced conformational transition of pectic acid. They are - 17 and - 26 $\text{cal} \cdot (\text{mol H}^+)^{-1} \cdot \text{K}^{-1}$ in water, and - 18 and - 20 $\text{cal} \cdot (\text{mol H}^+)^{-1} \cdot \text{K}^{-1}$ in aqueous 0.05 M NaClO_4 , respectively [19]. Upon strong bonding of cations, e.g., H^+ or Ca^{2+} ions, the liberation of the immobilized solvent molecules - necessary to allow for an intimate ion-uronate bonding - becomes the driving force of the bonding process, both in the acid gel formation and in the calcium-induced pectate gelation, from the initial single-ion “tilted egg-box” calcium bonding to the longer sequences of calcium ions in the cooperative “shifted egg-box” bonding mode [61]. As to the interaction with Ca^{2+} , one can write (Eq. (2)):



Scheme 2. Schematic representation of the relative position of the OH groups on C2 and C3 in different *trans*-1 \rightarrow 4 linked glycuronans. a. β -D-glucuronic acid in β -glucuronans; b. α -D-galacturonic acid in galacturonans; c. β -D-mannuronic acid in alginates; d. α -L-guluronic acid in alginates.

The volume change for calcium bonding, $\Delta \bar{V}_{bond}$, is very large: it is +29 mL · mole complex [61]; it corresponds very closely to twice the value of $\Delta \bar{V}_p$ (= +14.0 mL/mol H^+ or, more appropriately, expressed in mL · (mole neutralized uronate charge by protonation)⁻¹). In fact, given the accepted stoichiometry of charged uronate groups per bound calcium ion in the core of the egg-box model (i.e. 2:1) and the demonstrated charge annihilation within the resulting complex (i.e. $2 COO^- + Ca^{2+} \rightarrow [Ca(COO)_2]^0$) [61], this means that the by-far-largest hydration component involved in the egg-box formation with Ca^{2+} is that pertaining to the dehydration of the uronate group. All the (compensated) hydration variations of the small ions involved (H^+ , Na^+ , Ca^{2+}) altogether account for a bare 3.6% ($(\Delta \bar{V}_{Bond} - 2 \cdot \Delta \bar{V}_p) / (2 \cdot \Delta \bar{V}_p) = 1/28 = 3.6\%$). It can easily be anticipated that this conclusion holds also for other cases involving divalent ion-pectate interactions (Pb^{2+} , Zn^{2+} , Cd^{2+} and Cu^{2+}), all showing similar - always positive - values of volume change of interaction, [62] with the ensuing liberation of a large number of water molecules and increase of configurational entropy. With reference to the data of the latter paper, an interesting point pertains to the comparison between the reported enthalpic and dilatometric results on binding calcium ions by alginate and pectate (see, in particular, their Figs. 3 and 4 for $\Delta \bar{H}$ and Figs. 5 and 6 for $\Delta \bar{V}$). A rough estimate of the observed effects at, say, a value of the molar ratio, R, of added calcium to uronate repeating unit of 0.4, returns the values for the enthalpy change of mixing of -0.5 kcal · mol⁻¹ and -1.3 kcal · mol⁻¹ for alginate and pectate, respectively. Still at R = 0.4, the corresponding values of the volume change of mixing are +3.7 cm³ · mol⁻¹ and +7 cm³ · mol⁻¹ for alginate and pectate, respectively. In both cases the observed effect for pectate is substantially larger (twice as large, or more) than that for alginate. With specific reference to the volume change, the smaller value of volume change of mixing calcium with alginate finds a clear counterpart in the smaller value of the volume change of protonation of alginate ($\Delta \bar{V}_p = +11.5$ mL/mol H^+) with respect to pectate (+14.0 mL/mol H^+).

To reach a detailed description of the role of hydration in the stability of the pectate-calcium (and alginate-calcium complexes as well) will require resorting to more sophisticated molecular dynamics simulations and advanced NMR methods. In fact, if it is undoubted that, considering three model neutral disaccharides (maltose, sucrose and trehalose) as significant sample cases, “evidence is accumulating in support of the notion that it is not the amount or overall hydration but rather the detailed individual carbohydrate-water interaction that is likely to determine carbohydrate structure and functionality.” [63], nevertheless, in the case of the ionotropic gelation of polyuronates, it is the very change of overall hydration to be so important as to become the driving force for junction formation, both in the H^+ and in the Ca^{2+} case, eventually modulated by the subtleties of the conformational features of the facing chains in the junction.

3. Expansion

As openly stated in the Introduction, one of the goals of this paper is to contribute assessing the “intrinsic” conformational features of the chain of poly(galacturonic acid) in its acid (uncharged) and salt (fully negatively charged) forms, as a result of the complex interplay of short- and long-range interactions. It is to be remarked that such stringent condition ruled out a number of literature experimental data, which fail providing the possibility of assessing the two limit conformations. Ideally and whenever possible, extension from the limit cases to intermediate values of charging can add further insight into the process of charge-induced conformational transformation. Theoretical predictions, from this group and from others, will be compared with experimental results. Different properties can be used to measure polymer dimensions, [64,65] the radius of gyration, R_g (defined as the root-mean square of the z-average of its squared value, $R_g \equiv \langle R_g^2 \rangle^{1/2}$), being the most straightforward. However, not all theoretical calculations provide such values; rather, they always provide properties like the characteristic ratio, C_n (or its value for the infinitely long chain, C_∞), or the persistence length, L_p . Both properties are excellent measures of chain expansion:

recalling the dictionary's definition of expansion (from “to expand: to increase the extent, number, volume, or scope of”) one realizes that they account not only for the “static” overall chain dimensions, but also for the “dynamic” contribution that short-range (e.g. from conformational energy constraints) or long-range (e.g. from excluded volume or from polyelectrolytic interactions) effects produce on the galacturonan molecule size.

The experimental data will derive from capillary viscometry experiments obtained as a function of the degree of charging as α , the degree of ionization of the weak acid function, limiting those obtained as a function of the degree of methyl-esterification to the ancillary testing of the corresponding theoretical modeling. In fact, the latter approach (very often used in the study of galacturonan) will be demonstrated to unavoidably introducing additional types of biases [66] that may strongly affect the polymer behavior and the derived conclusions. Viscometric data in aqueous salt-free solution (“water”) and in the presence of supporting 0.05 M NaClO₄ (“salt”) - namely at different values of ionic strength, I - will help assessing the polyelectrolyte contribution to conformation.

3.1. Modeling expansion of galacturonan

Table 2 A reports the theoretical values of the persistence length of

Table 2

Persistence length (L_p) of both the uncharged (U) and the charged (C) forms of pectic acid in water [71].

MODELING			
state of charging	Uncharged(U)	Charged(C)	C/U ratio
L_p (Å)			
A.			
Boutherin, 1997 - Molecular Mechanics (MM) and Metropolis Monte Carlo [69]	370 [44] ^a	581 [55] ^a	1.57
Noto, 2005 - Molecular Dynamics (MD) and Monte Carlo [9]	120±20	300±50	2.50
Noto, 2005 - adiabatic map of the dimer (MM) and Monte Carlo [9]	121	131	1.08
Guidugli, 2010 Molecular Dynamics (MD) and Monte Carlo [12]	79.5±1.1	135.0±1.9	1.70
average	173±133	287±211	1.7±0.6 1.7±1.3
B.			
Cros, 1996 - Molecular Mechanics (MM) and random Boltzmann weighted distribution method [70]	135	n.a.	n.a.
Braccini, 1999 - Molecular Mechanics (MM) and Metropolis Monte Carlo [71]	166	n.a.	n.a.
average A. + B.	166±95	287±211	1.7±1.3
C.			
state of charging	Uncharged(U)	Charged(C)	C/U ratio
Characteristic ratio (C_∞)			
Ruggiero, 1995 - Molecular Mechanics (MM; *: calculated with different force fields and potential energy barriers) and Monte Carlo [67]	197*	171*	0.87
	208*	177*	0.85
	234*	193*	0.83
average	213±19	180±11	0.85±0.02 0.85±0.12

L_p and C_∞ values from molecular mechanics (MM) calculations are not italicized, values from molecular dynamics (MD) in italics.

^a “the calculated chain dimensions for 55 might be assumed to be dimensions at very low salt concentration and to represent a maximal limit of extension. On the other hand, dimensions calculated for 44 represent the other extreme, the dimensions that in reality may represent the high salt concentration behavior for homopolymer linked with the α -(1 → 4) linkage.” [69].

both the charged (C) and the uncharged (U) forms of pectic acid in water, calculated according to the methods indicated in brief in column 1, collected from literature in a historical order together with their ratio (C/U ratio, last column). Averaging of the values of the latter parameter immediately points to a larger expansion of the pectate chain in the charged over the uncharged form, beyond error (1.7 ± 1.3). By chance, the value of the C/U ratio of reference [12] from Table 1 coincides with the average. In general, there is large spread of values, for a given state (U or C), which is substantiated by the large standard deviation values of the U and C averages. The C/U ratio values based on MD calculations are larger than those from MM. Subset B. reports additional values of L_p , calculated for the uncharged case only. Nevertheless, taking the average value of all L_p data for the uncharged form from both subsets A. and B. leaves the picture unchanged.

The only case in which the C/U ratio is (just slightly, though) smaller than 1 is that of Ruggiero et al. [67]; this peculiarity led us to group Ruggiero's data in a separate subset, namely subset C. Their data clearly show how the characteristic ratio depends on the different force fields and potential energy barriers used. Interestingly, the Authors do not comment on this rather peculiar result, but for reporting that "In the regions of low and high degrees of ionization, the chain dimensions are dependent on the potential function used but are less dependent in regions of intermediate degrees of ionization." They underlined the observed "convexity of the curves, in which C_∞ decays to half of its initial value as the galacturonate content increases from 0 to 50%. After this value, the unperturbed dimensions gradually rise as the percentage of charged groups increases.", which Ruggiero et al. claim to find support in the static light-scattering results obtained by Plashchina et al. [68] on sodium pectate and pectinate (at different levels of esterification, though, not of protonation) in 0.1 M NaCl aqueous solutions.

3.2. Viscometry and expansion

Passing to experimental data, Fig. 2 reproduces the reduced

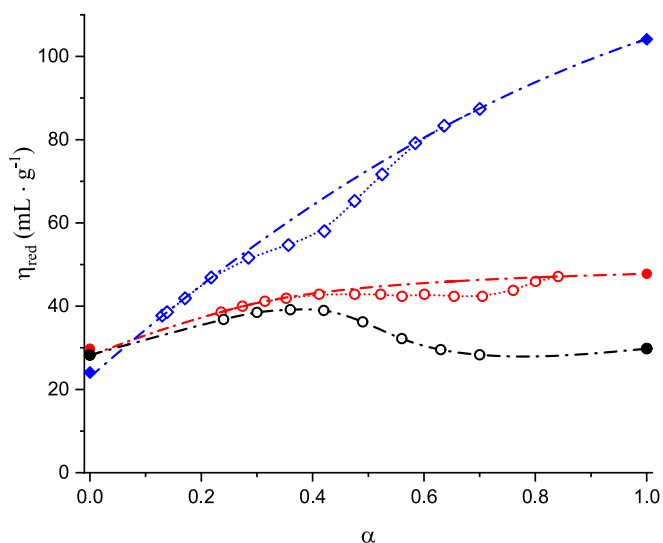


Fig. 2. Dependence of the reduced (specific) viscosity, η_{red} (in $\text{mL} \cdot \text{g}^{-1}$), of pectic acid on the degree of dissociation, α , at 25 °C. Pectic acid in water (blue open lozenges connected by blue short-dotted line in the range of transition) and in aqueous 0.05 M NaClO_4 (red open circles connected by red short-dotted line in the range of transition); the dash-dotted curves represent the theoretical behavior in the absence of the $3_1 \rightarrow 2_1$ conformational transition, allowing for determining the limit values at both $\alpha = 0$ and $\alpha = 1$ (full symbols). The black symbols (open and full circles and dash-dotted curve) represent the extrapolated value at $I \rightarrow \infty$ (see Supporting Information for details regarding all dash-dotted curves). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

(specific) viscosity, η_{red} , of pectic acid as a function of the degree of dissociation, α , both in water ("salt-free" condition) and in 0.05 M NaClO_4 . The experimental $\eta_{\text{red}}(\alpha)$ curves (Fig. 2) show a marked inflection - resulting in a sigmoid shape - in the central part of the range of α , that correspond to the pH-induced conformational transition [19]. The sigmoid is very closely resembling the pH-dependence of the viscosity of poly(L-glutamic acid) across the helix-coil conformational transition (e.g. for the intrinsic viscosity dependence, see reference [72]). The first analysis of the viscometric results as to chain dimensions pertains to that between the η_{red} at the smallest - initial - value of α , α_{min} , and that at the highest available value, α_{max} , both in "water" and in "salt", namely the experimentally available extreme values; they have been reported in box a of Table 3. They can be assumed as partially representative of the polymer state in the limit of "almost-no charge" and "almost-complete charging", respectively. It is immediately evident that the well-known increase of acidity of weak polyacids brought about by an increase of ionic strength, produces an increase of both α_{min} and α_{max} . Still, the interval of α spanned in the two sets of data is very similar (0.57 in "water" vs. 0.60 in "salt"), thus making the relative comparison very reasonable. The values of the C/U ratio from the first set of data are both larger than one, the ratio in "water" being larger than that in "salt" (i.e. 1.3 vs. 1.1).

Next, it was decided to perform two, sequential, extrapolation procedures from the data of Fig. 2. The first one was aimed at providing estimates of the η_{red} data in the limit of $\alpha = 0$ and $\alpha = 1$ for both "water" and "salt" conditions. The extrapolated values for both cases ("water" and "salt"), whose details have been reported in the Supplementary Information, are reported in Table 3, box b. As expected, the values at $\alpha = 0$ are smaller than the corresponding values of box a; likewise, the values at $\alpha = 1$ are larger than the corresponding values of box a. One might note an apparently odd result for the $\alpha = 0$ case: on passing from "water" to "salt", the value of the former case is smaller than that of the latter one. Actually, this result would suggest an increase of ordering (and hence of chain stiffening and expansion) brought about by an increase of the ionic strength in the low α range, with a "tightening" of a partially "loose" 3_1 helix prevailing in "water", recalling what found in the case of poly(L-glutamic acid) [73]. The values of the C/U ratio for this set of conditions confirm the previous trend: in both cases the values of the C/U ratio not only are larger than 1, but both show an increase upon increasing the range of α , still the ratio in "water" being larger than in "salt" (i.e. 1.7 ± 0.1 vs. 1.19 ± 0.02). Interestingly, the length-ratio for the case of the absence of added simple electrolyte - at finite polymer concentration - is equal to that predicted by the theoretical calculations of Guidugli et al. (namely, 1.7), also carried out in the salt-free conditions [12]. Finally, it was tried to evaluate the extrapolated η_{red} values for the fully protonated ($\alpha = 0$) and for the fully ionized ($\alpha = 1$) forms in conditions of infinite value of the ionic strength, I , so to get rid of all electrostatic contributions of polyelectrolyte origin. The extrapolation procedure - performed as usual in the polyelectrolyte literature as a function of $I^{0.5}$ - has been briefly outlined in the Supplementary Information; the results have been graphically reported in Fig. 2, and the limiting values at the α extremes in box c of Table 3.

From inspection of both Figure and Table, two main points are worth being highlighted: the $(\eta_{\text{red}})_{I \rightarrow \infty}$ curve enhances the sigmoid behavior already apparent in the curves at finite I . The curve connects the region of prevalence of the 3_1 helical conformation (for $\alpha \leq 0.35$) and that of the 2_1 one (for $\alpha \geq 0.7$), respectively [61]. The values of $(\eta_{\text{red}})_{I \rightarrow \infty}$ at the two limits are extremely close: the (linear) C/U ratio is 1.02.

Probably the most comprehensive investigations on the conformation (and geometry) of galacturonan chain come from the group of Pérez et al. [2,74]. As to the helical conformation of the polysaccharide they indicate four low energy conformations which would generate integral helices; in the three cases of the right and left handed 3_1 helix and of the 4_1 one, the values of h , namely of the translation of the galacturonate residue along the helix axis, is 0.43 nm, and in the case of the 2_1 helix is 0.435 nm. Still from Reference [74]: "These different models can be

Table 3

Values of η_{red} at the lowest (U) and at the highest (C) value of α , both for the case in “water” and in “salt”, as well as the limit values at $\alpha = 0$ and at $\alpha = 1$.

EXPERIMENTAL									
state of charging	η_{red} (mL · g ⁻¹)				C/U ratio				
	Lowest (“U”)		Highest (“C”)		(volume (l ³))		length (l ¹)		
ionic strength	“water”	“salt”	“water”	“salt”	“water”	“salt”	“water”	“salt”	
a. values at extreme α (exper.)	$\alpha = 0.13$	37.7	$\alpha = 0.70$	87.5	(2.3)		1.3		
	$\alpha = 0.24$	38.6	$\alpha = 0.84$	47.1	(1.2)		1.1		
b. values at limit α values	$\alpha = 0.00$	22.9 ± 0.5	$\alpha = 1.00$	104.1 ± 4.5	(4.5 ± 0.2)		1.7 ± 0.1		
	$\alpha = 0.00$	28.3 ± 0.5	$\alpha = 1.00$	47.8 ± 0.0	(1.6 ₉ ± 0.0 ₄)		1.19 ± 0.0 ₂		
c. $I \rightarrow \infty$ limit of same α values	$\alpha = 0.00$	28.3 ^a	$\alpha = 1.00$	29.8	(1.0 ₆)		1.0 ₂		

^a set equal to η_{red} in “salt” at $\alpha = 0.00$, assuming 100% of ordering in analogy with the PLGA case [72] and no further dependence on I .

compared with experimental data obtained on both acidic and esterified pectins. Fibre diffraction performed on Na⁺ and Ca²⁺ pectate gels yielded models [10,11] of three-fold, right-handed helices repeating in 1.3 nm ($n = 3$, $h = 0.43$ nm). On the basis of circular dichroism, calcium stoichiometry and competitive inhibition, a two-fold helix having a repeat of 0.870 nm ($n = 2$, $h = 0.435$ nm) was proposed for calcium gel [6,75].” Very interestingly, is one takes the ratio of the - experimentally determined - values of h for the charged (2₁ helix) over the uncharged (3₁ helix) forms, one obtains 1.01, namely only 1% lower than the present C/U ratio from extrapolation of viscometry data to $I \rightarrow \infty$ (i.e. 1.02)!

Such conclusion on the coincidence of the values of the average distance between charges projection of the helix axis, h , for the 3₁ and the 2₁ conformations of pectate sheds new light on the statement in the previous Section that $\Delta\bar{V}_{tr}$ of pectate is zero. At variance, the other two cases quoted, namely κ -carrageenan and poly-L-glutamic acid, show non-zero values of the variation of the linear charge density parameter, ξ , accompanying the intramolecular transition. In fact in the former case this variation is $\Delta\xi = \xi_{\text{helix}} - \xi_{\text{coil}} = 0.87 - 0.69 = 0.18$ [56], in the latter one is $\Delta\xi = \xi_{\text{helix}} - \xi_{\text{coil}} = 4.76 - 2.10 = 2.66$ [76]. In galacturonan $\Delta\xi = \xi_{3_1} - \xi_{2_1} = 1.65 - 1.64 = 0.01 \approx 0$ (having taken $b_{3_1} = 4.33$ [10,11] Å and $b_{2_1} = 4.335$ Å [6,75]).

This allows concluding that:

- the value of $\Delta\bar{V}_{tr}$ of charged biopolymers largely reflects the change of charge density accompanying the transition; its sign could be very likely correlated with the type of hydration of the charged group on the biopolymer. For example, a “hard” group like the sulfate one in κ -carrageenan show negative values of $\Delta\bar{V}_{tr}$, whereas a “soft” group like COOH gives positive $\Delta\bar{V}_{tr}$ in PLGA;
- the $\Delta\bar{V}_{tr} \approx 0$ of pectate well correlates with the difference in charge density of the corresponding limit conformations. Being the “intrinsic” difference in elongation of the two helical conformations of galacturonan practically zero, it is reasonable to describe its “intrinsic” 2₁ → 3₁ conformational transition just as a screw-like rotation of the polymer around its helical axis with practically no concomitant change of pitch.

3.3. Carboxylate charge annihilation by protonation versus methoxyl-esterification

The quoted results by Ruggiero et al. [67] are the only theoretical data on the change of dimension (expansion) of galacturonan as a function of α . In their Fig. 4, the Authors provide (without commenting) the “Characteristic ratio C_n for $n = 200$ units as a function of the content (%) of charged groups. The curves are normalized with respect to the value of C_n with 0% charged residue content” for two systems: one in which the reduction of charge is modelled by protonation (hereafter: “PROT”) and one in which the reduction of α is obtained by methyl esterification of the carboxylic group (hereafter: “ESTER”). To allow for direct comparison with the viscometric data of Fig. 2 in water, it was decided to re-normalize their data at $\alpha = 1$ (100% GalA⁻). To allow for a congruent

comparison with the persistence length values (whose dimension is linear), the ratio was elevated to the third power to allow accounting for volume dimensionality: $\left(C_n/C_n(100\% \text{GalA}^-)\right)^3$.

A very large discrepancy is immediately apparent from the comparison of both the theoretical curves with the experimental one: the marked parabola-like trend with α in the central part of the curve and, consequently, the wholly diverging behavior at low α values are totally at odd with experiment, in particular - and surprisingly - for the “PROT” case. Essentially, the calculation fails to predict the monotonic decrease of dimensions of PGA upon protonation, not to say the sigmoid behavior stemming from the parallel 2₁ → 3₁ conformational transition.

Two investigations are present in the pectin literature, reporting experimental data on dimensions (elongation) of pectic acid as a function of the degree of chain charging, in both cases obtained by methyl-esterification of the COOH groups. The former one is the light scattering study of Plashchina et al., providing values of the radius of gyration, [68] the latter one is an investigation of the solution conformation of pectin polysaccharides using small angle neutron scattering, viscometry, and molecular modeling, giving values of intrinsic viscosity [70]. In both cases the ionic strength of the systems was $I = 0.1$ M. All available data on partially methylated PGA from experiment [68] and theory [67] have been reported in Fig. 3 b, after proper elevation to the third power of the radius of gyration and of the characteristic ratio data to allow for comparison with viscosity. All data having scaled so as to coincide at $\alpha = 1$, which is the situation of present interest.

At variance with the PROT case, the agreement between theoretical predictions and the two independent sets of experimental data can be considered very good, all cases showing a similar trend with a minimum at around $\alpha = 0.6$ which is revealed by a simple parabolic fit (with no other scope than guiding the eye) for all three cases. This not only highlights the agreement between light scattering and viscosity results, but, possibly more important for the present discussion, also the semi quantitative agreement of the theoretical modeling by Ruggiero et al. of the galacturonan chain in the ESTER way of achieving charge neutralization.

This result suggests possible explanations of the observed discrepancy in the PROT case: they can be tentatively traced back to the substantial difference between a COOCH₃ group with respect to a COOH one, both as to charge fractions on the oxygen atoms (and the related donor- acceptor-properties) and as to steric hindrance. In fact, Fig. 1 has visually addressed the relevant role of O(C6) in effective H-bonding.

Moreover and more peculiar to the polyelectrolyte nature of the galacturonate polycarboxylate, an additional explanation could be found in the “static” approach of MM, corroborated by the unexpected similarity of trends between the “proton” and the “methoxyl” theoretical curves. In fact, in both cases calculation assumes a (random) distribution of fixed (fully-)charged and uncharged residues, with the only bound of the total fraction of charging. This is unescapable for the “methoxyl” case, but it is unrealistic for the “proton” case. It is well known that the proton of the carboxylic group has a very high mobility, being in continuous exchange with the solvent and then with other carboxylic

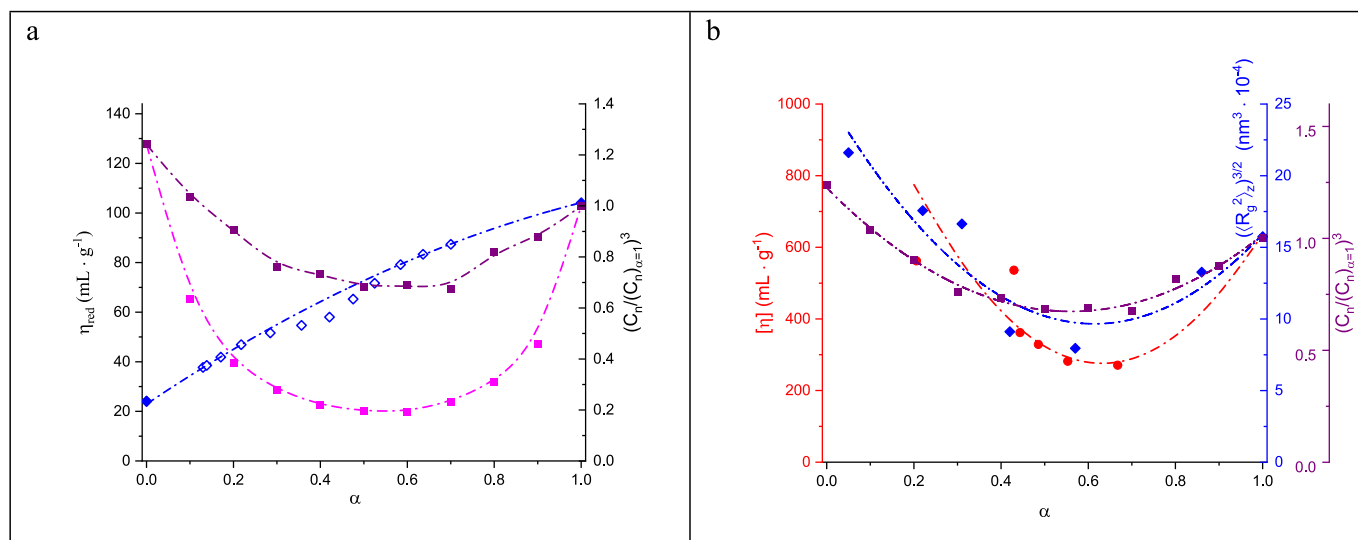


Fig. 3. a. Comparison of the dependence on α of the reduced (specific) viscosity, η_{red} (in $\text{mL} \cdot \text{g}^{-1}$), of pectic acid (blue symbols) and of the third-power of the ratio of the characteristic ratio at α over that at $\alpha = 1$ for the PROT (pink color) and the ESTER case (purple color), respectively, redrawn from Fig. 4 of reference [67]; all cases refer to the salt-free water solution. b. Comparison between the third-power of the ratio of the characteristic ratio at α over that at $\alpha = 1$ for the ESTER case (purple color, r.h.s. outer scale), the radius of gyration (blue color, r.h.s. inner scale) and the intrinsic viscosity (red color, l.h.s. scale) of pectin samples at different values of the % degree of esterification, DE, as a function of the degree of charging, α ($\alpha = (1 - \text{DE})/100$). The data have been redrawn from references [67,68,70]. The dash-dotted parabolic curves have been drawn to guide the eye. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

groups on the polyelectrolyte. In practical terms, at any given value of α (with $\alpha \neq 1$, being the case at $\alpha = 0$ irrelevant for the absence of charge) a more realistic picture of the charge state of the polymer repeating units would be that of an equal (average) value of the fractional charge α on each galacturonic group. It amounts assuming that the timescale of proton fluctuation is much shorter than that of any overall change of polymer conformation. This approach proved to be quite successful in treating the binding of mobile counterions on carboxylated polyelectrolytes [77]. Moreover, such an averaging of the fractional charges on the polyuronate chain (in the absence of events of charge annihilation like in the Ca^{2+} -pectate complexation [61]) conforms very well with the assumptions at the root of the counterion-condensation theory of linear polyelectrolytes [77,78], which proved very effective in describing most solution properties of ionic carbohydrate polymers [19,56,61,79].

A final word of caution then pertains to the conclusion often reported in pectin literature, namely that “the effect of esterification on the polymer conformation is similar to that of protonation” [67] or that “there is no significant influence of the methoxyl group on the conformational behaviour of both the disaccharide and the polysaccharide.” [74]. Whereas the fully uncharged form of poly(galacturonic acid) seems to be only slightly or even not at all affected by the presence of either a proton or by an esterifying methoxyl group (both as agents of charge reduction), this is clearly not any longer true for all partially charged forms of the polymer: “chemistry matters”!

3.4. Charging effects on chain expansion

Altogether, considering the whole of the experimental data and of the theoretical calculations, it is possible to state that:

- in water, the unscreened polyelectrolyte interactions tend to expand the fully charged chain: in salt-free solution, the relative increase of viscosity with respect to the uncharged one is 4.5-fold and that of the persistence length is 1.7-fold. For $I = 0.05$ M, those values reduce to 1.7 and 1.2, respectively. However and besides polyelectrolyte effects, a contribution to expansion in water certainly comes from partial breakdown of the tight 2_1

conformation due to the reduced disordering effect of I in the absence of supporting electrolyte (the “loose” 2_1 helix [61];

- the 3_1 helix is the most stable conformation at low pH values in all conditions of ionic strength, and the “loose” 2_1 helix is the conformation at neutral-to-basic pH values;
- modeling uronate charged groups by carboxyl esterification can lead to very misleading conclusions as to the theoretical conformational behavior of polyuronates.

4. Conclusions

The suitable combination of theoretical modeling and of experiments has allowed achieving the initial goals of the paper, namely to shed more light on hydration and expansion of pectic acid as a function of the fraction of charging of the weak polyacid.

The charged form of galacturonan (namely Na^+ pectate) is much more hydrated than the neutral one: the largest effect pertains to the galacturonate carboxylate group rather than to rest of the pyranose ring. This clearly explains also the driving force for bivalent ion bonding by Na^+ pectate.

Likewise, the charged form of the polyuronide is more expanded than the neutral form, in water. However, by increasing the ionic strength, I , of the medium, the picture progressively changes up to largely reducing the chain expansion gap between the uncharged and the charged forms. At any event, the tendency to behave like a rather expanded chain by galacturonan - already successfully described as a worm-like chain [68] - holds for both the uncharged and charged form. Using the above quoted experimental values of the projected distance between monomers along the axis chain [6,10,11,75], the calculated L_p values in Table 1 imply that, starting from any given monomer, these chains keep memory of direction by about 18 and 29 monomeric units for the neutral and ionized chains in water, respectively. This property can be very easily correlated with propensity to give linear stretches of interchain junctions both in acid and in neutral pectate gels, by interaction with H^+ or Ca^{2+} ions, respectively.

The comparison between theoretical and experimental data has shown that the process of polyuronate charge reduction by protonation and that by methoxyl esterification of the carboxylate groups are NOT

equivalent from the conformational standpoint, thus sending a clear message of warning on the reliability of extrapolation from one type of chemical structure to the other one.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.bpc.2023.106960>.

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