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POLYELECTROLYTIC EFFECTS IN CARBOXYLIC DERIVATIVES OF NATURAL POLYSACCHARIDES

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ABSTRACT

Physicochemical properties of some ionic derivatives obtained by selective oxidation of amylose, scleroglucan, and cellulose are presented. The study concerns the energetics of proton dissociation of these glycouronans in aqueous solution, by potentiometry and microcalorimetry. In addition, characterization of the samples has been carried out by u.v.-circular dichroism spectroscopy and by dilatometry. The dependence of the apparent dissociation constant and of the enthalpy of dissociation as a function of the degree of dissociation is discussed and compared with that obtained by a simple model based on the polyelectrolytic theory.

INTRODUCTION

Ionic polysaccharides are widespread in Nature, and are often related to the binding of a large quantity of water. In the presence of suitable counterions, the special affinity for water may become macroscopically evident by formation of a gel phase. The interactions of water with the polymeric ions in the presence of different counterions have accordingly been studied extensively[1–3].

The wide range of properties arise from at least two variables: (*a*) the chemical structure and sequence of sugar residues in the chain, and (*b*) the nature, density, and distribution of charged groups. Variations in the degree of ionization of the latter, and the ionic strength of the solution, may additionally modify the conformation of the molecular chains by changing the mutual charge repulsion and the extent of couterion screening.

The main theme of our research is the physicochemical investigation of (ionic) polysaccharides under conditions ranging from very dilute aqueous solutions to gels[4–6]. In the present paper, the ionization of some new derivatives is investigated by potentiometric titration, calorimetry, dilatometry, and u.v.-circular dichroism spectroscopy. The new derivatives were obtained by selective oxidation of the primary hydroxyl groups of amylose, cellulose, and scleroglucan[7–9]. Scleroglucan is a (1→3)-linked β-D-glucopyranan having a single, β-D-glucopyranosyl group attached to O-6 of every third unit in the main chain. This kind of derivatiza-

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tion is especially interesting, because it brings about dramatic changes in solution properties without introducing bulky substituents in an irregular manner, as is necessary, for example, in the preparation of carboxymethyl derivatives The absence of this complication makes it possible to discuss the thermodynamics of the ionization process with greater confidence, and to compare these data with theoretical results obtained from current polyelectrolytic theory.

Theory

The polyelectrolytic model. — Central to the nature of ionic polymers are the interactions between the charged polymers and their counterions. These interactions are far more complicated than those occurring in solutions of simple salts, because the charge density on the chain (*i.e.*, local concentration) may reach much higher values. This explains the use of such terms as "ion-atmosphere" or "ion-binding" to describe the accumulation of counterions in the polyelectrolyte domain. An important consequence is that many ionic polymers (including polysaccharides) in aqueous solution undergo conformational transitions between states having different degrees of intramolecular order when, for example, the temperature, the ionic strength of the solution, or the charge density of the polymer is changed. These processes may notably change the formal charge-density on the polyelectrolyte chain as monitored by experimental thermodynamic results.

Although molecular polyelectrolytic theories have been proposed since the early fifties, they have only recently been fully accepted within the different fields of biopolyelectrolytes. Such a theory can be regarded as the polyelectrolytic counterpart of the Debye–Hückel theory for simple electrolytes Among all theories, the most attractive from the formal aspect of the analytical equations is that proposed by Oosawa[10] and by Manning[11,12].

This theory provides a simple equation for the electrostatic part of the free energy of a chain in solution, under the following assumptions: (*1*) the real chain of finite spatial dimensions is replaced by an infinite line of charge; (*2*) the actual charge-distribution is replaced by a linear charge-density with a constant charge-spacing, b, along the line; (*3*) the interactions between two or more linear, charged arrays are neglected (infinite dilution state); (*4*) the dielectric constant, D, of the solution is taken as equal to that of the bulk solvent; (*5*) the free ions in solution are treated under the Debye–Hückel approximation; and (*6*) the reference state for any thermodynamic property is that of a solution containing small ions at a concentration equal to the total charge-concentration of the polyelectrolyte solution. The contribution of mobile ions to the electrostatic, excess free-energy of the solution is neglected. Therefore, the free energy calculated is an excess free-energy relative to the reference state already defined.

Under these conditions, the excess electrostatic Gibbs energy, G^{el}, can be evaluated in terms of Debye–Hückel potentials, summed over all pairs of charges on the poly-ion, to give, for the simple case of monovalent counterions and monovalent fixed charges,

$$G^{el} = F^{el} = -RT\,\xi^{n}\ln(1 - e^{-kb}), \qquad (1)$$

where k is the Debye screening parameter ($k^2 = 4\pi e^2 N(C_+ + C_-)/10^3 Dk'T$), and ξ is the so-called charge-density parameter ($\xi = e^2/Dk'Tb$); ($C_+ + C_-$) is the sum of the concentrations of counter-ions and co-ions, N is the Avogadro number, k' is the Boltzmann constant, and T is the absolute temperature. In Eq. *1*, $n = 1$ when $\xi < 1$, and $n = -1$ when $\xi > 1$. The critical condition $\xi = 1$ arises as a convergence requirement in the procedure of potential summation. The consequence is that, for $\xi > 1$, a fraction, $r = 1 - \xi^{-1}$, of counterions per charged group on the chain will "condense", to lessen the effective, linear charge-density, to $\xi = 1$. For $\xi > 1$, a free energy of "mixing" term, accounting for the entropy change upon ion condensation, is to be added to the right-hand side of Eq. *1*, to give the total excess, polyelectrolytic free-energy.

Analytical expressions can be derived[13–15] from Eq. *1* to evaluate the changes of free-energy, as well as other state functions for such processes as dilution, mixing with ions, and dissociation.

Proton dissociation. — From the expression derived for the electrostatic contribution to the enthalpy of dissociation of a weak polyacid for any condition of ionic strength, it was found that the enthalpy function noticeably depends upon charge density (*i.e.*, upon α), especially in the absence of added salt[14]. The theoretical results given in ref. 14 show a discontinuity of first order in the plot *vs.* α, at the value of $\xi = 1$, similar to that found in the pK_a function and reported by Manning[16]. Because no experimental evidence has ever been produced to confirm this prediction, we decided to redraw an expression for pK_a and ΔH_{diss}, without imposing the equilibrium condition $r = 1 - \xi^{-1}$ in the starting expression for the total polyelectrolytic free energy[17]. Starting from Eq. *1*, the derivative with respect to α has been obtained for any value of the independent variable r, and only thereafter is the equilibrium value[11] of r (*i.e.*, $r = 1 - \xi^{-1}$) inserted in the derivative. This procedure makes the assumption that the same minimum condition holds for both G^{el} and $G_{diss} = 2.303\,RT\,(pK_a - pK^0)$.

The equations for G_{diss} are

$$\xi \leqslant 1$$

$$\bar{G}^{el}_{diss}(\alpha) = \frac{\partial G^{el}}{\partial(\alpha_{nm})} = -RT\alpha\xi^{0}\left\{2\ln(1 - e^{-kb}) + \frac{kb}{e^{kb} - 1}\left[\frac{1}{2}\frac{\alpha}{(2R + \alpha)} - 1\right]\right\}$$

$$k^2 = \lambda\, C_m\,(2R + \alpha)$$

$$\xi \geqslant 1$$

$$\bar{G}^{ion}_{diss} = \bar{G}^{el}_{diss} - \bar{G}^{mix}_{diss} = -RT\frac{1}{\alpha\xi^{0}}\left\{2\ln(1 - e^{-kb}) + \frac{kb}{e^{kb} - 1}\cdot\right.$$

$$\cdot \left[\frac{1}{2} \frac{1}{(2R\xi^0 + 1)} - 1 \right]\Bigr]\Bigr\} + RT \left\{ \left(1 - \frac{1}{\alpha\xi^0} \right) \ln \frac{(\alpha - 1/\xi^0)}{V_p C_p (R + \alpha)} + \right.$$

$$\left. + \frac{1}{\alpha\xi^0} \ln \frac{R + 1/\xi^0}{(R + \alpha)(1 - V_p C_p)} + 1 - \frac{1}{\alpha\xi^0} \right\}$$

$$k^2 = \left[\lambda\, C_m \left(2R + \frac{1}{\xi^0} \right) \right] = \lambda\, C_m [2R + \alpha(1 - r)]$$

where

$$V_p C_p = \frac{(1 - e^{-kb})^2 (\alpha - 1/\xi^0)}{R + 1/\xi^0} \exp \left[\frac{1}{2} \frac{kb}{e^{kb} - 1} \frac{1}{2R\xi^0 + 1} + 1 \right]$$

$$R = \frac{C_s}{C_m} \qquad b = \frac{b_0}{\alpha}$$

$V_p C_p$ represents the volume fraction of the polyelectrolytic phase, including polymer and condensed ions, and where R is the ratio of the molarity of univalent salt to the molar concentration of polymer repeating units, both charged and uncharged; b^0 is the structural distance between the projection of charges on the line axis in the fully ionized form of the polyacid.

In the case of $\xi > 1$, the derivative includes the mixing term due to the condensation process of a fraction of counterions onto the polymer in order to screen the excess charge density to the effective equilibrium value of $\xi = 1$.

An important result of the equations just reported is that they coincide at the value of $\xi = 1$, as expected for a thermodynamic function of state; it is reported graphically in Fig. 5.

Analogously, taking the proper temperature derivative of the electrostatic free energy, followed by insertion of the equilibrium condition, produces two different equations for the two cases ($\xi < 1$, and $\xi > 1$) just reported.

$\xi \leq 1$

$$H^{el}_{diss} = -RT\alpha\xi^0 \left(1 + \frac{d \ln D}{d \ln T} \right) \left\{ 2 \ln(1 - e^{-kb}) + \right.$$

$$\left. + \frac{kb}{e^{kb} - 1} \left[1 + \frac{1}{2} \left(\frac{1}{2} \frac{\alpha}{2R + \alpha} - 1 \right) \left(3 - \frac{kb}{e^{kb} - 1} e^{kb} \right) \right]\Bigr] \right\}$$

$\xi \geq 1$

$$\bar{H}^{\mathrm{el}}_{\mathrm{diss}} = -RT\,\frac{1}{\alpha\xi^0}\left(1+\frac{\mathrm{d}\ln D}{\mathrm{d}\ln T}\right)\Bigg\{2\ln(1-e^{-kb})+$$

$$+\frac{kb}{e^{kb}-1}\Bigg[1+\frac{1}{2}\left(\frac{1}{2}\,\frac{1}{2R\xi^0+1}-1\right)\left(3-\frac{kb}{e^{kb}-1}e^{kb}\right)\Bigg]\Bigg\}$$

Also, in the case of the enthalpy of dissociation state function, numerical coincidence at $\xi = 1$ is now achieved.

EXPERIMENTAL

Materials. — Samples of amylose (A-2 and A-3), cellulose (C-1), and scleroglucan (S-2) were oxidized as previously reported[8,9], following the procedure outlined by Painter[7]. All derivatized samples were stored as freeze-dried solids in the Na salt form. Solutions were freshly prepared from the solid by dissolution in twice-distilled water. Aliquots of the A-2, A-3, and S-2 solution were passed through an ion-exchange resin to convert the polymer into the protonated form, the concentration of which was determined by potentiometric titration. The titer of the C-1 solution was determined after dialysis (four times) against 0.1M aqueous acetic acid, and extensive dialysis against water, until disappearance of traces of acetic acid in the outer compartment of the dialysis system. The cloudy solution of C-1 was homogenized, and an aliquot was titrated, while other aliquots were brought to neutral pH by addition of known amounts of M aqueous NaOH.

Methods. — Calorimetric and potentiometric experiments were performed under controlled conditions by using an LKB 10700-1 flow-type microcalorimeter and a Radiometer PHM52 pH meter, respectively. Calorimetric experiments on C-1 were carried out by using a batch-type LKB 10700-2 microcalorimeter. In all cases, the degree of ionization, α, and the $\mathrm{pK_a}$ were calculated from the degree of neutralization, $\bar{\alpha}$, and from the potentiometric pH value, while the enthalpy change of dissociation was evaluated from the heat response and the corresponding ionization change, $\Delta\alpha$, calculated from the potentiometric data. Analogously, dilatometric experiments were performed by using Carlsberg type dilatometers, as already described[18]. All procedures have been reported in detail elsewhere[4].

Optical activity and circular dichroism measurements were performed with a 141M Perkin–Elmer polarimeter and with a Jasco J-500A dichrograph, respectively.

Osmometric and viscometric measurements were carried out with a Melabs CSM-1 recording, membrane osmometer and an automatic AVS Schott–Geräde Viscometer, respectively.

RESULTS

Characterization of the products. — Samples A-2, A-3, and S-2 were com-

TABLE I

CHARACTERIZATION OF THE SAMPLES[a]

Sample	*Equiv. wt* [b]	*Glucuronic acid (%)*	$M_n/10^3$ *(dalton)*	$[\eta]$ *(dL* g^{-1}*)*
		[c] [d]		
A-2[e]	460	0.40–0.45	8.7	0.072
A-3[e]	360	0.52–0.56	11.1	0.157
S-2	425	0.40	14.5[f]	1.187
C-1	208	0.84	12.1	0.919

[a] Na^+ salts. [b] Free-acid form. [c] Calculated as molar fraction from the equivalent weight [d] Calculated as molar fraction from the sugar analysis. [e] From ref 8 [f] The value refers to sample S-1, obtained in the same way

pletely soluble in water, both as the Na salt and as the free-acid form. On the other hand, sample C-1, which was soluble in the Na^+ salt form, gave turbid solutions with visible, bright *microgels* at low pH. Viscosity and membrane osmometry experiments were carried out on the soluble polymeric forms in salt solutions (to suppress configurational, polyelectrolytic expansion). The values of intrinsic viscosities and number-average molecular weights are reported in Table I, together with some other characteristics of the samples. The values of the molecular weight ($\sim 10^4$ compared to $>2.10^5$ for the parent polymers) do not appear to be related to the degree of oxidation, but rather to the composition of the reaction medium. Comparison of the values of the intrinsic viscosity clearly show that the intrinsic chain extension is greater for samples S-2 and C-1 than for samples A-2 and A-3.

Potentiometric titrations. — Potentiometric titration curves are shown in Fig. 1 for two different polymer concentrations ($\sim 4.10^{-3}$ and 8.10^{-3} eq. L^{-1}, respectively) and ionic strengths (water and 0.05M $NaClO_4$). The apparent pK_a *vs.* α curves reported in Fig. 1a–c show almost regular increase with α, as expected for weak polyacids. Moreover, the addition of salts, as well as increasing polymer concentration, enhance the dissociation, as monitored by the decrease of the pK_a value. Quite different trends are shown by the pK_a curves of sample C-1, reported in Fig. 1d, where a continuous decrease of pK_a is observed as a function of α, although the pK_a is still suppressed by increasing the salt or polymer concentration. While an increase of pK_a with α is understandable for polyelectrolytes that undergo an increase in charge density, it is apparent that the decreasing pK_a values for C-1 must be related to the aggregation process occurring at low pH.

It is also noteworthy that, although solutions of C-1 at low pH were almost opaque, with visible aggregates, the experimental values collected were completely reproducible, provided that the acid C-1 solution was prepared by using the dialysis procedure described in the Experimental section. The experimental pK_a behavior of C-1 has, therefore, an immediate qualitative explanation in a disaggregation process which occurs upon ionization of carboxyl groups, by generating a net

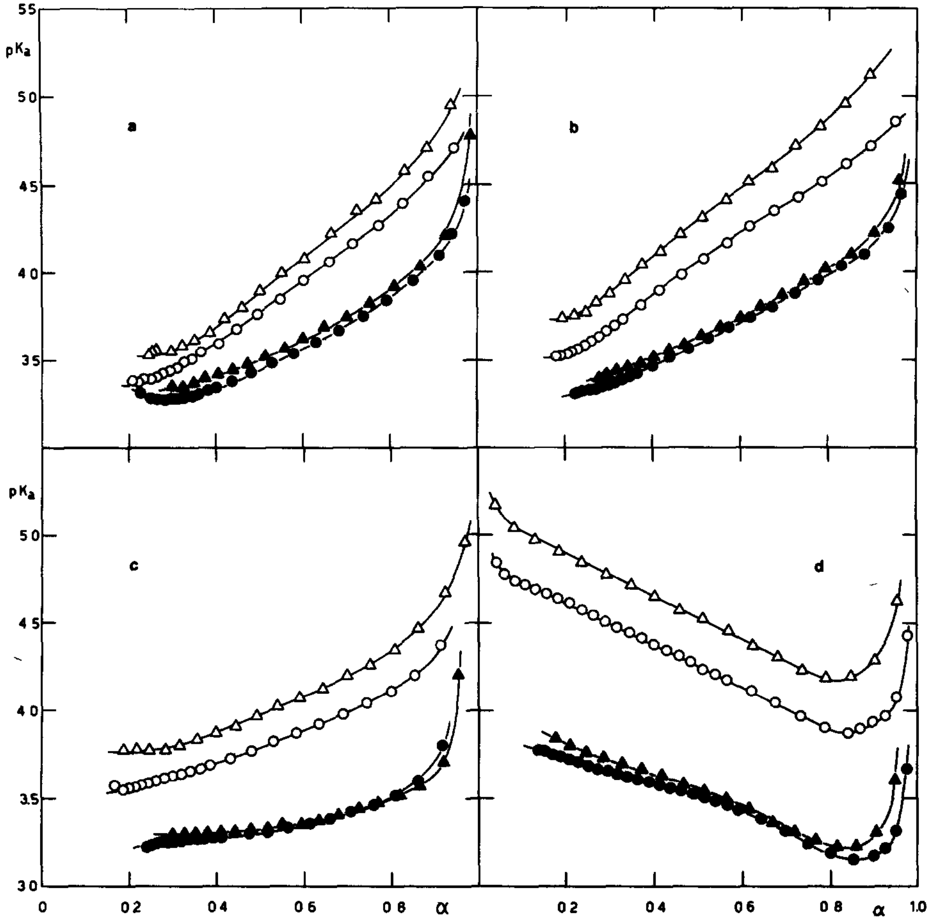

Fig 1. Dependence of the pK$_a$ as a function of α, the degree of ionization, for polyuronides titrated with NaOH in aqueous solution at 25°. (a) A-2, (b) A-3, (c) S-2, (d) C-1 Clear symbols (water), full symbols (aqueous 0.05M NaClO$_4$) Equivalent concentration: 8.0 10^{-3} mol L^{-1} (○,●), 4.0 10^{-3} mol L^{-1} (△,▲)

decrease of charge density on the polymeric (multi)aggregates.

Enthalpimetric and dilatometric titrations. — Differential enthalpies of dissociation, measured as a function of α, are reported in Fig. 2. All curves shown a minimum, located between ($0.5 < \alpha < 0.7$), except for the case of C-1. The experimental ΔH values are the sum of two contributions: one is the "intrinsic" enthalpy change due to the ionization of the carboxyl group; the other is the enthalpy change due to the changes of the charge density on the polymer, including all other contributions which are a consequence of this process (conformational transitions, disaggregation, *etc.*).

While the first contribution may safely be assumed to be constant, provided that all ionizable groups are equal, many topological changes will contribute to the

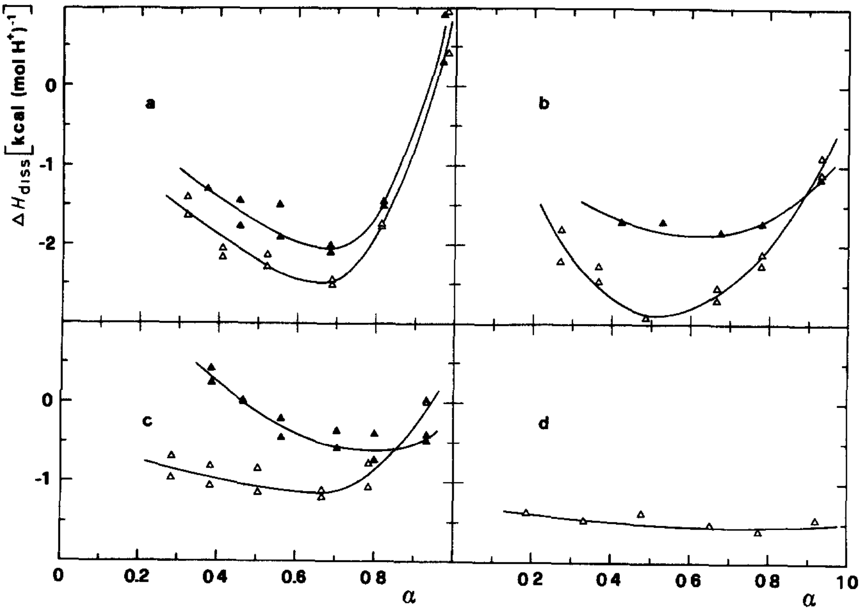

Fig 2 Dependence of the dissociation enthalpy as a function of the degree of dissociation at 25° (For symbols, see legend to Fig 1)

dependence of ΔH upon α. Furthermore, it is not unsafe to assume that the intrinsic enthalpy of dissociation is almost zero, according to literature values for small, carboxylic molecules. As a consequence, most, if not all, of the enthalpic effect would be due to the second contribution It is interesting that, independent of the theoretical equation used, it can be predicted that the α value corresponding to the minimum in the ΔH curve is the value of α where the critical charge density is reached, a result which is evident from the present experimental data.

Quite different, and seemingly more "normal", behavior is exhibited by C-1 (see Fig. 2d), whose values of ΔH are almost constant. Although independence of the intrinsic ΔH upon α could be claimed, it is evident that this constancy arises from a more complex compensation phenomenon, which includes the aggregation occurring at low α values.

Without presenting the complete ΔS curves, which can be calculated from the enthalpic and free-energy data, it suffices to say that all entropic values for the dissociation process are negative, and range between -30 and -15 cal.mol^{-1}.K^{-1}, presenting a minimum corresponding to the minimum observed in the ΔH curves.

Experimental data for the volume changes upon dissociation, treated similarly to the enthalpic data, are reported in Fig. 3. The set of data collected for samples A-1, A-2, and S-2 are practically indistinguishible and, moreover, show a linear dependence upon α, with a slope giving the value of -14 mL/mol H$^+$ for ΔV_{diss}.

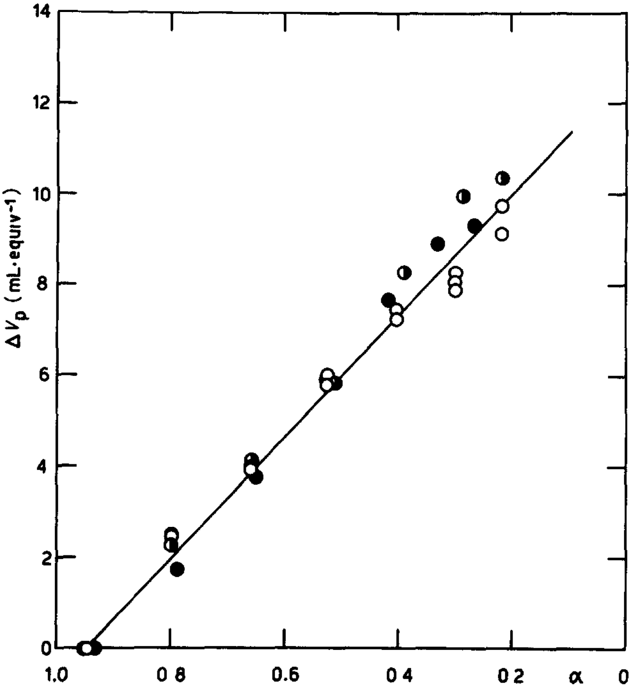

Fig 3 Volume changes upon protonation of polyuronates in water at 25°; polymer concentration: 4.0 10^{-3} mol L^{-1}; A-2 (◐), A-3 (●); S-2 (○)

Circular dichroism. — Circular dichroism (c.d.) spectra were recorded as a function of the degree of ionization in water and in aqueous 0.05M $NaClO_4$, on samples A-2, A-3, and S-2. The dependence of the c.d. spectra upon α is almost linear in all cases, and reflects exclusively the titration of the carboxyl group to carboxylate, presenting an iso-dichroic point centered at 220 nm for A-2 and A-3, and at 230 nm for S-2, respectively (see Fig. 4). No difference in the trend or intensity value was detected between the spectra in water and those in salt solution. The few spectra recorded for sample C-1 (because of the increasing turbidity upon decreasing the pH of the solution) show not only a dependence but also the same shape of the dichroic bands, similar to that of samples A-2 and A-3. Interestingly, both C-1 and samples A-2 and A-3 show a small, positive band at ~230 nm, and a negative band at lower wavelengths in the salt form, showing that the dichroic bands of the carboxyl and of the carboxylate groups do not depend on the configuration of the anomeric carbon atom for the (1→4)-D-glucans. Comparison with the spectra collected for S-2, where a single negative band occurs in the range of wavelength explored, suggests that the positive band at 230 nm may be associated with the perturbation arising from substituted O-4 in samples A-2, A-3, and C-1, but not in sample S-2.

Theoretical results. — Following the procedure outlined in the theory section, the excess free energy, in the form of pK_a, and the electrostatic enthalpy of the

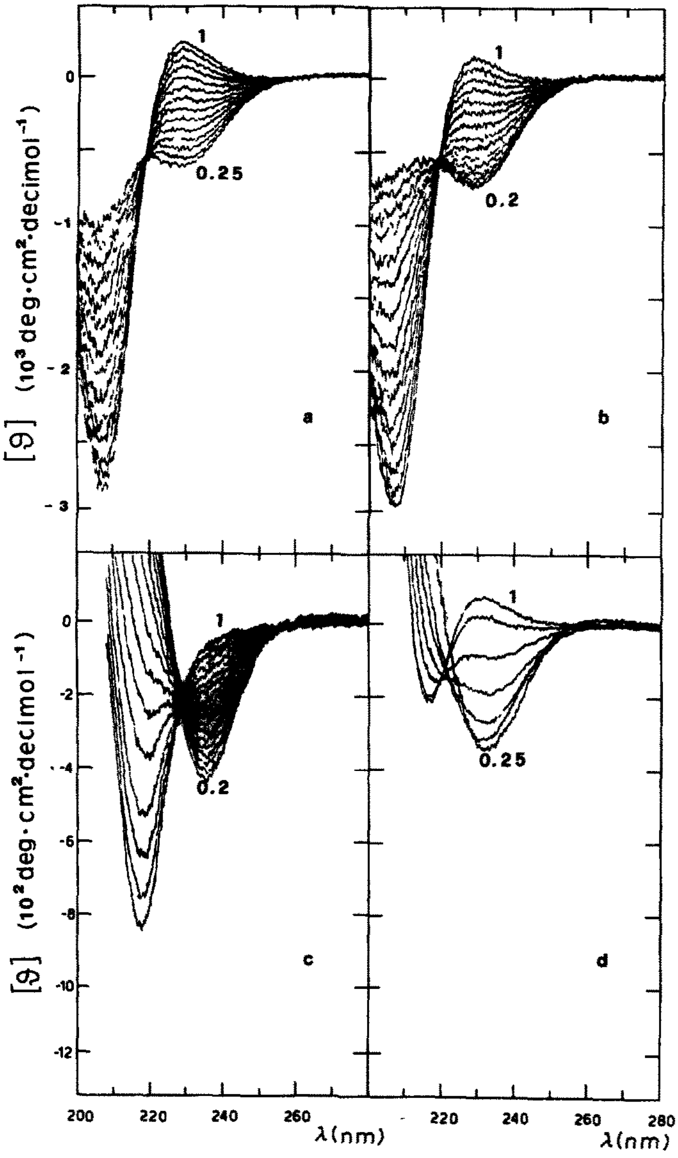

Fig. 4. Circular dichroism spectra of polyuronides as a function of the degree of dissociation in water. (a) A-2, (b) A-3, (c) S-2, (d) C-1. Equivalent concentration (a), (b), and (c) 4.0 10^{-3} mol.L^{-1}; (d) 10^{-2} mol.L^{-1}

dissociation can be calculated for various experimental conditions. In Fig. 5, the values of $pK_a - pK^0$ and of ΔH_{diss} are reported for model polymers corresponding to samples S-2, A-2, and A-3. All three polymers, in both the experimental and calculated results, show an increase of about two units in ΔpK_a in passing from $\alpha = 0$ to $\alpha = 1$. Also, the experimental enthalpic data are in qualitative agreement with

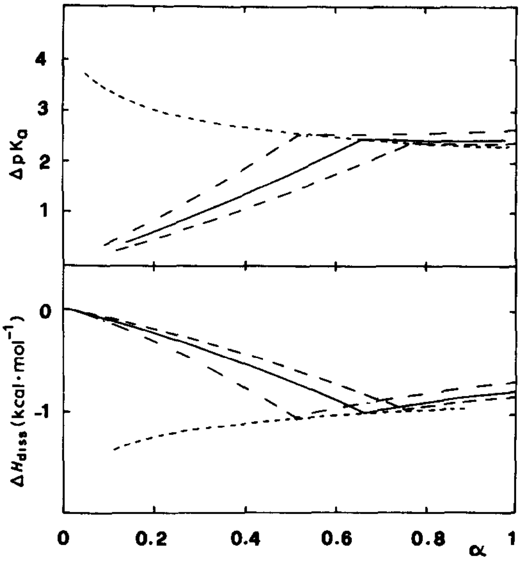

Fig 5. Dependence of the electrostatic part of the dissociation constant (a) and of the dissociation enthalpy (b), calculated with Eqs *2, 3,* and *5, 6,* respectively for the following model chains: (——) charge spacing b = 4.76 at α = 1, (—·—) b = 3.70; (——) b = 5.38, (----) b = 6.13.

the predicted trend of the calculated electrostatic contribution, by showing a minimum in $\Delta H_{\rm diss}$ at the value of α corresponding to a formal, linear charge-density for the value of $\xi = 1$. In fact, by taking the value of 2 Å per residue as the average separation of monomers projected on the axis of the amylose chain in a realistic, solution conformation[19], the value of b^0 for samples A-2 and A-3 (at α = 1) would be 4.76 and 3.70 (on the basis of the measured d.s. of 0.42 and 0.54), respectively. Therefore, it is predicted that samples A-2 and A-3 reach the critical value $\xi = 1$ at a degree of ionization of $\alpha = 0.67$ and $\alpha = 0.52$, respectively. This result is in full agreement with the minimum observed in the plot of $\Delta H_{\rm diss}$ (see Fig. 2a and 2b). Similarly, for sample S-2 it can be deduced from solid-state fiber diffraction data[20] and known d.s. (see Table I) that b^0 = 5 Å. A minimum should be observed in the enthalpy plot at $\alpha = 0.70$, which agrees with the results of Fig. 2c.

The actual value of $\Delta H_{\rm diss}$ and some difference in the experimental trend may well reflect other non-electrostatic or non-modellable contributions taking part in the process. In its present form, the theoretical prediction is a good representation of the real cases, allowing for a continuous increase in the pK$_a$ value as a function of α, although consideration of some previous comments concerning the intrinsic conformational flexibility would notable improve the agreement, both at low α and across the α values for which $\xi = 1$.

Concerning the sample C-1, it is evident that the theoretical model cannot fit the experimental data, because condition 3 of the theoretical section is not met. Nonetheless, one can make the simplest hypothesis that a disaggregation process occurs upon ionization, so that the average distance between charges on each chain aggregate is constant and equal to a minimum b value ($b$min) up to a value of α corresponding to $b$min(Å)/7.134, and decrease as $b = b\text{min}/\alpha$ beyond that. The results calculated for $b\text{min} = b^0 = 6.13$ Å ($\xi = 1.0$ for sample C-1) are reported in Fig. 5. The values of pK_a and of ΔH_{diss} have been calculated from Eqs. *2* and *5*, respectively, for $\alpha < b^0$(Å)/7.134 and from Eqs. *3* and *6*, respectively, for larger α values.

DISCUSSION AND CONCLUSIONS

Most neutral polysaccharides having a regular chain-structure are insoluble in water, behavior which is not ascribable, *a priori*, to the enthalpy of mixing for the polymer and the solvent. Polysaccharide and water experience a large variety of interactions leading to generally small, absolute ΔH values, so that predictions of sign and magnitude are impossible. If the crystalline rigidity is partially retained in solution, the entropy change may be insufficiently favorable, and preclude isotropic solutions. On the other hand, favorable contributions to ΔS are usually significant in the presence of ionic interactions, as the introduction of fixed charges on the polymer chain is the most efficient way of decreasing the free energy of mixing with water. The presence of uronate residues in certain polysaccharide structures has stimulated thermodynamic studies of the energetics of the process of ionization[1–5,21–23]. Literature data provide a value of 3.23 for the pK_a of glucuronic acid[22,23], while no data are available on the enthalpy of dissociation. Only recently have a few calorimetric data been collected and reviewed[24], showing that proton dissociation of polyuronides in water is almost constantly exothermic with values of ΔH reaching approximately -1.8 kcal.(mol $H^+)^{-1}$ at $\alpha = 1$, except for a few "anomalous" cases. This result is extremely interesting in view of the fact that the enthalpy of protonation seems to be largely independent of the nature of the monomers. Deviations from this "base line" at -1.8 kcal also disclose a dependence of ΔH upon the degree of ionization α of the polymer. At least for two polysaccharides, poly(galacturonic acid) and xanthan, the trend to positive values of ΔH is paralleled by sigmoidal changes in other properties, and has been ascribed to conformational changes induced by the ionization of carboxyl groups[4,21].

The polyacids studied herein are representatives of an interesting group of "synthetic" glycuronans with physicochemical properties which partially reflect structural features of the parent polymers. Even if the reaction procedure could be improved, mainly in order to diminish the depolymerization, the products obtained show typical polyelectrolyte effects, at least for what concerns the proton dissociation here discussed.

Among the physicochemical properties reported, the integral volume changes upon protonation seem to be independent of the degree of ionization, the conformation, and the type of linkage. This behavior seems to be more a rule than an exception[25,26], as evidenced by the constancy of the volume change, even in the case of poly(galacturonic acid), where a cooperative, conformational transition takes place[4]. The position and the change of intensity of the two circular dichroic bands, in the region of carboxyl group absorption, depend on the pH and on the absolute configuration of the D-glucosyl residues in the polymeric chain, but not on the degree of oxidation. The present results show a negative band at 240 nm for the uncharged polyacids, which is in qualitative agreement with that reported in the literature on model methylglucuronic acids[27], but our results clearly show that the bands also depend on the enchainment, especially in the salt form. It is suggested that c.d. bands are sensitive to the local dissymetry, *e.g.*, configuration of carbon-5 and substituents on O-4 and -3, but not to long-range conformational features.

The other thermodynamic data for the dissociation of the oxidized derivatives of amylose and scleroglucan do not show "irregular" trends which are known to occur in the case of cooperative conformational transitions. They are good models for the application of theories aimed at exploring the structure–properties relationship in the polyelectrolyte field.

Data have also been reported for a sample of oxidized cellulose, for which the atypical behavior can be easily ascribed to, and simulated with, the occurrence of an aggregational process at low pH.

Although some of the theoretical derivations here reported may still suffer from the inadequacy of the assumptions discussed elsewhere[14], the theory is nonetheless able to give a consistent set of predictions as far as the energetic behavior of polyelectrolyte solutions is concerned. The possibility of factorizing the purely electrostatic contribution from the nonionic one appears to be a significant achievement in the study of ionization process, and enables one to disclose possible conformational transitions and to evaluate the nonionic contribution. As regards conditions *1* and *2* reported in the theory section, the charge parameter is usually calculated from the structural parameters of the chain in the solid state. The assumption of such a rigid conformation in solution may appear unrealistic when the polysaccharidic chain can experience a large set of conformational states. An example is given herein for the amylose derivatives, for which the charge density has been calculated from the realistic conformation of the chain in solution based on a statistical mechanical model, and not from one of the helical forms existing in the solid state. The agreement between theory and experiment as to the position of the minimum in the enthalpy of the dissociation curve further validates the present procedure.

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